THE IMPACT OF WASTEWATER QUALITY ON RECEIVING WATER BODIES AND PUBLIC HEALTH IN BUFFALO CITY AND NKONKOBE MUNICIPALITIES

by

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DECLARATION

I, the undersigned, declare that this dissertation submitted to the University of Fort Hare for the degree of Master of Science and the work contained herein is my original work unless cited and has not been submitted at any other university for any degree.

Signature:

Date:

SUMMARY

The Eastern Cape Province of South Africa is composed mainly of rural areas where the communities still rely heavily on surface water sources for their domestic, irrigation and recreational water needs. This fact places major importance on effluents discharged from wastewater treatment plants into the surrounding surface water bodies to adhere to stringent water quality standards. The chemical and microbiological quality of the effluents must therefore be closely controlled and diligently monitored so that these needs can be met without the communities in the region being put at risk of contracting waterborne diseases.

This study evaluated the efficiency of the various wastewater treatment plants for the removal of chemical and microbiological contaminants in order to establish the relationship between the quality of the final effluents and that of the receiving water bodies. To this end, four wastewater treatment plants in the region, i.e. Alice and Fort Beaufort (which both serve the Nkonkobe municipal region), Dimbaza and East London (which both serve the Buffalo City municipal region) were investigated. Wastewater samples were taken monthly from the individual plants analysed from the 6th August 2003 to the 24th March 2004. Wastewater samples were physicochemically characterised according to biological oxygen demand (BOD), chemical oxygen demand (COD), dissolved oxygen (DO), pH, phosphate, residual chlorine, temperature, total nitrogen and total suspended solids (TSS). Student's t-test was used to compare the physicochemical parameters in the effluent and receiving water body samples. The targeted pathogenic microorganisms under investigation in this study were *Salmonella typhimurium, Shigella dysenteriae* and *Vibrio cholera*. Standard methods were applied in all aspects of the analyses for the isolation and detection of these microorganisms and also for the identification of the general microbiological quality of the effluent and the receiving water bodies. Polymerase chain reaction (PCR) was used to confirm the presence of the target microorganisms. The risk assessment was conducted based on the outcome of molecular characterisation of isolates to study the impact of target microorganisms on the health of the communities.

All the four wastewater treatment plants, the quality of both the effluents and the receiving water bodies were acceptable with respect to the temperature (mean range: 16.52 - 23.33 °C), pH (mean range: 7.79 - 8.97), chemical oxygen demand (COD) (mean range: 7 - 20 mg/l) and total suspended solids (TSS) (mean range: 161.43 - 215.67 mg/l). However, the nutrients (orthophosphate - mean range: 3.70 - 11.58 mg/l and total nitrogen - mean range: 2.90 - 6.90 mg/l) were eutrophic. The dissolved oxygen (DO) (mean range: 3.26 - 4.57 mg/l) and the biological oxygen demand (BOD) (mean range: 14 - 24 mg/l) did not comply with the recommended limits which were 3.0 - 6.0 mg/l for BOD and 5 mg/l for DO.

Chlorine residuals were determined in the final effluent and the receiving surface water body samples. Although the South African Water Quality Guidelines do not specify any standard for the concentration of free chlorine residual in treated wastewater effluent, this study considered a baseline concentration of 0.1 mg/L. Based on this concentration, the free chlorine residual in the effluents indicated the availability of free chlorine residual in all the wastewater treatment plants. The exception in free chlorine residual concentration of 0.05 mg/L was noted at the Fort Beaufort wastewater treatment plant. Although the results of this study revealed that the free chlorine residual concentration fell within the 0.1 mg/L range, the occurrence of faecal coliforms such as *Salmonella typhimurium, Shigella dysenteriae* and *Vibrio cholerae* could still be detected in the final effluent. With the exception of the Alice plant, all others plants measured and kept records of the chlorine residuals.

In general, a gradual removal of presumptive bacterial pathogens was observed in the different zones of the wastewater treatment plants. Although, there were variations with regards to both the patterns and the efficiency of each plant for the removal of the target pathogens, about 71% of the total influent samples contained presumptive *Salmonella*, while 50% and 33.5% of the effluents and receiving water body samples were observed to contain presumptive *Salmonella* respectively. Similar observations were made for presumptive *Shigella* and *Vibrio* pathogens with decreasing incidences of the pathogens from influents to the receiving water bodies. The presence of these presumptive pathogens in the enriched cultures is indicative of the presence of at least one cell per 100 ml of the wastewater samples. Hence, the microbial qualities of the effluents in all locations exceeded the maximum safety limit for effluent discharge by the South African General and Special Standards of nil faecal coliforms/100mL. The microbiological quality of the effluent generated and in turn discharged into the receiving water body was poor, unsafe for recreational or irrigational use and not acceptable for human consumption.

Among the potentially dangerous pathogens microorganisms isolated were *Aeromonas hydrophila, Enterobacter cloacae, Escherichia coli, Klebsiella pneumoniae, Klebsiella ornithinolytica, Pasteurella pneumonia, Proteus mirabilis, Providencia rettgeri* and *Salmonella* spp. Some of the pathogenic microorganisms found in the influent of the wastewater treatment plants were retained in their respective final effluent and went on to impact on the microbiological quality of the receiving water bodies.

The Dimbaza wastewater treatment plant comparatively produced the final effluent quality microbiologically that contained only 6 (*Aeromonas hydrophila, Enterobacter aerogenes, Klebsiella pneumoniae, Klebsiella ozonae, Proteus mirabilis, Providencia rettgeri*) out of the 10 species of the pathogenic microorganisms identified in the influent being found also in the final effluent. This was in contrast with the results obtained from the other three treatment plants for which the data showed poor removal of the pathogens identified in the influent, with 9 out of 11 in Alice (*Aeromonas hydrophila, Enterobacter aerogenes, Enterobacter cloacae, Escherichia coli, Klebsiella pneumoniae, Morganella morganii, Proteus mirabilis, Providencia rettgeri* and *Serratia odorifera*), 8 out of 12 in East London (*Aeromonas salmonicida, Enterobacter cloacae, Klebsiella pneumoniae, Klebsiella oxytoca, Klebsiella ornithinolytica, Proteus mirabilis, Providencia rettgeri* and *Salmonella* spp.) and 7 out of 9 in Fort Beaufort (*Enterobacter cloacae, Escherichia coli, Morganella morganii, Pasteurella pneumoniae, Proteus mirabilis, Providencia rettgeri* and *Salmonella* spp.). In the various wastewater treatment plants, some pathogenic microorganisms found in the final effluent also reappeared in the receiving water bodies. Hence *Salmonella* spp. and *Vibrio cholerae* were genetically confirmed by PCR analysis.

The effluents from the four wastewater treatment plants have contributed to the microbiological contamination of their respective receiving water bodies.

The health risks were found to be high since the 95th percentiles of *Salmonella* and *Vibrio cholerae* infection were above the US EPA, 1994 acceptable risk limit of 0.01% which indicates no probability of infection. There are no acceptable risks for South Africa. The results showed that health effects were potentially associated with significantly higher occurrences of the target pathogens detected during the water quality monitoring period. There is therefore much need to address wastewater treatment problems in these communities as the effluents or the receiving water bodies are used for various purposes.

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DEDICATION

This dissertation is dedicated to the LORD Almighty and supreme God whose divine love and protection has brought me to this stage of my education. Thank you, Father, for remaining faithful to me in my unfaithfulness, for showing me how exalted, how lifted up and glorious you are.

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LIST OF ABBREVIATIONS

APHA	-	American Public Health Association
BOD	-	Biochemical Oxygen Demand
COD	-	Chemical Oxygen Demand
DFID	-	Department of International Development
DNA	-	Deoxyribonucleic acid
DO	-	Dissolved Oxygen
DWAF	-	Department of Water Affairs & Forestry
TSS	-	Total Suspended Solids
US EPA	-	United States Environmental Protection Agency
WHO	-	World Health Organisation
WRC	-	Water Research Commission

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CHAPTER 1

GENERAL INTRODUCTION

Access to safe water and to sanitary means of excreta disposal are universal needs and, indeed, basic human rights. Safe water and sanitation are essential elements of human development and poverty alleviation and constitute an indispensable component of primary health care. In developing countries, the lack of adequate water supply and sanitation services is one of the most important environmental issues. The problem is particularly acute in the densely populated peri-urban areas and rural areas where the large majority of the dwellers are typically low-income people. It is estimated worldwide that over half a billion urban people and over 2 billion rural people lack sanitation services (UNEP, 2002). Despite the efforts of the last two decades, investment in the sanitation sector has remained inadequate while the needs have continued to grow especially with regard to wastewater treatment. This situation is a result of the low priority given to treatment. It is roughly estimated that less than 5% of all the wastewater in developing countries receive any treatment before discharge into the environment (UNEP, 2002).

Wastewaters show different degrees of environmental nuisance and contamination hazard due to their chemical and microbiological characteristics (Bohdziewicz and Sroca, 2005). In municipal wastewater, the common chemical quality variables of concern are chemical oxygen demand (COD), suspended solids, ammonia nitrogen, phosphorus, salinity and a range of trace metals. The biological nitrogen and phosphorus removal system has been extensively investigated for municipal wastewater treatment. Excessive nutrients (primarily nitrogen and phosphorus), in wastewater, sludge and excreta, may contaminate surface waters and can cause eutrophication. Eutrophication of freshwater sources may create environmental conditions that favour the growth of toxin-producing cyanobacteria. Toxins produced by cyanobacteria can cause gastroenteritis, liver damage, nervous system impairment and skin irritation. Chronic exposure to cyanobacteria toxins has been associated with liver cancer in animals and may cause similar effects in humans (Chorus and Bartram, 1999).

Wastewater pathogens that most frequently cause disease include *Salmonella* spp., *Shigella* spp., pathogenic *Escherichia coli*, *Vibrio cholerae*, *Yersinia enterocolitica*, *Campylobacter jejuni*, Hepatitis A viruses, *Giardia* spp., *Cryptosporidium* spp., and *Entamoeba histolytica* (WHO, 1993). Most of these pathogens are distributed worldwide but outbreaks occur more frequently and endemicity is higher in areas where access to good quality water supplies and sanitation is limited (UNEP, 2002). In the industrialized world, reduction in the occurrence of waterborne diseases such as cholera, typhoid and other diarrhoeal diseases has contributed substantially to reducing the infant death rate. In contrast, developing countries continue to experience a devastating toll of illness and death in many of the most populous regions of the globe. It is estimated that 2.1 million people die every year from diarrhoeal diseases and the majority of these deaths are among children in developing countries and 65% of these fatalities could be prevented by water, hygiene and sanitation interventions (WHO, 2002).

The final effluent that is released to nature can be reused for agricultural and industrial purposes, or recycled and used as drinking water. The World Health Organization (WHO) Health Guidelines (WHO, 1989) and the United States Environmental Protection Agency (US EPA/USAID) Guidelines (US EPA, 1992) has influenced standards for wastewater reuse in many countries. The WHO Health Guidelines focus mainly on the presence of pathogens, while the Environmental Protection Agency (EPA) also includes physicochemical parameters such as organic load (Biological Oxygen Demand (BOD_5)/Chemical Oxygen Demand (COD), Total Suspended Solids (TSS) and residual chlorine concentration (US EPA, 1992). These effluents when released to the river constitute an important health risk for the population using this water for other purposes such as clothes cleaning, bathing or even drinking, if not disinfected.

The South African General and Special Standards stipulate that treated sewage effluent should comply with a standard of nil faecal coliforms/100mL (National Water Act No 45, 1999). This standard can only be achieved by disinfection. Disinfection methods available include physical process such as ultraviolet radiation (Carmimeo *et al*., 1994) and chemical processes such as chlorine, monochloramines, bromine and ozone (Aieta *et al*., 1980; Jacangelo *et al*., 1989; Pretorius and Pretorius, 1999). Although regulations require disinfection of effluents prior to discharges, it has been found that such effluents were either inadequately disinfected or not disinfected at all (Simpson and Charles, 2000).

Microbiological health risks remain associated with many aspects of water use from drinking water in developing countries (Lloyd *et al*., 1989) to irrigation reuse of treated wastewater (Rose, 1986) and recreational water contact (Grabow, 1991). Drinking water poses a very high health risk; however, the risk can be reduced by effective treatment and by applying drinking water criteria. Agricultural use means the irrigation of crops that may be eaten uncooked (Jagals, 2000). Recreational uses refer to activities with full bodily contact of the water such as swimming or intermediate contact such as water-skiing, canoeing or angling (DWAF, 1996b). During recreational contact, dermal absorption and accidental ingestion may pose a potential health risk.

With a growing human population and continued improvement of quality of life, water resources are under stress both quantitatively and qualitatively (Gleick, 2002). The world supply of freshwater is limited and threatened by pollution from various human activities. Rising demands for water to supply agriculture, industry and cities are leading to competition over the allocation of the limited freshwater resources (Gleick, 2002). In South Africa, the quality of surface water is of particular concern because 80% of the population relies on the surface water as their main source of water (Venter, 2001). The faecal contamination of South Africa's water resources is becoming an increasing threat (Momba and Mfenyana, 2005; Momba *et al*., 2006a). The major causes of faecal contamination of water sources such as rivers, dams, groundwater as well as drinking water are: i) release of partially treated sewage or sewage leakage ii) leaching of poorly maintained septic tanks iii) improper management of farm waste and iv) run-off of faecal matter during rainy periods (Isobe *et al*., 2004). It therefore becomes imperative to monitor the quality of effluent discharged into water sources in order to highlight the quality of water sources and to provide the impetus for sustained government intervention where necessary, which is the subject of this investigation.

The aim of this study was to evaluate the efficiency of the urban, semi-urban and rural wastewater treatment plants for the removal of chemical and microbiological contaminants in order to establish the relationship between the quality of the final effluent and that of the receiving water body which might further in case of pathogenic microorganisms, influence infection and disease in the community. To achieve this aim, the following objectives were pursued:

(i) To assess the performance of four wastewater treatment plants that serve the Buffalo City (Dimbaza and East London) and Nkonkobe municipalities (Alice and Fort Beaufort), in terms of nutrient removals (phosphate, total nitrogen) and physicochemical characteristics (biological oxygen demand (BOD), chemical oxygen demand (COD), dissolved oxygen (DO) and total suspended solids (TSS) which might lead to the pollution of water sources.

(ii) To evaluate the performance of the individual wastewater treatment plants for the removal of *Salmonella typhimurium, Shigella dysenteriae* and *Vibrio cholerae* and identify the origin and clinical relevance of microbiological contaminant responsible for the pollution of water sources and waterborne diseases and their impact on public health in general and the health risk these pathogens pose to the Eastern Cape community in particular.

This study was planned to generate valuable information that could be used by water engineers, wastewater treatment plant managers, microbiologists and especially the Department of Water Affairs and Forestry and the Department of Health in order to develop or review effective policy for the discharge of effluents into water sources.

CHAPTER 2

BACKGROUND OF THE STUDY

Theoretically and by law, raw sewage is treated in wastewater treatment plants before being discharged into surrounding surface waters. Treatment of wastewater aims at the removal of (i) Oxygen consuming organic matter (due to potential oxidation), (ii) Nutrients, (iii) Harmful hazardous and toxic substances, and (iv) Pathogens (WHO, 2004). The present chapter focuses on the chemical and microbial contaminants of wastewater and their effect on the health of the community once they are found in the effluent and receiving water bodies at the concentrations exceeding the recommended limits.

2.1 CHEMICAL CONTAMINANTS IN WASTEWATER

In municipal wastewater, the common chemical quality variables of concern are chemical oxygen demand (COD), suspended solids, ammonia nitrogen, phosphorus, salinity and a range of trace metals as illustrated in Table 2.1 and 2.2. These contaminants are derived from domestic sewage, the urban runoff, including wet and dry deposition from atmosphere and industrial discharges (Blanchard *et al*., 2001; Katsoyiannisi and Samara, 2004). Household wastewaters are derived from toilet, kitchen, bathroom, laundry and others. Wastewater from the toilet is termed 'blackwater'. It has a high content of solids and contributes to significant amount of nutrients (nitrogen, N and phosphorus, P). Blackwater can be further separated into faecal materials and urine. Each person on average excretes about 4 kg N and 0.4 kg P in urine and 0.55 kg N and 0.18 kg P in faeces per year (UNEP, 2002). Greywater consists of water from kitchen, bathing/showering and washing of clothes. The former may have a high content of solids and grease, and depending on its intended reuse/treatment or disposal can be combined with toilet wastes and form the blackwater (UNEP, 2002). In order to minimize environmental pollution it is necessary to treat wastewater before it is discharged.

Table 2.1: Chemical Compositions of Domestic Wastewater (Veenstra *et al*., 1997)

PARAMETER	DOMESTIC WASTEWATER
TSS (Total suspended solids) mg/l	160 – 1350
BOD (Biological oxygen demand) mg/l	120 – 1000
COD (Chemical oxygen demand) mg/l	280 – 2500
Kjeldahl nitrogen (Kj-N) mg/l	30 – 200
Total-Phosphorus mg/l	4 – 50

Table 2.2: Chemicals of Health Concern Found in Untreated Municipal Excreta and Wastewater and (Chang *et al*., 1995; National Research Council, 1998; World Health Organisation, 1999; ATSDR, 2000)

Chemical	Health Effect
Halogenated Compounds	
Chloroform	Skin irritation, nausea, embryo/fetotoxic
DDT	Nervous system damage, cancer
Di and tri-chlorobenzenes	Liver, kidney, and blood damage
Heavy Metals	
Arsenic	Gastrointestinal, skin, and nerve damage, cancer
Cadmium	Gastrointestinal, kidney and lung damage
Lead	Nervous and immune system, kidney damage, fetotoxic
Inorganic Chemicals	
Cyanide	Brain and heart damage, shortness of breath, death
Hydrogen Sulfide	Nausea, vomiting, mucous membrane irritation
Nitrate	Methaemoglobinaemia
Nutrients	
Nitrogen Phosphorus	Cause eutrophication which facilitates the growth of toxin-producing cyanobacteria and other harmful algae
Organic Chemicals	
Benzene	Anaemia, dizziness, leukaemia
Phenol	Irritation of skin, eyes, gastrointestinal tract, systemic toxicant
Xylene	Confusion, dizziness, memory loss, embryo/fetotoxic
Other Chemicals	
Endocrine disruptors and Pharmaceuticals	Reproductive/developmental effects in wildlife, various potential effects in humans

The most appropriate wastewater treatment is one that will produce an effluent meeting the recommended chemical guidelines both at a low cost and with minimal operational and maintenance requirements. Modern wastewater treatment plants can effectively accomplish carbon and nitrogen removal, as well as microbial pollution control (Johnson *et al*., 2000). However, conventional treatment technologies have not been specifically designed for the different organic contaminants of wastewater (natural or synthetic) Johnson and Sumpter, 2002). The removal efficiencies are influenced, apart from their physico-chemical properties, by microbial activity and environmental conditions (Katsoyiannisi and Samara, 2004). Several studies have shown that the elimination of organic contaminants is often incomplete (Manoli and Samara, 1999; Carballa *et al*., 2004) rendering wastewater treatment plants important sources of toxic chemicals to the receiving environment (Bergh and Peoples, 1977; Shannon *et al*., 1977; McIntyre *et al*., 1981; Barrick, 1982; Eganhouse and Kaplan, 1982).

Legislations are enforced to ensure that the recommended guidelines are achieved. The general wastewater limit values applicable to discharge of wastewater into a water source in South Africa, according to the amended Act 54 of 1956 are as follows: 75 mg/L for Chemical Oxygen Demand, 0.25 mg/L for chlorine as free chlorine and 10 mg/L for orthophosphate as phosphorus while soap, oil or grease has a limit of 2.5 mg/L (Water Act, 1956). The special limits for effluent disposal in catchments areas for parameters such as Chemical Oxygen Demand, Chlorine as free chlorine and orthophosphate as phosphorus are not allowed to exceed 30 mg/L, 0 mg/L and 1 mg/L respectively (National Water Act No 45 (1999). The effluent must not contain any substance capable of producing colour, odour or taste (Bitton, 1994).

2.2 MICROBIAL CONTAMINANTS IN WASTEWATER

Wastewater treatment plants discharge significant amounts of pollution-indicator and pathogenic micro-organisms, leading to the deterioration in the quality of water sources (Bahlaoui *et al*., 1997; Kühn, *et al*., 2000; Simpson and Charles, 2000, Momba and

Mfenyana, 2005). However the South African General and Special Standards stipulate that treated sewage effluent should comply with a standard of nil faecal coliforms/100mL (National Water Act 45 of 6 December 1999). The failure to properly treat and manage wastewater and excreta world-wide is directly responsible for adverse health and environmental effects (WHO, 2001). Human excreta have been implicated in the transmission of many infectious diseases including cholera, typhoid, hepatitis and cryptosporidiosis (WHO, 2001). The density and diversity of faecal pathogens can vary depending on the intensity and prevalence of infection in the sewered community. High concentrations of pathogens are often reported in raw sewage worldwide (US EPA, 1992). Table 2.3 shows the various classes of microbial pathogens found in untreated wastewater and their related diseases. The main focus of this study is largely on bacterial pathogens that cause waterborne diseases.

Table 2.3: Pathogens Found in Untreated Municipal Wastewater (Sagik *et al*., 1978; Edwards, 1992 and National Research Council, 1998).

Agent	Disease
Bacteria	
Campylobacter jejuni	Gastroenteritis, long term sequelae (*e.g*. arthritis)
Escherichia coli	Gastroenteritis
E. coli 0157:H7	Bloody diarrhoea, haemolytic uremic syndrome
Helicobacter pylori	Abdominal pain, peptic ulcers, gastric cancer
Legionella pneumophila	Legionnaire's disease
Leptospira (spp.)	Leptospirosis
Salmonella (various serotypes)	Salmonellosis, long term sequelae (*e.g*. arthritis)
Salmonella typhi	Typhoid fever
Shigella (3 serotypes)	Shigellosis (dysentery), long term sequelae (*e.g*. arthritis)
Vibrio cholerae	Cholera
Yersinia enterocolitica	Yersiniosis, long term sequelae (*e.g*. arthritis)
Protozoa	
Balantidium coli	Balantidiasis (dysentery)
Cryptosporidium parvum	Cryptosporidiosis, diarrhoea, fever
Cyclospora cayetanensis	Persistent diarrhoea
Entamoeba histolytica	Amoebiasis (amoebic dysentery)
Giardia lamblia	Giardiasis
Viruses	
Adenovirus (many types)	Respiratory disease, eye infections
Norovirus (several types)	Gastroenteritis
Enteroviruses (various types)	Gastroenteritis
Coxsackie A	Herpangina, aseptic meningitis, respiratory illness
Coxsackie B	Fever; paralysis; respiratory, heart and kidney disease
Norwalk virus	Gastroenteritis
Hepatitis A and E virus	Infectious hepatitis
Parvovirus (several types)	Gastroenteritis
Rotavirus (Groups)	Gastroenteritis

2.2.1 Waterborne outbreaks

An outbreak can be defined as an increase of cases of a particular infection above what would be normally expected (WHO, 2001). The potential for human exposure to the pathogens responsible for causing enteric disease is ever-present because of the proximity of humans to sources of faecal waste. Environmental conditions (heavy rainfall or runoff) may allow greater exposure to pathogens, giving rise to a sufficient dose and resulting in disease. The causative agent may become more important because of an increased source of pathogen dose (sewage spill) or increased virulence of the pathogens (mutation to create a more virulent strain). Exposure of immunocompromised individuals increases the risk of disease (Craun, 1991).

In developing countries, treatment of water and waste is often non-existent or grossly inadequate and until sanitation is improved it will be impossible to impact greatly on the level of waterborne disease. Deficiencies in treatment and delivery systems, anthropogenic impacts on source water and the emergence of resistant and more virulent microorganisms pose serious threats to human health in industrialised countries such as:

- Less immunity to pathogens (because of better sanitary conditions and a higher population of immunocompromised individuals) and the resulting higher susceptibility and risk of disease during systems failure
- Anthropogenic alterations of water systems that have stimulated eutrophication, changes in food chain structure and unrestricted growth of "nuisance species", creating breeding sites for vector-borne diseases
- Changes in agricultural production methods, including high-density animal operations carried out in proximity to urban development, leading to an increase in transmission of animal pathogens to humans. Ageing and deteriorating environmental infrastructure, particularly in inner cities (Craun, 1991).

Various reports on the impact of waterborne diseases in countries worldwide revealed thousands of outbreaks due to bacterial, protozoan and viruses associated with the consumption of treated and untreated water (Ford and Colwell, 1996; WHO, 1996; Hunter, 1997). The following section will deal with specific microbial pathogens relevant to outbreak case studies. Although protozoa and virus are not the main focus of the present study, a brief summary of the diseases caused by some of these microorganisms will be part of this section.

2.2.2 Wastewater pathogens and their related diseases

Most waterborne pathogens are in human and animal feces and enter water along certain pathways. Important pathways include defecation in water bodies, carried by overland flow and/or subsurface water flow. Human contamination or inadequacies at water treatment plants have been implicated in large-scale waterborne outbreaks while most current waterborne outbreaks are associated with swimming pools and recreational water bodies (lakes and rivers) (Levy *et al*., 1998; Upton, 1999).

2.2.2.1 *Hepatitis A Virus*

Hepatitis A virus belongs to the family of *Picornaviridae* (Hunter, 1997; Sobsey, 1999). Humans are the natural and primary reservoir of Hepatitis A virus, but other primates can be infected. The normal route of transmission for Hepatitis A virus is faecal-oral, typically person-person, but transmission can occur in water due to faecal contamination (Lemon, 1997). Hepatitis A virus is stable in water warmer than 60°C and is relatively stable in the environment, being resistant to heat (70°C for up to 10 min), extremes of pH (pH 1 for 2 h at room temperature) and microbial proteolytic enzymes naturally found in water (Hollinger and Ticehurst, 1996).

The infectious dose for Hepatitis A virus is unknown, but has been assumed to be in the range of 10 to 100 virus particles. After ingestion, the virus passes through the stomach and begins to replicate in the lower intestine, continuing its main replication after uptake in the liver. Bile transfers the virus to the intestinal tract to contaminate faeces. The incubation period is 3 to 5 weeks (Baron, 1996). The viral numbers in faeces increases in peak levels during one or two weeks before the onset of symptoms and decline rapidly after symptoms appear (Chin, 2000).

Hepatitis A virus causes “infectious hepatitis” with symptoms of acute inflammation of the liver (Sobsey, 1999). The fatality rate from Hepatitis A virus infection is low; typically 0.1 to 0.3%, but the illness can be severe in those over 50. The disease in children is often asymptomatic, but in adults, Hepatitis A virus infection shows an abrupt onset of fever, malaise, appetite loss, nausea, dark urine and abdominal discomfort, typically followed by jaundice with an enlarged and tender liver. It is usually a mild illness, lasting one to two weeks, but it may be severely disabling with duration of several months (Chin, 2000).

Hepatitis A virus causes at least 30 000 reported cases of hepatitis per year in the U.S., likely with substantial under-reporting (Sobsey, 1999). Although waterborne outbreaks have occurred, most cases arise from direct or indirect person-to-person transmission associated with poor housing and sanitation.

2.2.2.2 *Norovirus (Norwalk virus)*

This pathogen is a member of the family *Caliciviridae*, genus *Norovirus*. This family separates into four genera: *Lagovirus*, *Vesivirus*, "Norwalk-like virus" (NLV) and "Sapporo-like virus" (SLV). Only the latter two are responsible for human infections (Huffman *et al*., 2003).

Humans are the normal host and are the only known reservoir for these viruses (Chin, 2000). Infection is typically spread via faecal-oral transmission, following contamination of water or food or consumption of raw or lightly cooked contaminated shellfish. Infection by ingestion of food was implicated in 39%, person-to-person contact in 12% and water ingestion in 3% for 348 outbreaks attributable to Norwalk-like virus reported to Centres for Disease Control from January 1996 to November 2000; 18% could not be linked to a specific transmission mode (CDC, 2001).

Norwalk-like virus typically produces a self-limited, mild to moderate disease with symptoms of nausea, vomiting, diarrhoea, abdominal cramps, headache, low-grade fever or chills. Adults more commonly experience diarrhoea; while vomiting is more prevalent among children. Young patients may experience only vomiting (Chin, 2000; CDC, 2001). Norwalk-like virus is very contagious and rapidly spreads from person to person, with fewer than 100 virus particles necessary for transmission (Huffman *et al*., 2003). The incubation period from exposure to onset of symptoms ranges from 12 to 48 hours with symptoms typically lasting 12 to 60 hours. During this period, viruses are shed in vomitus and faeces, possibly for prolonged periods after acute symptoms have subsided (CDC, 2001).

2.2.2.3 *Rotavirus*

The rotaviruses are classified into *Reoviridae* family (Abbaszadegan *et al*., 1999; Chin 2000). There are seven known rotavirus groups classified A–G on the basis of their distinct antigenic and genetic properties. Human infection has been reported with groups A–C rotaviruses. Of these, group A rotavirus is the most important, being a major cause of severe gastroenteritis in infants and young children worldwide (Mulholland, 2004). Group A rotavirus is known to have the highest prevalence and pathogenicity, causing an estimated nearly one million deaths every year, predominantly in developing countries. Groups A, B, and C rotaviruses are found in humans and animals, and the interspecies transmission of rotavirus, including human infection by a bovine strain, has been reported (Abbaszadegan, 1999). Recent studies suggest that as global deaths from childhood diarrhoea have decreased during the past two decades, the proportion of diarrhoea hospitalizations attributable to rotavirus may have increased (Parashar *et al*., 2006).

Rotavirus produces a sporadic, seasonally occurring, often severe illness for infants and young children, involving symptoms of vomiting, abdominal distress, fever and watery diarrhoea that can lead to severe dehydration causing death (Abbaszadegan *et al*., 1999). These symptoms do not normally allow rotaviral disease to be readily distinguished from acute diarrhoea caused by other agents.

Rotaviruses are globally recognised as major pathogens of diarrhoea in children and adults (El-Sheikh and El-Assouli, 2001). Rotavirus is the cause of more than one-third of hospitalized cases of diarrhoeal disease in infants and children under five, responsible for an estimated 3.5 million cases of diarrhoea and 125 deaths among infants and young children in the United States and an estimated 600 000 to 870 000 annual deaths worldwide (Abbaszadegan *et al*., 1999; Chin 2000). Effectively, all children are infected with rotavirus in their first 3 years of life with the highest incidence in 6- to 24-month age group in Peru (Chin 2000).

Rotaviruses are transmitted by the faecal-oral route and have been implicated in at least nine documented waterborne outbreaks (Abbaszadegan *et al*., 1999). Rotaviruses are ubiquitous in human wastewaters (Abbaszadegan *et al*., 1999), so the conditions for waterborne rotavirus outbreaks are likely in place whenever human sewage contaminates drinking water. Widespread host immunity in the exposed population may limit the impact of such outbreaks.

The incubation period is typically less than 48 hours, but may range from 24 to 72 hours, with the duration of the illness typically in the range of 4 to 8 days (Abbaszadegan *et al*., 1999; Chin 2000). Huge numbers of rotavirus are excreted in faeces of infected individuals, as high as 10^{10} per g of faeces (Chin 2000). Excretion normally ceases by about the eighth day of infection of rotavirus, but excretion for more than 30 days has been reported for immunocompromised patients.

Rotaviruses are believed to resist inactivation at extreme lows or highs of pH (3.5 or 10), can survive sewage treatment and may survive for days to weeks in receiving waters, depending on water quality and temperature (Abbaszadegan *et al*., 1999).

2.2.2.4 *Giardia lamblia* (syn. *G. intestinalis, G. duodenalis*)

Giardia lamblia is the most widely used name for this common enteric protozoan parasite, but *G. intestinalis* has been proposed. *Giardia* exists in two forms, a dormant, robust and infective cyst for transmission in the environment and a trophozoite, which is the active living form in the host gut (Schaefer, 1999).

Hosts to *Giardia* spp include dogs, cats, sheep, cattle, deer and humans. Infection is spread by faecal-oral transmission including animal-to-human transmission, person-to-person transmission and faecal contamination of food and water (U.S. EPA, 1999b).

Giardiasis in humans may occur as an asymptomatic infection or as acute or chronic diarrhoea. Other symptoms may include bloating, flatulence, cramps, loss of appetite, vomiting, weight loss, fatigue, mucus or blood in stool, malabsorption of ingested fats leading to pale, greasy and foul-smelling stool, malabsorption of fat-soluble vitamins and, occasionally fever. If left untreated, symptoms may last from 10 days to 12 weeks or longer. *Giardia* has a relatively long incubation time of 7 to 10 days within a range of 1 to 75 days, depending on the ingested dose and health of the host. *Giardia* outbreaks typically have shown incubation periods of 1 to 3 weeks. When administered by ingestion of a gelatine capsule to healthy volunteers, as few as 10 cysts have been found to be infective, but a wide range of infectivity levels have been reported, suggesting differences in virulence among strains or other intervening factors that affect host susceptibility (Chin 2000).

Giardiasis is the most commonly reported intestinal protozoan parasite infection worldwide and prevalence surveys of infection among children range from 1 to 68% (Chin 2000). *Giardia* was commonly identified in the U.S. between 1971 and 1996, with 115 drinking water outbreaks causing 28 000 cases (Craun and Calderon, 1999). Surveys of wastewater, receiving waters, source waters and treated drinking water have found that *Giardia* is often found in surface waters of North America. A large survey of U.S. groundwater sites found that 34% of sites susceptible to contamination were positive for *Giardia* or *Cryptosporidium*, or both, as were 14% of moderate-risk and 4% of low-risk site (Moulton-Hancock *et al*., 2000).

Chlorine can achieve more than 99% inactivation of cysts at lower pH (7.0 to 8.0) and warmer temperatures (25ºC), but very long contact times are needed to achieve substantial inactivation at higher pH (8.5 to 9.0) and low temperatures (0 to 5ºC) (WHO, 2004b). Chloramines are much less effective than free chlorine for inactivating *Giardia* (Schaefer, 1999; U.S. EPA, 1999b).

2.2.2.5 *Cryptosporidium parvum*

Cryptosporidium parvum is a coccidian protozoan, an obligatory parasite of the intestinal tract of warm-blooded animals and is the species that produces human disease. *Cryptosporidium* became prominent as a serious human pathogen following reports of severe diarrhoea among AIDS patients in 1982. *Cryptosporidium* released in faeces, is disseminated and survives in the environment as a robust, double-walled oocyst, which contains 4 sporozoites. Following ingestion, the oocyst releases the sporozoites into the small intestine. These sporozoites invade the intestinal tract and each develops into trophozoites, then into merozoites, which reproduce asexually and lastly into zygotes. Zygotes reproduce asexually to complete the cycle by forming oocysts, which are released back into the environment in the faeces of the infected host (Sterling and Marshall, 1999).

The normal hosts and reservoirs include humans, cattle and other domestic animals. Environmental monitoring surveys have shown that *Cryptosporidium* oocysts are commonly found in surface waters in the range from 0.01 to 100 per L (Bukhari *et al*.,

1998) and they are also found in groundwater (Moulton-Hancock *et al*., 2000). In 1993, *Cryptosporidium* caused one of the largest recognised waterborne disease outbreaks in Milwaukee, USA, with an estimated 403 000 persons affected (Mackenzie *et al*., 1994).

The main symptom of cryptosporidiosis in humans is profuse, watery diarrhoea that may contain mucus or slime (Miliotis and Bier, 2003). Other symptoms include cramping, abdominal pain, nausea, vomiting (mainly in children), weight loss, mild fever and fatigue (New Zealand Food Safety Authority, 2001). Symptoms may reoccur after a period of recovery, but generally last less than 3 weeks (Chin, 2000).

The precise incubation time of cryptosporidiosis is unknown, but has been estimated to be 7 days within a range of 1 to 12 days (Bukhari *et al*., 1998) or a range of 4 to 28 days (Sterling and Marshall, 1999). The median infective dose was reported as 132 cysts in one volunteer study, but as few as 30 oocysts caused disease in one of five volunteers at that dose (Dupont *et al*., 1995). More comprehensive volunteer studies have shown a wide range of infectious dose for different strains of *Cryptosporidium*, with the dose for a median risk of infection as low as 10 oocysts for one strain or as high as 1 000 oocysts for another, along with differences in attack rate and incubation time (Okhuysen *et al*., 1999). Oocysts are very resistant to chlorine disinfection, making this process an inadequate barrier to *Cryptosporidium* (WHO, 2004b). The main breakthrough in disinfection practice has been the discovery that low dosages of UV will damage, but not kill, the oocysts, rendering them unable to reproduce within a host (Clancy *et al*., 2000).

2.2.2.6 *Campylobacter* spp

Campylobacter is a member of the family *Vibrionaceae* (Fricker, 1999). The genus *Campylobacter* includes 14 species, several of which are pathogenic to humans and animals. Human illness is caused by *C. jejuni*, *C. coli* and *C. upsaliensis* (Fricker, 1999). *Campylobacter jejuni* was recognized as one of the leading causes of gastroenteritis in humans (Blaser *et al*., 1986).

Campylobacter spp. maintains a wide range of animal hosts, including domestic and wild animals (Chin, 2000). Birds provide a major source of human infection including risk through uncooked, contaminated poultry (Chin, 2000). *Campylobacter* spp do not reproduce in waters at ambient temperatures (20 to 23ºC). *Campylobacter* spp are found in fresh and marine waters affected by birds or wildlife, domestic sewage and undisinfected sewage effluents. *Campylobacter* spp survive best in colder temperatures below 10 ºC (Blaser *et al*., 1986; Fricker, 1999). This group of pathogens is estimated to produce between 5% and 14% of all diarrhoeal disease worldwide (Chin, 2000).

Campylobacter produce acute gastroenteritis with diarrhoea which may be either profuse and watery, or dysenteric. Diarrhoea may be of variable severity and is often accompanied by abdominal pain, headache, fever, nausea and vomiting. The disease appears with sudden onset, but may be preceded by flu-like symptoms and has a typical incubation period of 2 to 5 days within a range 1 to 10 days, depending on the dose ingested (Fricker, 1999; Chin 2000). The illness usually lasts 2 to 5 days, but may last more than 10 days. Illness may be prolonged in adults and relapses may occur in up to 20% of cases (Blaser *et al*., 1986). Infected individuals who are not treated with antibiotics may shed organisms in faeces for 2 to 7 weeks (Chin, 2000). Infection may be caused by ingestion of fewer than 500 of these pathogens.

In a small number of cases, a typhoid-like syndrome or reactive arthritis may occur and in rare cases, fever-related convulsions, Guillain-Barré syndrome (a paralysis that lasts several weeks and usually requires intensive care) or meningitis may occur. Chronic complications have been reported in 1 to 2% of cases (Fricker, 1999). Guillain-Barré syndrome is reported in 0.1% of cases in the USA, but the relatively high incidence of campylobacteriosis allows the possibility that 40% of all Guillain-Barré syndrome cases in the US may be caused by campylobacteriosis. Some immunocompromised individuals may develop septicaemia, a life-threatening condition. Overall, an estimated 100 fatalities may be caused in the USA each year by campylobacteriosis (CDC, 2003).

Conventional disinfection using chlorine is easily sufficient to inactivate *Campylobacter* to an adequate degree in drinking water supplies. These bacteria appear to be somewhat more susceptible to chlorine than *E. coli*, and treated water systems that are maintained free of *E. coli* will also be free of *Campylobacter* (Fricker, 1999).

2.2.2.7 *Escherichia coli* (Enterohemorrhagic *E. coli*, Enterotoxigenic *E. coli*)

Escherichia coli is a member of the family *Enterobacterioceae* and are gram-negative rods (Todar, 2002). *E. coli* are facultative anaerobes, mobile by means of a flagellum and unable to form spores to survive unfavourable environmental conditions (Todar, 2002). These bacteria are a vital component of the intestinal flora of warm-blooded animals (mammals and birds) because they assist in the digestion of food (Todar, 2002). However, at least six groups of pathogenic strains of these otherwise beneficial bacteria are now recognised: enteropathogenic *E. coli* (EPEC); enterotoxigenic *E. coli* (ETEC); enteroinvasive *E. coli* (EIEC); enterohemorrhagic *E. coli* (EHEC), which includes *E. coli* 0157:H7; enteroaggregative *E. coli* (EaggEC); and diffuse adherent *E. coli* (DAEC) (Nataro and Kaper, 1998). The ETEC and EHEC groups have been identified as causing major waterborne-disease outbreaks (Chin, 2000).

The human gastrointestinal tract is the principal reservoir for pathogenic *E. coli* strains, except EHEC, which has cattle as its primary reservoir (Lejeune *et al*., 2004). Pathogenic strains of *E. coli* are spread by faecal-oral route, with food or water contamination as a primary cause of outbreaks, but secondary person-to-person transmission also occurs, in which case humans serve as a temporary reservoir (Chin, 2000).

The infective dose for EHEC is recognised to be much lower than for other toxic *E. coli*. One estimate for a median infectious EHEC dose is near 10^6 organisms (Haas *et al*., 2000), but another predicts infections possible at about 10^2 organisms (Strachan *et al*., 2001). The median infective dose for ETEC is 10^8 to 10^{10} organisms, unless stomach acids are neutralized, bringing the infective dose down to 10^6 organisms.

The symptoms of EHEC infection (most commonly *E. coli* 0157:H7 in North America, Europe and Japan) include diarrhoea that may range from mild and non-bloody to severe diarrhoea that is virtually all blood (Swerdlow and Griffin, 1997). Serious diarrhoea is accompanied by abdominal pain with little or no fever. Haemolytic uremic syndrome (HUS) may develop in 2 to 8% of cases with EHEC diarrhoea (Rowe *et al*., 1998). *Escherichia coli* 0157:H7 produces potent cytotoxins: the Shiga toxin(s), one of which is identical to the toxin produced by *Shigella,* which can also cause HUS (Chin 2000). The incubation time of EHEC has a median of 3 to 4 days, but the observed range has been 2 to 8 days (Chin 2000). The illness typically last about a week, but longer duration is possible (Lejeune *et al*., 2004). Complications like HUS will certainly lead to longer illness.

The symptoms of ETEC infection include a profuse, watery diarrhoea with neither blood nor mucus, similar to that caused by cholera. Other symptoms include: abdominal cramping, vomiting, acidosis, extreme exhaustion and sometimes low-grade fever. Enterotoxigenic *E. coli* (ETEC) strains produce either or both of two types of toxin: heat-labile toxins and heat-stable toxins (Levine, 1987). The two groups who commonly experience this disease are young children in tropical countries (typically aged less than two), and non-immune adults (typically travellers from affluent countries). The latter may account for 20 to 40% of all cases of travellers' diarrhoea, a condition affecting up to 60% of visitors to tropical countries. The incubation time of ETEC is typically 1 to 3 days, but may be as short as 10 to 12 hours, with illness usually lasting fewer than 5 days. Excretion of ETEC and risk of person-to-person transfer may be prolonged (Chin, 2000).

2.2.2.8 *Salmonella* spp

Salmonella species are the members of a genus of the family *Enterobacterioceae*, a large group of bacteria widely distributed in the environment. These bacteria are anaerobic; do not form spores and are usually motile, gram-negative rods (Chin 2000).

Salmonella are commonly found in animals such as poultry, birds and cattle. Humans are also carriers, both when recovering from infection and during asymptomatic infection. Transmission occurs through the faecal-oral route, mainly by ingestion of faecally contaminated and inadequately heated or disinfected food, unpasteurised milk and untreated water. *Salmonella* have been reported to survive from 1 to more than 100 days (Faechem *et al*., 1983). Factors affecting the survival of *Salmonella* spp include the presence of protozoa, organic matter, nutrients, ultraviolet (UV) light and temperature. *Salmonella* spp have been reported to survive for extended periods in contaminated surface waters, activated sludge effluents and other nutrient-rich waters.

A wide range of values have been reported for the median infective dose for non-typhoid salmonellosis, including estimates of 10^9 (Hunter, 1997) 100 000 (Moe, 1997), below 1 000 and possibly as low as 10 bacteria (Hunter, 1997). The range suggests that the interplay of factors leading to infection is not fully understood. Infants and immunocompromised individuals are expected to be more susceptible.

Salmonella spp typically invade through the bowel mucosa and multiply in the sub mucosa. *Salmonella* spp pass through the stomach, adhere to and penetrate the mucosa of the intestine. After penetration of the lamina propria and the sub mucosa, *Salmonella* spp are phagocytosed by the polymorphs and the macrophages. Some of the bacilli pass to the mesenteric glands, from where *Salmonella* spp enter the bloodstream and give rise to a primary bacteraemia. During this phase the bacteria are shed in the liver, gall bladder, spleen, bone marrow, lymph nodes, lungs and the kidneys; the organs where *Salmonella* spp further multiply. This is a transitory bacteraemia which occurs 24 to 72 hours after ingestion of the bacteria, is rapidly brought to an end by the removal of the bacilli by the reticuloendothelial cells, particularly those of the liver and the spleen. Active proliferation proceeds in the liver and the spleen during this phase. This is evident by the recovery of increasing numbers of bacilli from these organs; while the blood remains sterile. After intracellular multiplication, the bacteria re-enter the blood stream and a continuous secondary bacteraemia associated with a generalisation of infection throughout the tissues and as secondary invasion of the intestine is seen. This secondary bacteraemia may last from days to weeks (http://www.geocities.com/avinash_abhyankar/pathogenicity.html).

More than 2 000 *Salmonella* serotypes have been identified, but only about 200 are commonly encountered and their taxonomy is being revised according to current understanding of their DNA relationships (Chin, 2000). Although any serotype can cause salmonellosis, which is a collective description for infectious diseases caused by members of the bacterial genus, certain serotypes have been associated with specific presentations, e. g., *Salmonella enteritica* serotype e*nteritidis* and serotype t*yphimurium* are associated with gastroenteritis while serotype c*holeraesuis* and serotype d*ublin* are associated with bacteraemia (Goldberg and Rubin, 1988; Threlfall *et al*., 1992). Serotype *typhimurium* and other serotypes (mainly *paratyphimurium* A, B and C) cause enteric or typhoid fever, septicaemia and gastroenteritis (McConkey and McConkey, 2002).

Typhoid fever is caused by *Salmonella typhi*, now proposed to be called *Salmonella enterica* serovar Typhi or simply *Salmonella typhi* (Chin, 2000). Typhoid fever was once the most common form of waterborne disease in industrialized countries, occurring far more commonly than cholera. Globally, it is estimated that typhoid fever causes over 16 million cases of illness each year, resulting in over 600 000 deaths (Wain *et al*., 2003) but fewer than 500 cases are reported in the US and most of the cases are imported from endemic areas (Chin, 2000). Typhoid fever is endemic in most parts of Central America (Olarte and Galindo, 1973), Southeast Asia (Mirza *et al*., 1996; Ling *et al*., 2000) and the Indian subcontinent (Shanahan *et al*., 1998; Rahman *et al*., 2002) and recently increasing numbers of cases have been reported in Africa (Kariuki *et al*., 2000, Mills-Robertson *et al*., 2002). Typhoid fever is distressingly prevalent in developing countries, where it remains a major health problem (Arora *et al*., 1992). Typhoid fever remains endemic to many parts of South Africa, including Kwazulu-Natal, Northern Transvaal and the Transkei (Coovadia *et al*., 1992), with a recent outbreak occurring in Delmas, Mpumalanga. In this province, health spokesperson reported that there were 380 cases of diarrhoea, 30 suspected cases of typhoid fever and nine confirmed cases (Mail and Guardian, 8, September, 2005). The outbreak originated in the town's water supply, suspected to have been contaminated with human faeces. *Salmonella* spp will be found in surface water wherever there are animal populations and are frequently found in wastewater effluents and receiving waters. Chlorination inactivates *Salmonella* spp more readily than *E. coli*; so maintaining adequate chlorination should achieve disinfection for *Salmonella* spp.

Gastroenteritis typically involves acute inflammation of the small intestine and colon accompanied by sudden onset of headache, abdominal pain, diarrhoea and gastroenteritis (Chin, 2000). The predominant gastroenteric symptoms are caused by the pathogen invading only the surface layers of the gut. In some cases, deeper pathogen invasion occurs, allowing spread through the bloodstream, possibly leading to more serious conditions including blood poisoning, meningitis or abscess formation at remote sites (Hunter, 1997). The incubation period of *Salmonella* gastroenteritis ranges from 6 to 72 hours, but is most commonly 12 to 36 hours. Incubation periods will be shorter when higher pathogen doses are delivered. Bloody diarrhoea may occur in up to 30% of cases, but the disease is usually self-limiting within 2 to 5 days. In unusual cases, illness may persist for weeks (Hunter, 1997). Rural areas represent a dangerous reservoir of *Salmonella* spp (Casner, 2001).

2.2.2.9 *Shigella* spp

Shigella species are a genus in the bacterial family *Enterobacterioceae*. *Shigella* spp are gram-negative rods with a number of species or serogroups: *S. dysenteriae* (serogroup A), *S. flexneri* (serogroup B), *S. boydii* (serogroup C) and *S. sonnei* (serogroup D). Serogroup D has caused the majority of reported infections in the US. *Shigella* spp are anaerobic, do not form spores and are non-motile.

Shigella spp are transmitted by faecal-oral route through contamination of water, milk and food (from sewage or sludge on croplands or from infected food-handlers). Person-to-person spread is caused by inadequate sanitation, poor hand-washing practices and poor personal hygiene.

The median infective dose is very low for bacterial pathogens; as few as 10 to 200 bacteria may cause disease (Moe, 1997). Humans are the only significant host, although outbreaks have occurred in primates. *Shigella* can survive outside the human host for up to 4 days in river water and for more than 44 to 100 days or longer in clean cold waters (Hunter, 1997). *Shigella* survive best in very clean, but unchlorinated, water or in polluted water that contains nutrients but few competitor bacteria.

Shigella cause bacillary dysentery that is diarrhoea that contains blood and mucus (Hunter, 1997). The mechanism that leads to shigellosis requires bacterial penetration across the intestinal barrier via mucosal cells. Upon reaching the underlying lymphoid follicles, the bacterium is engulfed by resident macrophages. Inside the macrophage, *Shigella* escapes from a phagosome into the cytoplasm and that kills the cell by inducing apoptosis. In the process of a dying macrophage, mature interleukin-1β (1L-1β) and 1L18 are released (Guichon *et al*., 2001). These two cytokines are imperative in the initiation of inflammation. Another method of invasion, which is commonly employed by *Shigella*, is pathogen-directed endocytosis. Invasion of enterocytes and bacterial cell-to-cell spread enhance the tissue damage (Guichon *et al*., 2001). Symptoms of shigellosis include: fever, nausea, cramps and painful straining during attempted bowel movement. Shigellosis may range from mild, self-limiting diarrhoea to much more severe symptoms including HUS and convulsions in young children (Chin, 2000).

Worldwide, shigellosis is estimated to cause 600 000 deaths per year, with the majority of cases and deaths in children under ten years old. Epidemiological studies of shigellosis in Bangladesh have shown that various water sources, e.g. ponds, lakes, wells and rivers, can act as sources of infection (Islam *et al*., 1993a). In the United States, outbreaks of shigellosis have been attributed to swimming in contaminated water (Rosenberg *et al*., 1976). *Shigella flexneri* has been the most common cause of bacillary dysentery (shigellosis) in South Africa (Donald *et al*., 1987). However, *Shigella* type 1 (SD1), causes the most severe form of shigellosis due to its unusual virulence (Gangarosa *et al*., 1970). *Shigella* type 1 was an important cause of dysentery worldwide in the first part of the twentieth century (Mata *et al*., 1970). *Shigella* type 1 disappeared for 50 years, and reappeared in Central America in the late 1960s (Mata *et al*., 1970) and in Central Africa since 1979 (Frost *et al*., 1982). An outbreak was reported from Burundi, East Africa (Ries *et al*., 1994) and a number of cases of haemolytic uraemic syndrome in adults due to this bacterium were reported from South Africa (Bloom *et al*., 1994). When *Shigella* type 1 arrived in South Africa, the first cases was reported from Mpumalanga (Bloom *et al*., 1994). However, in 1995/1996 a *Shigella* type 1 epidemic in Kwazulu-Natal resulted in thousands of observed cases with many hundreds of deaths (CDC, 1996). *Shigella* type 1 causes a particularly virulent form of dysentery, which has emerged as an epidemic throughout Kwazulu-Natal and the Eastern Cape, with cases being observed in the Western Cape and Mpumalanga (Rollins, 1996).

The incubation time of shigellosis is usually 1 to 7 days, but may range from 12 to 50 or 96 hours with illness typically lasting 3 days to 2 weeks (Miliotis and Bier, 2003). *Shigella* spp are readily inactivated by chlorination. Drinking water outbreaks of shigellosis require both a source of human faecal contamination and inadequate chlorination or alternate disinfection.

2.2.2.10 *Vibrio* spp

Members of the genus *Vibrio* are defined as gram-negative, asporogenous rods that are straight or have a single, rigid curve. *Vibrio* spp are motile; most have a single polar flagellum, when grown in liquid medium. Most produce oxidase and catalase, ferment glucose without producing gas (Baumann and Schubert, 1984). Three species, *V. cholerae*, *V. parahaemolyticus* and *V. vulnificus* are well documented pathogens (Kaper *et al*., 1995; McLaughlin, 1995; Osawa *et al*., 1996). *Vibrio mimicus* is a recognised pathogen with similar characteristics to *V. cholerae*, except an ability to ferment sucrose. Other species within the genus, such as *V. aginolyticus, V. fluvialis, V. furnisii, V. metschnikovii* and *V. hollisae* are occasional human pathogens (Rippey, 1994). *Vibrio cholerae*, the causative agent of cholera, has been studied mainly because of its medical importance.

Cholera is transmitted primarily by the faecal-oral route and indirectly through contaminated water supplies (Weber *et al*., 1994). Direct person-to-person spread is not common. Food supplies may be contaminated by the use of human faeces as fertilizer or by freshening vegetables for market with contaminated water (Popovic *et al*., 1993).

Cholera outbreaks in several countries are thought to have resulted from the consumption of raw, undercooked, contaminated, or recontaminated seafood such as shellfish and oyster. *Vibrio cholerae* has since been detected in seawater and other environmental sources around the world, both in areas where cholera is endemic and in cholera-free areas (Jiang and Fu, 2001). It has been determined on the basis of volunteer trials that, depending on the health of a given individual, the ingestion of approximately 10^4 – 10^6 *V. cholerae* 01 organisms are likely to produce clinical cholera (Cash *et al*., 1974).

Vibrio cholerae pathogenesis involves the co-ordinated expression of a number of virulence factors including cholera toxin (CT), which is directly responsible for the disease symptoms and toxin-co-regulated pilus (TCP), which is required for intestinal colonization. Cholera toxin consists of two subunits (Spangler, 1992). Subunit A is responsible for adenylate cyclase inactivation, including tremendous loss of fluids during illness. The B subunit is involved in binding the toxin to the epithelial cell surface acceptors in the small intestine. The chromosomal genes encoding the A and B subunits are designated ctxA and ctxB, and are expressed as a single transcriptional unit (Guidolin and Manning, 1987). The ability to produce cholera toxin is an important step in the diagnosis of cholera because only toxin-producing strains have been associated with severe, watery diarrhoea and epidemics (Finkelstein, 1988). Various cholera toxin genes PCR assays, using primers that amplify regions of either *ctxA* or regions covering both *ctxA* and *ctxB* have been described (Koch *et al*., 1995; Varela *et al*., 1993).

Clinical manifestations begin with onset of massive watery diarrhoea, speckled with flakes of mucus and epithelial cells (rice-water stools) and contain enormous numbers of *Vibrio* cells. Infection is often mild or without symptoms but sometimes severe. The bacteria bind to the intestinal wall, multiply and secrete an enterotoxin, which attacks the intestinal mucosa. This interferes with the normal processes of salt and water balance across the gut wall, causing severe diarrhoea, vomiting and impairment of the normal function of many organs. Patients may lose as much as 10 – 12 litres of protein-free fluid and associated electrolytes, bicarbonates and ions, a day and become severely dehydrated (Pelczar *et al*., 1999). Death may result from reduced fluid levels and salt imbalance in the body, shock and delay. However, 50–60% mortality rates frequently results from untreated cholera (Todar, 1999). Treatment involves replacing lost fluid and salts.

Cholera occurs worldwide. In 1991, an epidemic occurred in Bangladesh which was responsible for an estimated 8 000 deaths in a 12-week period (Siddique *et al*., 1995). Since 1991, the world has witnessed extension of the seventh pandemic into South America and South Africa, as well as the appearance of a previously unknown pathogenic serogroup of *V. cholerae* (O139) (WHO, 2001). In 1992 to 1993, 21 countries in the Western Hemisphere, mostly in coastal areas, reported 800 000 cholera cases with more than 8 000 cases resulting in death (Tauxe *et al*., 1994). In July 1994, 14 000 deaths from cholera were reported in refugee camps in Rwanda (Siddique *et al*., 1995). In April 1997, a total of 1 521 deaths were recorded during a cholera outbreak among 90 000 Rwandan refugees residing in temporary camps in Democratic Republic of Congo (Nabeth *et al*., 1997). Recent cholera outbreaks started in February 2006 in Luanda, Angola. Further cases have been detected and confirmed in the provinces of Bengo, Benguela, Bie, Kuanza Norte, Kuanza Sul, Huambo, Huila, Malange, and Democratic Republic of Congo. As of 3rd May, 2006, 26 979 cumulative cases and 1 085 deaths had been registered by UNICEF (UNICEF, 2006). The underlying cause of the outbreak across the affected communities and municipalities in all affected provinces is as a result of the cramped living conditions within the affected areas. Furthermore the appalling condition of sanitation, environmental conditions and inappropriate hygiene practices greatly exacerbate problems in the areas (UNICEF, 2006).

In 1974, the first epidemic of cholera in South Africa broke out in the western part of the Transvaal Province now Limpopo province (Isaäcson *et al*., 1974; Isaäcson and Koornhof, 1981; Küstner *et al*., 1981). Between 1974 and 1980, the disease was repeatedly introduced into Limpopo province from the north but was kept under control by appropriate control measures coupled with good surveillance (Küstner *et al*., 1981). In 1980, however, the disease reached epidemic proportions, and since then cholera has been regarded as being endemic in Limpopo and Kwazulu Natal (Isaäcson *et al*., 1981) with an annual epidemic occurring each summer. In October 2001, a cholera epidemic started in KwaZulu-Natal and between January and March 2002, the disease spread to other provinces. A total of 17 890 cases of cholera were reported compared to the 106 389 cases reported during the previous epidemic. Most of the cases and deaths were in KwaZulu-Natal and the Eastern Cape. The Eastern Cape is the second largest province in South Africa, covering an area of 169 580 square kilometres, but also one of the poorest of nine provinces. The Eastern Cape was the second most affected province where the epidemic started in the Oliver Tambo District and lasted for six months. The epidemic was mainly attributed to unsafe water as a result of untreated wastewater that was emptied into the Umtata River (National Department of Health, 2003).

2.2.3 Health risk assessment of waterborne diseases

Microbiological health risks remain associated with many aspects of water use; from drinking water in developing countries (Lloyd *et al*., 1989) to irrigation reuse of treated wastewater (Rose, 1986) and recreational water contact (Grabow, 1991). Drinking water poses a very high health risk; however, the risk can be reduced by effective treatment and by applying drinking water criteria.

Ingestion of faecally polluted water has long been recognised as a primary cause of diarrhoea. Predicting the risk of infection that can lead to waterborne diarrhoea should be part of managing the health-related microbiological quality of water (Haas *et al*., 1999). Current practices to predict a possible risk of infection related to the microbiological quality of water include environmental health practitioners and water quality managers generally testing water for the presence of indicator microorganisms. If present, a negative health effect can be expected with increasing risk expected as organisms numbers increase. This approach can be referred to as an observed-adverse-effect-level approach (OAELA) based on the occurrence of microbiological indicator organisms instead of actual pathogens. It does not provide a quantitative value for the microbiological waterborne health hazards that threaten water users since it can, at best, indicate the potential risk of infection by diarrhoea-causing pathogens occurring in the same water should a person ingest it (Genthe and Seager, 1996; Fewtrell and Bartram, 2001).

Quantitative health risk assessment is an emerging tool in the field of microbial food and water safety (ILSI, 1996). In its simplest form it consists of four main steps, namely: hazard identification, dose-response assessment, exposure assessment and risk characterization.

2.2.3.1 Hazard identification: Hazard identification is a step where potential contaminants of concern and their effects on human health are identified. The likelihood that the exposure to a pathogenic microorganism under specific exposure conditions poses a threat to human health could be assessed. The hazard characterization step of risk assessment is the one most amenable to a common approach (Genthe and Seager, 1996; Fewtrell and Bartram, 2001).

2.2.3.2 Dose-response assessment: Dose-response assessment involves the collection of data on the relationship between the dose of microbial contaminants (i.e. the amount of the target pathogenic microorganisms taken into the body through skin contact, ingestion or drinking) and the incidence of an adverse health effect in the exposed population. This relationship is characterized by the infectivity and there are three ways to characterize infectivity: (i) The ID_{50} or the infective dose is the dose that causes infection in 50% of the persons exposed to the pathogen; (ii) The Pinf is the probability of infection following the exposure to a single organism; (iii) The most complete form for characterizing infectivity is with dose/response curves, giving the probability of infection as a function of the dose. The criteria used for infectivity differ from one study to another thereby providing a quantitative basis for assessing health risks (Genthe and Seager, 1996; Fewtrell and Bartram, 2001).

2.2.3.3 Exposure assessment: Exposure assessment establishes the pathways along which the microbial contaminants may be released. The intensity, frequency and duration of human exposure to each of the targeted pathogenic microorganism in potentially exposed populations can be measured or estimated. As a default number, two litre/person/day is used to estimate drinking water exposure, (Macler and Regli, 1993) although this may be conservative (Roseberry and Burmaster, 1992). During contact recreational exposure, 100 mL/day has often been assumed as an exposure measure but actual data to validate this number are lacking (Haas, 1983). The purpose of an exposure assessment is to determine the microbial doses typically ingested by the direct user of a water (or food). In the case of water microbiology, this may necessitate the estimation of raw water microorganism levels followed by estimation of the likely changes in microbial concentrations with treatment, storage and distribution to the end-user (Regli *et al*., 1991). A second issue arising in exposure assessment is the amount of ingested material per 'exposure'.

2.2.3.4 Risk characterisation: The final step, risk characterization, combines the information concerning the hazard identification, dose-response and exposure assessment and uses it to characterize and describe the extent of the overall individual or population risk. It is very important to note that lack of sanitation service or inadequate treatment of wastewater has a negative impact on the health of a community (Fewtrell and Bartram, 2001). The following section focuses on the treatment of wastewater.

2.3 WASTEWATER TREATMENT

The world supply of freshwater is limited and threatened by faecal pollution as a result of indiscriminate discharge of untreated wastewater effluents. In developed countries, municipal wastewater systems are well organized and cover most parts of the regions but this is not the case in developing sewage countries. In South Africa, water is scarce and there is need to protect the available water resources from discharges of untreated wastewater. While sewage articulations exist in nearly all urban areas, rural areas as well as most peri-urban areas are generally devoid of effective facilities. A range of wastewater treatment technology options is discussed below.

2.3.1 On-site wastewater treatment systems

On-site wastewater treatment system relies on the decomposition of the organic wastes in human excreta by bacteria. This can take place in a single pit in the ground or in specially designed tanks to promote bacterial decomposition of waste. Pit latrine, pour flush latrine, composting toilet, septic tank and two improved treatment units represent the major types of the system (UNEP, 2002). This type of treatment can pollute the groundwater and surface water sources that may be in close proximity to its location. Although variations of these systems exist the treatment principles are the same. In this study, we will emphasise the impact of these systems on water sources.

2.3.1.1 Ordinary Pit Latrine

This is the most common sewage system employed in the rural communities of South Africa. The ordinary pit latrine is an onsite wastewater treatment system that collects excreta in a pit dug in the ground beneath a toilet structure. Its advantage is that it is generally built by the stand occupier and is better than having no sanitation at all. The disadvantages include the smell, insect nuisance cannot be readily controlled, the potential to pollute groundwater and its unsuitability if the ground is rocky or if the groundwater is close to the surface (Wood *et al.*, 2001).

2.3.1.2 Ventilated Improved Pit Latrine

The ventilated improved pit latrine is generally hygienic as a basic sewage system, cheap and easy to build. Its improved ability ventilate odour nuisance and minimize insect nuisance. The disadvantage is that the pit can flood if positioned in flow paths of storm water causing surface water pollution and health threats (Wood *et al.*, 2001).

2.3.1.3 Ventilated Improved Double Pit Latrine

This is suitable for higher population densities and designed for alternative modifying of shallow pits. The advantage is that shallow pit is easier to excavate and less likely to pollute groundwater. The disadvantage of the system is that it is more difficult to construct and support (Wood *et al.*, 2001).

2.3.1.4 Bucket Toilet

This is affordable and better than having no sanitation at all. However it has a dissuading use, encourages disease potential, odour and insect nuisance (Wood *et al.*, 2001). Buckets tend to get emptied close to the house, if not regularly collected causing health risks and surface water pollution (Wood *et al.*, 2001).

2.3.1.5 Chemical Bucket Toilet

It is generally provided as a service by local health authority at no cost to communities. Chemical additives such as formaldehyde are used to disinfect the waste reducing odour and insect nuisance (UNEP, 2002). The disadvantage of this system is that it is expensive for the local authority to provide service regularly. Also buckets often get full and overflow, when service frequency is inadequate, causing health risks and surface water pollution. Disposal of bucket contents creates handling and treatment problems at the sewage works. The system cannot handle large urine volumes, requiring alternative practice (Wood *et al.*, 2001).

2.3.1.6 Aqua-Privy

It is relatively cheap and easy to install as an on-site disposal system. Various configurations and capacities are available, some of which can be upgraded to full-bore sewage service. Small amount of water is used for flushing (solid free sewer) and minimal maintenance is required if correctly used and installed. The disadvantage of this system is that it requires frequent emptying to prevent the system getting too full of sludge; water has to be carried to the flushing tank and because the system uses small amounts of water, it may not properly flush (Wood *et al.*, 2001).

2.3.1.7 Compost Toilet

It is a dry system requiring no, or little flush water. This system composts, or stabilizes, sewage solids for possible use as soil conditions and fertilizer and odour and fly nuisance is controlled. Shallow excavation depth of pre-fabricated compost units minimizes risk of groundwater pollution and it is relatively attractive compared to basic out-house systems. The disadvantage is that pre-fabricated systems are relatively expensive while effective composting requires careful user attention. Compost value is minimized by presence of plastics, papers, etc (Wood *et al.*, 2001).

2.3.1.8 Septic Tank

This system generally has adequate capacity for a large family with full borehole water supply and it can be installed where there are no sewers. Its large capacity allows desludging of three to five years. The disadvantage is that the system is relatively expensive to construct due to septic tank size requirements and can only be installed where sites are large and groundwater pollution is not a problem (Wood *et al.*, 2001). In the peri-urban areas, septic tanks have been found to be widely in used.

2.3.1.9 Low Flush Toilet

This system has the convenience of a full flush toilet but uses less water and may cost less to install. Sewage is transported away from the community to off-site treatment and disposal. The disadvantage of the system is that it needs an off-site system to treat and dispose off the sewage, which is generally expensive. Although it uses less water, solids cannot always be totally flushed away and blockages can result causing spillage. Flush mechanisms tend to be problematical and difficult to maintain often resulting in the system being abandoned (Wood *et al.*, 2001).

2.3.1.10 Full Flush Toilet

It is most convenient system for users in urban areas. It can responsibly and reliably treat and dispose of sewage. It also removes the problem of sewage handling and disposal for the immediate community area. The disadvantage of the system is that it is the most expensive system to build and uses the most water. Spillages from blockages and leaks in full flush system can represent a significant local pollution problem because the volumes and quality of sewage are now concentrated in one pipeline system (Wood *et al.*, 2001).

2.3.2 Off-site wastewater treatment systems

Off-site treatment is the treatment of wastewater that has been conveyed using a sewerage system. Different degrees of treatment, in order of increasing treatment level, are used in off-site wastewater treatment systems. These include: preliminary, primary, secondary, tertiary and/or advanced treatment.

2.3.2.1 Preliminary treatment

Wastewater undergoes preliminary treatment, which relies on physical forces called unit operations and these include screening, sedimentation and filtration (Bitton, 1994). These processes could be mechanically aided and increase efficiency in the downstream process by removing large solid materials from influent wastewaters that could hinder the flow of sewage through the plant. The objective of preliminary treatment is the removal of coarse solids and other large materials often found in raw wastewater. Removal of these materials is necessary 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. In grit chambers, the velocity of the water through the chamber is maintained sufficiently high, or air is used, so as to prevent the settling of most organic solids. Grit removal is not included as a preliminary treatment step in most small wastewater treatment plants. Comminutors are 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. Flow measurement devices, often standing-wave flumes, are always included at the preliminary treatment stage. This initial process normally removes 35% of the biological oxygen demand (BOD), 30% of the chemical oxygen demand (COD), 60% of the suspended solids (TSS) but only about 10 – 20% of the total nitrogen and total phosphorus (Radojević and Bashkin, 1999). This is dependent on the concentration, the retention time in the sedimentation tank and the evenness of distribution and flow in the tank. This is the most rudimentary treatment, which can remove most of the solids in the water and moderately lower the BOD.

2.3.2.2 Primary treatment

The objective of primary treatment is the removal of settleable organic and inorganic solids by sedimentation, and the removal of materials that will float (scum) by skimming. Approximately 25 - 50% of the incoming biochemical oxygen demand (BOD_5), 50 - 70% of the total suspended solids (SS), and 65% of the oil and grease are removed during primary treatment (Radojević and Bashkin, 1999). Some organic nitrogen, organic phosphorus, and heavy metals associated with solids are also removed during primary sedimentation but colloidal and dissolved constituents are not affected. The effluent from primary sedimentation units is referred to as primary effluent.

2.3.2.3 Secondary treatment

This secondary treatment step is generally considered environmental biotechnology as it harnesses natural self-purification capacity in enclosed reactors for the biodegradation of organic matter and bioconversion of soluble nutrients in wastewater. It utilises biological processes to remove additional suspended material and further lower BOD. Biological treatment methods are mostly preferred because these methods are environmentally friendly and cost effective compared to the chemical methods (Momba, 1995; Muyima *et al*., 1997). The effectiveness of this treatment system is dependent upon the biochemical changes carried out by microorganisms. Approximately 90% of the suspended solids and BOD can be reduced by secondary treatment.

2.3.2.4 Tertiary and advanced wastewater treatment

Tertiary and/or advanced wastewater treatment is employed when specific wastewater constituents, which cannot be removed by secondary treatment, must be removed. Individual treatment processes are necessary to remove nitrogen, phosphorus, additional suspended solids, refractory organics, heavy metals and dissolved solids. However, advanced treatment processes are sometimes combined with primary or secondary treatment (e.g., chemical addition to primary clarifiers or aeration basins to remove phosphorus) or used in place of secondary treatment (e.g., overland flow treatment of primary effluent). Conversion of dissolved substances into a solid settleable form can be achieved with alum; lime or iron salts usually ferrous sulphate, ferric sulphate or ferric chloride and addition of polyelectrolyte (Nacheva *et al*., 1996). These chemicals mix with the wastewater to form an insoluble gelatinous floc which settles rapidly, carrying with it most of the suspended solids in the wastewater. Chemical coagulants are used extensively in sludge treatment to thicken the solids and promote dewatering. To prevent odours, iron compounds and hydrogen peroxide are used.

Various off-site wastewater treatment systems have been described. Activated sludge treatment is now considered the conventional means of large-scale off-site treatment of sewage. Rotating biological contactors is a common example of the system. Trickling filter is an alternative that was developed earlier than the activated sludge process. Traditionally, there have been simpler and effective methods of treating sewage, which include the use of ponds or lagoons, land based treatment and aquaculture (UNEP, 2002).

2.3.2.4.1 ***Trickling Filter***

In the trickling filter liquid sewage is evenly distributed over the upper surface of the filter bed made of rocks and trickles down to under drain system, which removes the effluent to the secondary sedimentation basins and allows free circulation of air throughout the bed to support the growth of organism upon which the process depends. The thickness of the biofilm increases as new organisms grow. Trickling filter offers effective land utilization, low initial capital outlay, low operation and maintenance cost, no specialized mechanical equipment, non-clogging configuration, efficient biological oxygen demand (BOD) and anaesthetic advantage (Gray, 1989; Characklis and Marshall, 1990; Le Tallec *et al*., 1997). However the system is not readily adaptable where climate conditions include severe winter. Figure 2.1 illustrates a trickling filter.

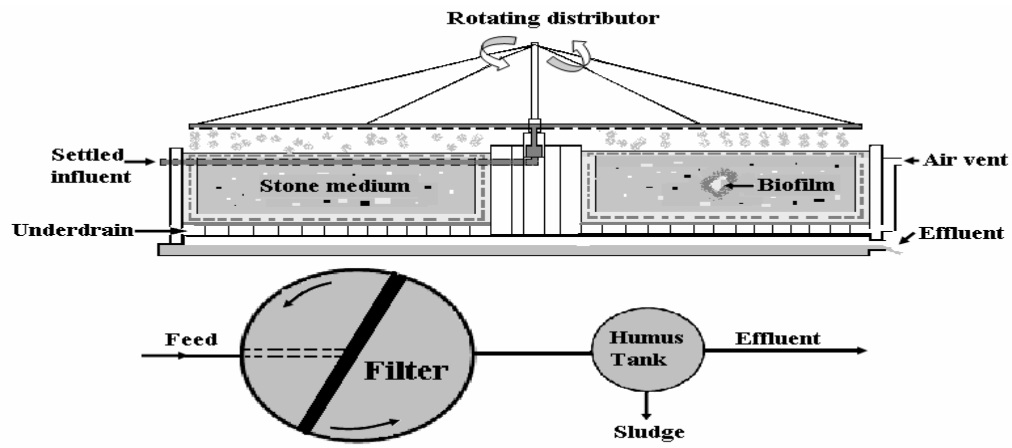


Figure 2.1: Schematic diagram of a trickling (biological) filter (UNEP, 2002).

2.3.2.4.2 *Rotating Biological Contactors*

Rotating biological contactors (RBC) consist of a series of closely spaced discs partially immersed in a tank in which wastewater flow. Oxygen is supplied to the attached biofilm from the air when the film is out of the water and from the liquid when submerged, since oxygen is transferred to the wastewater by surface turbulence created by the discs' rotation. Its biological principle is based on the metabolic activities of complex microbial communities that grow on the disc surface, forming a biofilm (Bishop and Kinner, 1986). RBC is flexible enough to endure fluctuating organic loads, requires little personal attention, is cheap to run and does not require too much land. The RBC has been used in treating winery wastewater and has been used in the treatment of effluents produced by various industries such as gold mining (Stott *et al*., 2001) and domestic sewage treatment (Tawfik *et al*., 2002). Its efficiency depends heavily on parameters such as the hydraulic retention time (Yeh *et al*., 1997), disc rotational speed and disc submergence (Lu *et al*., 1997), and the composition of the discs (Apilánez *et al*., 1998). Figure 2.2 illustrates a rotating biological contactor.

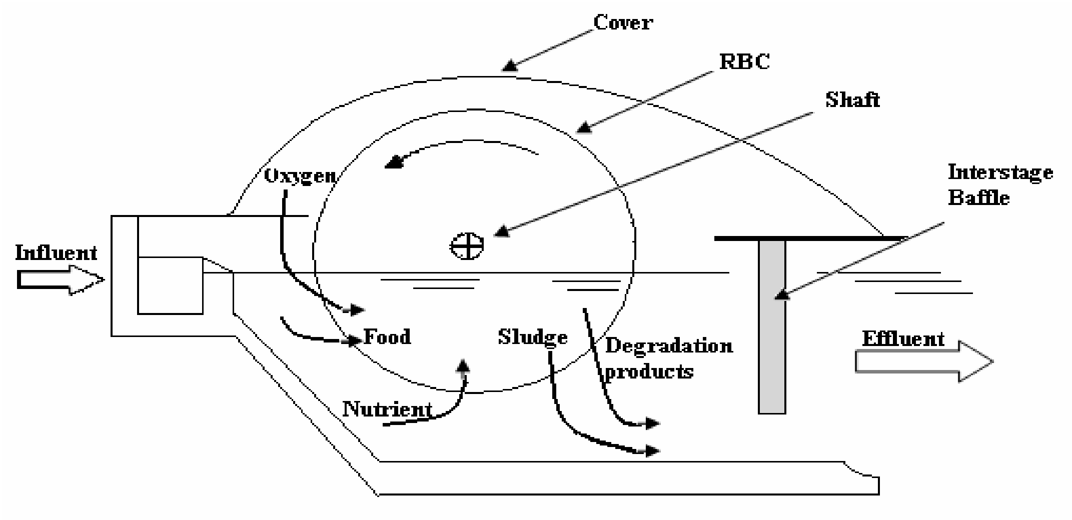


Figure 2.2: Rotating Biological Contactor schematic process (Grady *et al.*, 1999).

2.3.2.4.3 *Lagoons*

Lagooning is effective in treating wastewater and can reduce biological oxygen demand (BOD) and suspended solids to the same levels as mechanical treatment plants (e.g. Activated Sludge Treatment). In addition because of the longer residence time of wastewater in the lagoon (in the order of days), removal of pathogenic bacteria and viruses by natural die-off is greater than in an activated sludge treatment plant (residence time of the order of hours). Cysts of parasites and helminthes eggs are also usually removed through sedimentation in the lagoons.

A lagoon is a shallow excavation in the ground (1 to 2 m deep). It is generally unlined and percolation of wastewater into the soil and groundwater takes place. With time the percolation rate will reduce, because of formation of a sediment layer. Evaporation loss of water can be significant in arid climate regions. The soil itself is, however, not involved in the physical and biochemical wastewater treatment processes taking place in the lagoon. A lagoon can therefore be lined with a layer of clay or with an impermeable plastic membrane if protection of groundwater is desired, without affecting the performance of the lagoon. Wastewater lagoons are also called 'waste stabilization lagoons', because the organic substances in the wastewater are converted to more stable (less degradable) forms (Wood *et al.*, 2001).

The following processes take place in a lagoon. As wastewater enters a lagoon, sedimentation of solids occurs. Due to the long residence time of the wastewater in the lagoon system, much of the solids in the original wastewater are removed. Aeration of the water from the atmosphere occurs by a process of diffusion aided by turbulence caused by wind movement on the surface of the water. Oxygen is supplied by algae in the lagoon, which thrive on the nutrients (nitrogen and phosphorus) released by the decomposition of the organic wastes. The photosynthetic activity of algae, however, only takes place when there is sunlight. Thus oxygen produced by photosynthesis is only available during this period. A symbiotic relationship exists between the bacteria and the algae. Bacteria take up oxygen and release carbon dioxide, while algae take up carbon dioxide released by the bacteria and produce oxygen for the bacteria. Depending on the oxygen demand of the bacteria in the lagoon, the following conditions occur:

***Anaerobic lagoon*:** The oxygen demand of the bacteria exceeds oxygen supply by surface aeration and algal photosynthesis. Biodegradation of the organic wastes is by anaerobic bacteria. Methane gas is a by-product. Odorous gases are produced, but impact is reduced when a layer of scum forms at the water surface (Wood *et al.*, 2001).

***Facultative lagoon*:** The oxygen demand of the bacteria is met by surface aeration and algal photosynthesis, but is not met when the latter is not active. The water environment is aerobic during the day, but turns anaerobic at night. Biodegradation of organic wastes is by facultative bacteria, which can operate under both aerobic and anaerobic conditions (Wood *et al.*, 2001).

***Aerobic lagoon*:** The oxygen demand of the bacteria is met by surface aeration and algal photosynthesis (Wood *et al.*, 2001).

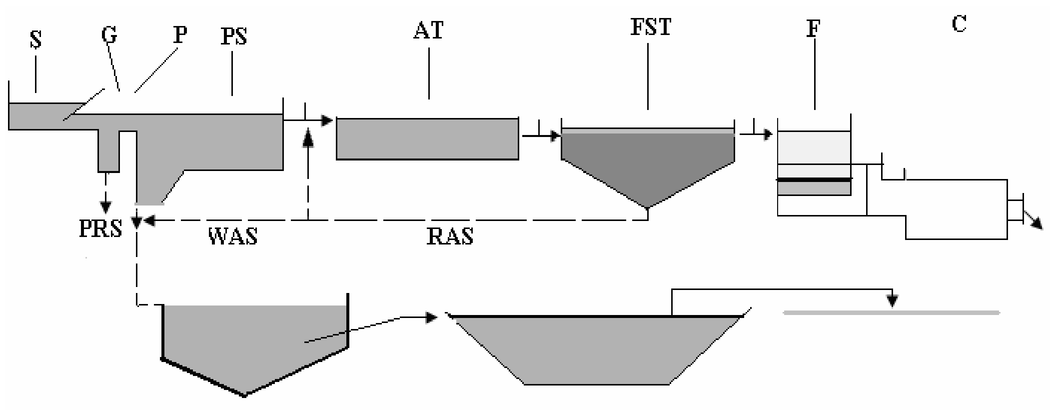
It is common to have a series of lagoons with the first one or two being anaerobic lagoons, the middle ones facultative lagoons and the last few aerobic lagoons. The sediment at the bottom of lagoons is anaerobic, and undergoes anaerobic bacterial decomposition. The first lagoon in a series will eventually be filled with solids. The sludge produced can be removed and treated for re-use or disposal or allowed to undergo further biodegradation in the lagoon prior to re-use. Anaerobic lagoons can be made deeper so that more sludge can be accommodated and the need to remove sludge made less frequent.

Lagoon performance is affected by temperature (UNEP, 2002). At a higher ambient temperature (e.g. in the tropics) a shorter residence time (less than 12 days) of wastewater in the lagoon is required to achieve the same level of treatment compared to when the temperature is below 15ºC. Because algae are present in treatment lagoons, they leave with the treated effluent. One way of harvesting the algae is through aquaculture (Wood *et al.*, 2001).

Oxygen transfer from the atmosphere into lagoons can be increased by mechanically agitating the surface of the water. This can be done by using a vertically mounted impeller and the lagoon becomes more like the aeration tank of an activated sludge process. The agitation can be provided using a horizontally mounted rotor. A configuration that can be used to apply this is a circular ditch, and the water is continuously circulated around the ditch so that its movement is like that in a river. Combination of two of these processes in series (e.g., biofilter followed by activated sludge) is sometimes used to treat municipal wastewater containing a high concentration of organic material from industrial sources (UNEP, 2002).

2.3.2.4.4 *Activated sludge*

According to Grady and Lim (1980), the expression "activated sludge" alludes to a slurry of microorganisms, which remove organic compounds from wastewater and these microorganisms are removed themselves, by sedimentation under aerobic conditions. In activated sludge systems, soluble organics are degraded by bacteria in an aerated basin and biomass is carried over with the influent into a clarifier where solids are allowed to settle, concentrate and are then removed. Part of the settled sludge is drawn off as waste; the rest is recycled to the aeration basin in order to maintain a high concentration of bacteria (Eckenfelder *et al.*, 1985). The full detail of this wastewater treatment system is given in section 2.7.3.



Legend

S- screen
G- grit chamber
P- pre-aerator
PS- primary sedimentation
AT- aeration tank
FST- final sedimentation tank
F- tertiary tank
C- chlorination
PRS- primary sludge
WAS- waste activated sludge
RAS- return activated sludge

Figure 2.3: Schematic diagram of a conventional activated sludge process (UNEP, 2002).

2.3.3 Activated sludge system as a method of choice for the treatment of wastewater.

The activated sludge process is the most widely applied biological wastewater treatment process in the world. The primary objective of the activated sludge system is the removal of soluble biodegradable compounds. It is capable of achieving equal reductions in soluble substrate in reactors of much smaller volume while producing an effluent relatively free of suspended solids (Grady and Lim, 1980).

Wastewater treatment plants are usually designed to efficiently remove biological oxygen demand compounds and nutrients, but seldom were planned specifically to remove pathogenic microorganisms from wastewaters. The removal efficiency of pathogenic and indicator microorganisms in wastewater treatment plants vary according to the treatment process type, retention time, other biological flora present in activated sludge, oxygen concentration, pH, temperature and the efficiency in removing suspended solids (Yaziz and Lloyd, 1979; De Zutter and Van Hoof (1984). The activated sludge process generally reduces enteric bacteria population particularly *Escherichia coli* by 91-99 percent (Kabler, 1959; Curds and Fey, 1969). The system has been shown to significantly remove *Giardia* and *Cryptosporidium* (oo) cysts (Stadterman *et al*., 1994).

There is a quantitative reduction of viruses by the activated sludge treatment process, although the mechanism by which they are removed or deactivated remains to be clearly explained (Grabow, 1968).

The activated sludge process has been renowned to produce an effluent of high quality (chemical and microbiological) at reasonable cost (Doorn *et al*., 2006). Moreover, the activated sludge system is controllable, because through adjustment of the amount of sludge wasted, the operator is able to regulate the mean cell retention time (MCRT) to obtain the desired effluent quality. Activated sludge reactors are relatively resistant to shock loads and can achieve acceptable effluent in spite of dynamic inputs (Grady and Lim, 1980), although these systems do perform better under more stable conditions. In order for the activated sludge process to operate successfully, it is essential that the resident microflora form flocs, which settle out readily, thereby producing a clear effluent with low suspended solid concentrations (Curds and Hawkes, 1975).

Like most systems, the activated sludge process presents some problems such as: long sludge age, vast quantities of sludge production and high consumption of energy. The process can be operationally unstable when the microorganisms in the aeration tank cannot survive a continuous series shock loading (Eckenfelder and Grau, 1998). Another problem associated with this system is a direct result of its controllability. It requires relatively sophisticated operation in order to achieve the desired results, hence very expensive. The activated sludge system removes certain priority pollutants at efficiency of 95% or more (Eckenfelder *et al*., 1985). To understand the process, the description of the different stages is necessary. The predominant physiological types of bacteria involved may shift during the various stages of the wastewater treatment. Conditions can range from highly aerobic to strictly anaerobic. Activated sludge process has undergone various modifications to be able to meet most wastewater treatment needs (Toerien *et al*., 1990). Briefly, these changes relate to the flow regime in the reactor, the size, the number and configuration of the reactors, the recycled flow, the influent flow and others incorporated either emphatically or present inadvertently or unavoidable.

Over the past two decades, significant advances have been made in the area of engineering (design) and technology (implementation and operation) (E&T) of the single activated sludge system. Implementation at full scale includes aerobic COD removal and nitrification, anoxic denitrification and anaerobic/anoxic/aerobic biological excess phosphorus removal (BEPR). This implementation has been aided by the development of a suite of steady state design models (e.g. Wentzel *et al*., 1990; Maurer and Guejer, 1994) and kinetic simulation models (e.g. Wentzel *et al*., 1992; Henze *et al*., 1995).

Aeration is one of the key operational designs, which contribute to the efficient degradation of organic matter. This is done by bottom air blowers such as porous diffusers or surface aerators, which oxygenate the mixed liquor for aerobic degradation. Aerobic conditions must be maintained in the reactors as this keeps the biomass in suspension. Other operational parameters, which affect the process efficiency, include mixed liquor suspended solids, organic loading, food to microorganisms ratio, sludge residence time and sludge recycle ratio. Although the process has undergone various changes, the basic principles remain the same: the removal of soluble biodegradable organic compounds from wastewater, the recycle of a small amount of return sludge from the clarifier underflow to the reactor and the high performance in the systems hinging on the mean cell resistance of microorganisms (Grady and Lim, 1980).

The activated sludge process has been designed primarily for carbon, nitrogen and phosphate removal under specific conditions (Ehlers and Cloete, 1999). The principle factors which determine the ability of the purification process to remove organic pollutants, and in that way, the quality of the effluent are: (i) the reaction time between the wastewater and biomass in the aeration tank, (ii) the types and speed of reactions that take place during wastewater treatment, (iii) concentration of the contaminants in the wastewater and (iv) the biomass in each moment during the reaction. Thus for optimal treatment using activated sludge system, it is important that the influent wastewater have certain characteristics that make it treatable, namely the wastewater must contain essential plant nutrients such as nitrogen and phosphorus, be between pH 7.0 - 8.5 and free of substances that inhibit the growth of aerobic microorganisms (Jenkins *et al*., 1993).

In addition, the wastewater stream should be constant in terms of quality and composition, in order to avoid shock loads that could negatively influence the process and yield. However, the constant agitation in the aeration tanks and sludge recirculation are deterrents to the growth of higher organisms. Observation of mixed populations of microorganisms can now be explained on the basis of growth kinetics, their settling properties and operational characteristics of the plant. The diagram below describes an activated sludge wastewater treatment process with all the stages involved during the treatment (Jenkins *et al*., 1993).

2.3.3.1 Stages of activated sludge system

Figure 2.4 below illustrates the various stages of a typical activated sludge wastewater treatment system.

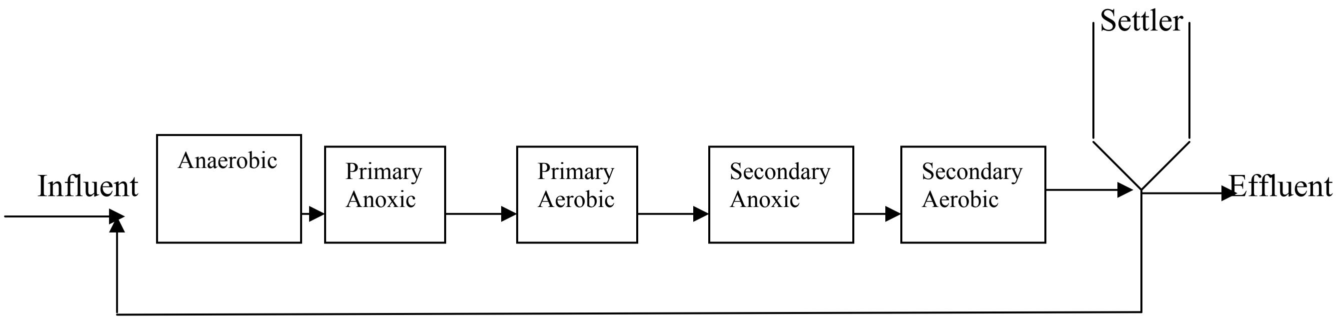


Figure 2.4: Schematic of a typical activated sludge wastewater treatment system (Toerien *et al*., 1990).

2.3.3.1.1 *The anaerobic zone*

The anaerobic zone is considered to be one in which both dissolved oxygen and oxidized nitrogen are absent (Barnard, 1976; Buchan, 1984). In this zone, sludge from the clarifier flows in jointly with the influent wastewater. It has been reported that for this zone to operate efficiently, oxygen and nitrates must be absent. This zone is responsible for the release of phosphate.

2.3.3.1.2 *The primary anoxic zone*

In this context, anoxic refers to the presence of nitrates and absence of dissolved oxygen (Pitman, 1984; Streichan *et al*., 1990). The primary anoxic zone is the main denitrification reactor in the process. It is fed by the effluents from the anaerobic zone and mixed liquor recycled from the aerobic zone. The presence of nitrate or nitrite and absence of oxygen leads to the enrichment of denitrifying bacteria, which reduces nitrate or nitrite to molecular nitrogen. Thus soluble and colloidal biodegradable matters are readily removed in this zone. It has been reported that phosphate release occurs under anoxic conditions if suitable carbon substrates are added (Muyima *et al.*, 1997).

2.3.3.1.3 *The primary aerobic zone*

Primary aerobic zone functions mainly to oxidize organic material in sewage, ammonia into nitrates and also provides an environment to take up all the phosphate released in the anaerobic zone (Kuba *et al*., 1997). The removal of ammonia, it must first be oxidized to nitrites by nitrifying bacteria such as *Nitrosomonas, Nitrospira* and *Nitrosolobus*. Nitrites are then oxidized to nitrates by *Nitrobacter, Nitrospira* and *Nitrococcus*. These nitrates are removed in the primary anoxic zone by denitrifying bacteria. Phosphate uptake is based on the enrichment of the activated sludge with bacteria capable of taking up excess orthophosphate. Certain bacterial species such as *Acinetobacter*, *Pseudomonas*, *and E. coli* have been associated with the enhanced phosphate removal in activated sludge (Muyima *et al*., 1997).

2.3.3.1.4 *The secondary anoxic zone*

The purpose of the secondary anoxic zone is to further convert excess nitrates into nitrogen, which was not removed in the zone preceding it. Due to the slow denitrification rate in this zone, the quantity of nitrate removed is small. The retention time in the anoxic zone is relatively 48 h because of the lower chemical oxygen demand (COD) (Muyima *et al*., 1997).

2.3.3.1.5 *The secondary aerobic zone and clarifier*

This zone removes additional phosphate, which was not removed in the primary aerobic zone. Residual ammonia is also oxidized in this zone. The secondary aerobic zone increases the level of the dissolved oxygen between 2 and 4 mg/l in the mixed liquor before it enters the clarifier. Aeration should be enough to promote phosphate uptake and maintain good aerobic conditions. Phosphorus is retained in the biomass as long as aerobic conditions prevail (Pitman, 1984). This zone prevents the development of anaerobic conditions in the clarifier and phosphate release before clarification (Muyima *et al*., 1997). In the clarifier, treated wastewater, free of organic matter and dissolved solids is released. Thereafter the treated wastewater is disinfected and discharged into the surrounding receiving water body.

2.3.3.2 Dynamic community of the activated sludge system

In the activated sludge process, wastewater is treated aerobically by a microbial consortium dominated by heterotrophic bacteria, which are flocculated in the mixed liquor of the aeration tank to form discrete clumps of microorganisms. Denitrification by heterotrophic bacteria in activated sludge treatment is of particular interest in that nitrates and nitrites are eutrophic (Gray, 1989); hazardous to human health as well as inhibit phosphorus removal during activated sludge treatment. Furthermore, denitrifying heterotrophic bacteria are often implicated in enhanced biological phosphorus removal (EBPR) both under aerobic as well as anoxic conditions (Kavanaugh and Randall, 1993). Activated sludge comprises a diverse population of microorganisms, which include eubacteria, filamentous bacteria, rotifers, protozoa and algae (Jenkins *et al*., 1984). Metazoans such as nematode worms and human enteric viruses may be found in sewage influent, but a large percentage appears to be removed by the process. In activated sludge systems, degradation of organic matter takes place in an aeration tank in which heterotrophic active biomass; essentially protozoa and bacteria (5% and 95% of total biomass, respectively) are responsible for the purification process (Ministry of Technology, 1968). These microorganisms are capable of surviving in activated sludge thus increasing the stability of the process.

2.3.3.3 Bacterial population of the activated sludge system and their respective roles

Sewage influent contains some tens of mg/l of bacterial cells, a level that can vary markedly according to the type of sewer, the weather conditions, the time of the year, etc. Activated sludge systems therefore consist of different bacteria species, which co-exist, and function, together in a complex microbial community. Both heterotrophic and autotrophic bacteria reside in activated sludge.

The dominant bacteria in the activated sludge system are aerobic heterotrophs that degrade and eventually mineralise organic compounds present in wastewater to carbon dioxide and water. It is the small size of bacteria and their resultant large surface area to volume ratio, which makes them efficient in terms of nutrient and catabolic exchange (Gray, 1989). Heterotrophic bacterial populations remain relatively stable throughout the plant with various environments in the three zones allowing different bacteria to dominate in terms of metabolic activity (Lötter and Murphy, 1985). Heterotrophic bacteria obtain energy from carbonaceous organic matter in influent wastewater for the synthesis of new cells. At the same time, heterotrophic bacteria release energy via the conversion of organic matter into compounds such as carbon dioxide and water. Important genera of heterotrophic bacteria include *Achromobacter, Alcaligenes, Arthrobacter, Citromonas, Flavobacterium, Pseudomonas* and *Zoogloea* (Jenkins *et al*., 1993).

Autotrophic bacteria in activated sludge reduce oxidized carbon compounds such as carbon dioxide for cell growth. These bacteria obtain their energy by oxidizing ammonia nitrogen to nitrate nitrogen in a two-stage conversion process known as nitrification. Due to the fact that very little energy is derived from these oxidization reactions, and because energy is required to convert carbon dioxide to cellular carbon, nitrifying bacteria represent a small percentage of the total population of microorganisms in activated sludge. In addition, autotrophic nitrifying bacteria have a slower rate of reproduction than heterotrophic carbon-removing bacteria (Jenkins *et al*., 1993). Two genera of bacteria are responsible for the conversion of ammonia to nitrate in activated sludge, *Nitrobacter* and *Nitrosomonas* (Brodisch and Joyner, 1983) and a number of bacteria have been involved in the enhanced phosphate removal (Momba and Cloete, 1996; Muyima *et al*., 1997).

Acinetobacter were frequently isolated from activated sludge plants operated to achieve the biological removal of phosphate. A number of reports have suggested that the dominant organism is either *A. calcoaceticus* or *A. lwoffi* (Murphy and Lötter, 1986). This organism became the model organism for studying mechanisms of biological removal of phosphate. Other bacteria such as *Aeromonas (*Brodisch and Joyner, 1983), *Klebsiella pneumoniae*, *Pseudomonas* spp. and *Escherichia coli* have all been implicated in the removal of phosphate (Momba and Cloete, 1996). Both aerobic and anaerobic bacteria may exist in the activated sludge, but the preponderance of species is facultative.

Facultative bacteria are an important factor in the survival in activated sludge when dissolved oxygen concentrations are low or perhaps approaching depletion. Bacteria are responsible for the stabilization of wastes coming into a treatment plant. Many of these bacteria form floc particles or clusters, which serve as sites on which waste can be absorbed and broken down. The majority of bacteria found in floc particles are spherical, rod-shaped or spiral shaped. Filamentous bacteria form the floc macrostructure known as trichomes, which provide a backbone for the floc particles, allowing the particles to grow in size. Imbalances between filamentous and floc forming has been correlated to activated sludge settleability as filamentous bacteria are important for the formation of robust flocs that are capable of withstanding agitation and aeration (Jenkins *et al*., 1993). When filamentous bacteria are present in excessive numbers or length, they often cause settleability problems known as bulking.

2.3.4 Disinfection of the Final Effluent

Regardless of what type of treatment used, municipal wastewater generally requires disinfection to meet specific microbiological units before discharge into the environment. Although regulations require disinfection of effluents prior to discharges, it has been found that such effluents were either inadequately disinfected or not disinfected at all (Simpson and Charles, 2000). It is important to note that the disinfection of wastewaters provide the first line of defence for drinking water from surface water sources. To meet this objective, the disinfectant must inactivate a wide range of microorganisms in a variety of wastewater qualities. Disinfection may be accomplished chemically (chlorination, chloramination and ozonation) or physically (ultra-violet radiation). Although Ozone and ultra violet (UV) irradiation can be used for disinfection of wastewater, these methods of disinfection are not in common use in South Africa.

2.3.4.1 Chlorination

According to White (1992) the most prevalent practice of wastewater disinfection is free chlorine (HOCl + OCl-), which dissociates into molecular hypochlorite (HOCl) and the hypochlorite ion (OCl-). This is also the practice in South Africa as was confirmed by a survey recently conducted by Momba, Thompson and Obi (2006a). Chlorine has a high oxidation potential and is thus, a much more efficient disinfectant, although it requires a relatively long contact time (U.S EPA, 1992). It has been found a disinfectant of choice as a result of its being effective, inexpensive and reliability. Chlorine is a very reactive chemical and does not only disinfect, but also rapidly reacts with contaminants such as NH4+, NO2-, H2S, Fe++, Mn++ and other organic compound (Yamamoto *et al*., 1988, Teefy and Singer, 1990). These compounds create a chlorine demand so that chlorine is applied until the demand is met and free chlorine appears. This practice is called breakpoint chlorination and is wasteful in that it consumes more chlorine than is required for disinfection alone.

Disinfection normally involves the injection of a chlorine solution at the head end of a chlorine contact basin. The chlorine dosage depends upon the strength of the wastewater and other factors, but dosages of 5 to 15 mg/l are common. Chlorine contact basins are usually rectangular channels, with baffles to prevent short-circuiting, designed to provide a contact time of about 30 minutes. However, to meet advanced wastewater treatment requirements, a chlorine contact time of as long as 120 minutes is sometimes required for specific irrigation uses of reclaimed wastewater. Generally, bacteria are readily susceptible to chlorine-based disinfection process. The bactericidal effects of chlorine and other disinfectants are dependent upon pH, contact time, organic content, and effluent temperature. In order to be effective, it is considered that the residual chlorine in the final stages of sewage treatment should be 0.1 mg/L (Voysey *et al*., 1988).

However, the reaction of free chlorine with the above-mentioned organic compounds present in wastewater leads to the formation of a group of compounds called trihalomethanes (THM's), which are considered a health hazard (Johnson and Jensen, 1986; Reynolds *et al*., 1989). Trihalomethanes have been proven to be toxic and possibly carcinogenic. Most viruses, the cysts and oocysts of protozoa are moderately susceptible to chlorine disinfection. These facts could be a concern in South Africa where treated sewage effluent is often reused as drinking water.

2.3.4.2 Chloramination

Chloramination is another widely used, chlorine-based disinfection method. Chloramines act as an oxidising agent but have lower oxidation potential. Benefits of using chloramines include a significant reduction in the formation of trihalomethane which is a product of the interaction of free chlorine with nitrogen containing organic compounds (Lykins *et al*., 1992) and greater disinfectant stability resulting in a reduction in disinfectant demand. This makes chloramination popular to many treatment authorities responsible for wastewater treatment, especially where the wastewater is high in organic compounds. Monochloramine has been confirmed as an effective disinfectant for wastewater especially in the properly nitrified effluents common to South African wastewater treatment effluents (Pretorius and Pretorius, 1999).

Disadvantages of chloramines are the relatively long lifetime after discharge to the receiving environment, possibly with toxicity problems, compared to free chlorine (Yamamoto *et al*., 1988) and their detrimental effect on kidney dialysis patients (Kreft *et al*., 1985). Studies have shown that free chlorine is a more effective disinfectant than the chloramines (Berman *et al*., 1992) while some field reports, that observe naturally occurring bacteria and water with a chlorine demand, have shown that chloramines are adequate, and in some cases superior to free chlorine in terms of indicator organism reductions (Reynolds *et al*., 1989).

2.3.4.3 Ozonation

It is one of the most common disinfection methods examined for its efficacy to replace chlorination. Ozonation has very good bactericidal and virucidal activities and requires shorter contact time to inactivate microorganisms. It can only be used on secondary treated wastewater and most suitable for medium to large-scale treatment process. It is a very efficient method for disinfecting wastewater and does not have problems with suspended solids, colour etc.

Despite these advantages, ozonation cannot maintain a residual in the water following treatment. Ozone reacts with organic substances to produce low-molecular weight oxygenated by-products that are generally more biodegradable than their precursors. These substances will promote biological growth in the distribution system (“regrowth”). Due to these reasons, ozone should be used in combination with other disinfectants that maintain an active residual for longer periods and it should be combined with some methods of filtration for removing biodegradable material (Glaze *et* *al*., 1987). Because of its instability, ozone must be generated on site. It is an expensive and energy intensive process, which is more complex to operate and maintain than the other systems available (U.S. EPA, 1992).

2.3.4.4 Ultraviolet light (UV)

Ionising radiation using ultraviolet light is a common physical disinfection method. Ultraviolet light radiation for treatment of water is basically a disinfectant technology. Ultraviolet light is electromagnetic radiation, lying between visible and X-rays wavelengths. This kind of radiation can be artificially generated by monochromatic low pressure mercury lamps, and causes severe bactericidal action in the wavelength band lying between 200 and 310 nm. The inactivation of microorganisms is essentially based upon photochemical reactions in the DNA, which result in errors being introduced into the DNA bacteria. There are some limiting factors for UV disinfection of wastewater such as absorption by suspended solids, microbial initial concentration, UV absorbance, and hydraulic delivery systems (U.S. EPA, 1992).

Ultraviolet light has very good bactericidal and virucidal activity. It disinfects primarily by interacting with the nucleic acids of the pathogen and by inducing damage, which interferes with the nucleic acid replication processes that are prerequisites for functional cell division (Setlow, 1967). Ultraviolet light requires a short contact time when compared to chlorination and is best suited for small to medium scale treatment processes. However ultraviolet light cannot maintain a residual in water following treatment (U.S. EPA, 1992).

Efficacy of both ultraviolet light and chlorine has been found to produce a disinfection level in wastewater (i.e. inactivation of bacteria, bacteriophage and poliovirus), and ultraviolet light did not have the problem of toxic by-product formation, which occurred with chlorination (Oppenheimer *et al*., 1997). Faecal indicators are very sensitive to ultraviolet light while specific F-RNA bacteriophage is the most resistant (Moreno *et al*., 1997). Although efficient in the rapid removal of contaminants, the chemicals required in treatment are expensive, increased volume of sludge is produced and often results in sludge with poor settling and dewatering characteristics. The disinfection efficiency of ultraviolet light is affected by the amount of turbidity, colour, microbial load, dissolved organic and inorganic matter in the wastewater. Increases in any of these factors therefore decrease the disinfection efficiency (Wolfe, 1990). Monitoring the quality of effluents before discharge therefore remains important.

2.4 MONITORING OF WASTEWATER QUALITY

Monitoring the physical, chemical and biological markers of a particular water source provides a means to determine the overall quality of the source water (Reynolds, 2003). Typical indicators used in wastewater quality monitoring include nitrogen and phosphorous (markers for the presence of nutrients); chlorophyll-a (a marker for algal blooms); suspended solids and turbidity (water clarity indicator); dissolved oxygen (an indicator of the oxygen available to aquatic organisms); pH (a measure of the acidity or alkalinity of the water); conductivity (a measure of the salinity of water), and coliform bacteria, among others. Monitoring of bacterial population is important for ecological studies in order to determine which bacteria have a significant role in the wastewater treatment process. It is also to demonstrate that the wastewater is properly disinfected during treatment in terms of sanitary quality and to evaluate some overall measure of the presence of indicators and, from that, the possible degree of risk of contracting waterborne diseases. This section mainly focuses on the monitoring of the bacterial population of wastewater.

2.4.1 Conventional method

Conventional wastewater quality monitoring involves measuring some chemical constituents and biological indicators on a fixed monitoring grid where samples are taken at an arbitrarily established frequency, usually for a period of a year. Even though the last decade has seen a molecular revolution in microbiology, the standard methods for monitoring wastewater treatment plants still rely on the tools available to the researcher at the beginning of this century, the microscope and agar plates.

2.4.1.1 Microscopic technique

The direct enumeration of bacteria in wastewater treatment plants has relied on the use of epifluorescence microscopy, typically after acridine orange or 4', 6-Diamidino-2-phenylindole (DAPI) staining and then passing through a membrane filter. Other microscope techniques that have been used to date include electron microscopy; autoradiography, infrared film photography and confocal laser microscopy. Microscope methods require skilled personnel and often present difficulties in distinguishing between living and dead cells, microbial cells from debris on the basis of morphology. Due to the failure to distinguish between living and dead cells or non-living particles, the direct count may result in an overestimation of the viable cells present (Kogure *et al*., 1979). It does not permit differentiation of bacterial cells on the basis of taxonomy and metabolic activity. Microscopy observance of microorganisms in activated sludge is a useful tool for monitoring the biological process. The microscopic examination of mixed liquor has characterized the evolution of microbial biomass and flocs characteristics for a given period of time. Direct total cell counts of bacteria in wastewater usually exceed counts obtained from heterotrophic plate counts and most probable number methods because, unlike those procedures, direct counts preclude errors caused by viability-related phenomena such as selectivity of growth media, cell clumping and slow growth rates (APHA, 1998).

2.4.1.2 Culture-based methods

Most investigations of bacteria in wastewater treatment plants have relied on indirect methods to isolate and enumerate. This includes traditional plate counting methods and other culture-dependent techniques. Approved methods and media for the isolation of bacterial pathogens from wastewater are well established. Traditionally water and wastewater treatment plants monitor heterotrophic bacteria, faecal and total coliform bacteria and other indicator organisms to evaluate efficacy of removal or inactivation of pathogenic microorganisms and assessing the related health risk. The total coliform group of bacteria includes genera such as *Enterobacter, Klebsiella, Citrobacter* and *Escherichia*. Although this section will focus on the detection of bacteria particularly *Salmonella* spp, *Shigella* spp and *Vibrio* spp., general methods used for heterotrophic bacteria and coliforms will be underlined.

It is recommended that combination of indicators, each with their own advantage and disadvantages, be used when assessing microbial water quality. Many groups of coliforms are poor indicators of faecal pollution as they can exist and grow naturally in both soil and water and are thus more useful as co-indicators of the general microbial condition of water. The faecal coliforms are a subgroup of total coliforms and are a much better indicator of faecal pollution (Hurst *et al*., 1997). Faecal coliforms have a very high correlation with faecal pollution, and can be used with confidence to directly infer faecal pollution of water (Hurst *et al*., 1997).

Approved traditional methods for the coliform detection include multiple tube fermentation technique (in lactose and lauryl tryptose broths incubated at 35ºC for 48 h) and membrane filter technique using different specific media and incubation conditions such as 35ºC for 24 h in m-Endo (APHA, 1998) and 37ºC for 24 h or 44ºC for 48 h in Tergitol-TTC (AFNOR, 1990). These methods have limitations such as duration of incubation, antagonistic organism interference, lack of specificity and poor detection of slow-growing viable but non-culturable (VNBC) microorganisms. To test for coliforms and faecal coliforms, a variety of simple and more specific tests have been developed. These include the membrane filtration technique, the presence-absence (P-A) test for coliforms, the most probable number technique and the related colilert defined substrate tests for detecting both coliforms and *E. coli*. The detection of coliforms based on specific enzymatic activity such as beta-D galactosidase and beta-D glucuronidase on many chromogenic and fluorogenic substrates have improved the sensitivity of these methods. Membrane filter has a low recovery compared to the most probable number and presence-absence techniques as described by APHA (1998). The various culture-based methods that can be used for the detection of bacteria are discussed below.

2.4.1.2.1 *Pour plate/Spread plate method*

The heterotrophic plate count may be determined by pour plate, spread plate, or membrane filter method (APHA, 1998). Together with the pour and spread plate methods and the membrane filter methods, the recommended media that could be used are Plate count agar (tryptone glucose yeast agar), R2A agar and NWRI agar (HPCA) while m-HPC agar can be used only for membrane filter method. The heterotrophic plate count provides an approximate enumeration of total numbers of viable bacteria that may yield useful information concerning water quality and may provide supporting data on the significance of coilform test results. The heterotrophic plate count is useful in judging the efficiency of various treatment processes and may have significant application as in-plant control test (APHA, 1998). The pour plate method can accommodate volumes of sample or diluted sampling, ranging from 0.1 to 2.0 ml producing colonies that are relatively small, compact and cannot be transferred. The spread plate method can accommodate small volumes of sample ranging from 0.1 to 0.5 ml and yields colonies that can be distinguished readily and transferred quickly for further purification and storage. The membrane filter method permits testing large volumes of low-turbidity water and requires coloured filters or contrast stains (APHA, 1998).

2.4.1.2.2 *Membrane filtration method*

The membrane filtration method has gained wide acceptance because the procedure is simple, rapid, and precise and gives definitive results (Geldreich *et al*., 1976). This method enables the enumeration of microorganisms present in a sample of water. This method consists of filtering a measured volume of water through a sterile filter with a pore size of 0.45 µm. The pore size of the membrane is such that the bacteria are retained on or near the surface of the membrane. The membrane is aseptically transferred to either a solid medium or to an absorbent pad saturated with a liquid medium such as m-ColiBlue24 Broth (APHA, 1998). On incubation at a specific temperature for a specific time, growth will occur. Colonies of characteristic morphology and colour, depending on the medium used, are counted and the number of organisms per 100 ml is calculated. The results are expressed in colony forming units per 100 ml (Rompré *et al*., 2002). Membrane filtration methods can also be used for the detection of coliforms.

2.4.1.2.3 *Fermentation tube technique for members of the coliform group*

The fermentation tube technique involves inoculating a measured volume of water, such as 10 ml in a test tube with a broth containing lactose. If fermentation occurs and gas is produced, this is taken as a positive presumptive test for coliforms. If one fermentation tube per sample is used, the test is called a presence-absence (P-A) test (Pipes and

Christian, 1984). If several fermentation tubes are inoculated with portions of the sample, the test is called the multiple tube fermentation (MTF) test. The original multiple tube fermentation tests that was used for coliforms involved the presumptive, confirmed and completed tests. The results may be used for calculation of an estimate of the coliform density of the sample, which is called the most probable number (MPN). The multiple tube test is frequently called MPN. The completed tests including the confirmed and completed tests require at least 4 days of incubation and transfers (Prescott *et al*., 1999).

2.4.1.2.4 ***Most probable number technique***

The enumeration of coliforms and *E. coli* is usually arrived at by the most probable number (MPN) method. When multiple tubes are used in the fermentation technique, results of the examination of replicate tubes and dilutions are reported in terms of the most probable number (MPN) organisms present. This number, based on certain probability formulae, is an estimate of the mean density of coliforms in the sample (Pipes and Christian, 1984).

2.4.1.2.5 Presence/absence (P-A) coliform test

Presence/absence (P-A) tests are a simple modification of MPN method in which a larger volume of water sample (50 or 100 ml) is incubated in a single culture bottle containing an appropriate medium. The Presence/absence test provides qualitative information on the presence or absence of microorganisms in the tested sample. The Presence/absence test also provides the opportunity for further screening of the culture to isolate other indicators (faecal coliforms, *Aeromonas*, *Staphylococcus*, *Pseudomonas*, faecal *streptococci* and *Clostridium*) on the same qualitative basis. Additional advantages include the possibility of examining a large number of samples per unit time (APHA, 1998).

2.4.1.2.6 ***Colilert test for the presence of total coliforms and E. coli***

The colilert defined substrate test can be used effectively to test for both coliforms and *E. coli*. A 100 ml-water sample is added to a specialized medium containing O-nitrophenyl-β-D-galactopyranoside (ONPG) and 4-methylumbelliferyl--β-D-Glucoronide (MUG) as the only nutrients. If coliforms are present, the medium will turn yellow within 24 h at 35ºC due to the cleavage of ONPG. To confirm the presence of *E. coli* the medium is observed under long wavelength UV light for fluorescence. In the presence of *E. coli* is present, the MUG is modified to yield fluorescent product. If the test is negative for the presence of coliforms, the water is considered suitable for human consumption (Prescott *et al*., 1999). The advantage of the test is that confirmatory tests are not required, results are easy to interpret and the test is very easy to inoculate.

2.4.1.2.7 Enzymatic methods

The biochemical tests used for bacterial identification and enumeration in classical culture method are generally based on metabolic reactions. These biochemical tests are not fully specific and many additional tests are sometimes required to obtain precise confirmation. The use of microbial enzyme profiles to detect indicator bacteria is an attractive alternative to classical methods. Moreover, reactions are rapid and sensitive. Chromogenic and fluorogenic substrates produce colour and fluorescence, respectively upon cleavage by a specific enzyme. Several authors such as Bascomb, (1987) and Manafi *et al*., (1991) have reviewed chromogenic and fluorogenic substrates used for bacterial diagnostics. The authors noted that the use of these substrates has led to improved accuracy and faster detection. Methods for the detection or enumeration may be performed in a single medium, thus by passing the need for a time-consuming isolation procedure prior to identification.

2.4.1.2.8 ***Culture-based method for the recovery of Salmonella spp; Shigella spp and Vibrio cholerae***

The various culture media used to enrich and selectively recover *Salmonella* spp; *Shigella* spp and *Vibrio cholerae* are discussed below.

2.4.1.2.8.1 ***Detection of Salmonella spp***

The conventional method for detecting *Salmonella* spp involves culture enrichment and plating procedures. Although *Salmonella* spp can be recovered readily from water and sewage, these bacteria may be present in low numbers and may be sub-lethally injured by exposure to such environments. Due to this reason the bacteria have to be incubated in pre-enrichment media such as Buffered Peptone Water (BPW) or lactose broth for 18-24 hours at 37°C, although some studies using naturally contaminated sewage samples have shown 43°C to be superior. Volumes of pre-enrichment media are added to a selective broth such as Rappaport-Vassiliadis (RV), Selenite or Tetrathionate broths. Many studies have shown Rappaport-Vassiliadis broth to be generally superior to the others although *Salmonella typhimurium* grows poorly on this medium.

Following enrichment, the broths are plated onto selective or diagnostic agars such as Xylose-Lysine-Deoxycholate (XLD), Deoxycholate Citrate (DCA) or Brilliant Green (BG) agars. *Salmonella*-like colonies on selective agars need to be confirmed by biochemical testing, and serology and phage-typing where appropriate. It may take up to five days to complete sample examination. This delay has led to the investigation of more rapid methods of *Salmonella* spp detection such as the Polymerase Chain Reaction (PCR). This will be discussed in section 2.4.2. Impedance enumeration of microorganisms is another method where the relative or absolute change in conductance of a medium is used as a signal to determine the metabolic activities of *Salmonella* (Ruan *et al*., 2001).

2.4.1.2.8.2 *Detection of Shigella spp*

The standard procedure for *Shigella* spp detection is based on isolation on selective culture media followed by identification by biochemical tests and agglutination assays (June *et al*., 1993). Methods that have resulted in isolation of *Shigella* spp include membrane filtration and centrifugation with or without subsequent broth enrichment (APHA, 1998). Detection in wastewater requires the samples to be centrifuged, after which the pellets are resuspended and inoculated into MacConkey and Xylose-Lysine-Deoxycholate (XLD) plates. Enumeration of suspected colonies is performed while biochemical identifications and serotyping follows (APHA, 1998).

Shigella spp can be recovered by enrichment on selective medium such as Selenite F broth, which minimizes accumulation of contaminants. Membrane filtration procedure involves filtering water samples through 0.45 μm pore size membrane and placing the filters Xylose-Lysine-Desoxycholate (XLD) or MacConkey agar plates.

Plates are incubated at 35ºC overnight. Red/pink colonies, which indicate lactose fermenters, are further cultured on Triple Sugar Iron Agar (TSI) and Lysine Iron Agar (LIA) slants at 35ºC. This process may take 48-72 h or even longer to obtain results. Since *Shigella* spp are very fastidious organisms, appropriate collection, rapid transport to the laboratory and rapid plating of the sample are important for isolation. Such conditions are often difficult to attain in developing countries.

2.4.1.2.8.3 *Detection of Vibrio spp*

Methods employed to detect the presence of *Vibrio cholerae* in environmental and clinical samples include the multilocus enzyme electrophoresis (MEE) and pulsed-field gel electrophoresis (PFGE). These techniques may have been used extensively, but their use has been limited since they are time consuming and labour intensive.

However, other simple and cost effective detection methods exist such as the concentration techniques in which Moore swabs are anchored for two days in the water source to be tested. This is followed by placement of swabs into an enrichment medium at 1:1 (weight/volume) ratio. Enrichment medium can be in a liquid alkaline peptone medium containing 1% sodium chloride or electrolyte supplement (1% sodium chloride, 0.4% magnesium chloride and 0.4% potassium chloride). Thereafter, incubation at an appropriate temperature, 37°C, followed by spread plating on a solid medium for selective growth. The medium of choice is Thiosulfate-Citrate-Bile-Salt-Sucrose (TCBS) Agar. This medium differentiates between sucrose-fermenting *Vibrio* (*V. cholerae*) and non sucrose-fermenting V*ibrio* (*V. parahaemolyticus* and *V. vulnificus*). Suspected colonies appear yellow, which is an indication of sucrose fermentation. Enumerations therefore require most probable number (MPN) method using alkaline peptone water, or in the case of large numbers of *Vibrio* spp, direct inoculation onto TCBS. Presumptive tests can be run to differentiate *V. cholerae* from other species. A non-selective medium such as trypticase soy agar or nutrient agar may be used at this stage for plating isolated colonies. Characterizations of isolates of V. cholerae require several biochemical tests to be run. If required serological and serogroup identification tests can be conducted (APHA, 1998; SABS, 2001).

2.4.2 Molecular-based methods

Several molecular methods have increasingly become the preferred tools for detection of bacteria pathogens in wastewater. Methods based on genotyping tend to have a high discriminatory power and offer rapid and sensitive subtyping, complementing conventional approaches that are based on phenotype, for example, serotyping and phage typing. Additional factors for consideration in the choice of molecular-based methods include reproducibility, ease of interpretation and time and cost efficiency. Recent methods for the analysis of microbial population in wastewater include molecular techniques such as Polymerase Chain Reaction-Denaturing Gradient Gel Electrophoresis (PCR-DGGE), fluorescent labelled and rRNA-targeted oligonucleotide probes specific for different taxonomic levels. Recently developed is an additional genus and species-specific probes for important filamentous and floc-forming bacteria (Wagner *et al*., 1994). Some of the known molecular-based methods are discussed below.

2.4.2.1 Polymerase chain reaction

The basic principle of polymerase chain reaction is amplification (up to a million times) of the specific DNA sequence of interest within a few hours. The Taq polymerase can amplify DNA from lysed cells without any further purification. In a PCR reaction, a specific DNA template is amplified by a thermo-cyclicing process, in which the DNA is denatured by high temperature. Subsequently, two specific oligonucleotides are hybridised to the complementary strands at a temperature just below the melting temperature – the annealing temperature, and finally DNA polymerase will extend the oligonucleotides at a temperature optimal for its activity. By repeating the cycle several times, DNA between the two primers is amplified exponentially (Jensen e*t al*., 1993). It is essential to have prior knowledge of a unique DNA sequence in the target (suspect) bacteria, which might be specific to a genus, species, or strain.

The polymerase chain reaction (PCR) is a rapid technique of high specificity and sensitivity. This technique employs single short primers (decamers) with arbitrary nucleotide sequences in a polymerase chain reaction (PCR) to randomly amplify genomic DNA, which subsequently generates strain-specific arrays of amplified DNA fragments (Shangkuan *et al*., 1997). Disadvantage of the PCR method is that it is expensive and requires sophisticated equipment. The major obstacle in using PCR for the detection and identification of pathogenic organisms from clinical samples and environmental water samples is the presence of substances that are inhibitory to PCR (Rossen *et al.,* 1992). The procedure uses primers directed at sequences located on the invasion plasmid and chromosome of species.

2.4.2.2 DNA-DNA hybridization (Southern blot)

Hybridization of nucleic acid is one of the preferred approaches to confirm identification of a nucleic acid sequence in an organism. The blotting method suitable for identifying DNA sequences in bacterial pathogens is known as Southern blot or DNA-DNA hybridization. Genomic or plasmid DNA from the bacteria is digested using one or more restriction enzymes and digestion fragments are separated using agarose gel electrophoresis. After single-stranded fragments are blotted onto nitrocellulose or nylon paper, the gene probe of interest (labelled with radioactive or chemiluminiscent digoxigenin) is hybridised. The target DNA sequence can be identified and its fragment size measured using an apparatus to detect radioactivity or chemiluminescence (Wondwossen, 2003).

2.4.2.3 DNA fingerprinting (genotyping)

Fingerprinting, also referred to as genotyping, identifies an organism or strain on the basis of its nucleic acid content (most commonly DNA). By definition, the most sensitive and accurate way of fingerprinting is DNA sequencing. However, approaches that indicate variation in sequences are less costly and more rapid than sequencing, and have been used effectively. Genotyping methods are commonly based on identification of restriction fragments, for example, by Restriction fragment-based genotyping methods (PFGE); amplification by Polymerase chain reaction, for example, by amplified fragment length polymorphism (AFLP) or repetitive sequence Polymerase chain reaction (Rep-PCR); or DNA sequence, for example, by muoltilocus sequence typing (MLST) (Wondwossen, 2003).

2.4.2.4 Restriction fragment-based genotyping methods (PFGE)

The basic principle of Restriction fragment-based genotyping methods, which has been used since 1984, is digestion of genomic DNA with rare cutter restriction enzymes specific for the bacteria of interest (Schwartz and Cantor, 1984). After restriction enzymes digest intact genomic DNA embedded in agarose, digested fragments are separated in a pulsed-field gel electrophoresis apparatus. This method has been applied to identification of various pathogens and has been the standard genotyping technique adopted by the Centre for Disease Control and Prevention (CDC) (Wondwossen, 2003).

2.4.2.5 Amplified fragment length polymorphism (AFLP)

Amplified fragment length polymorphism, a recently developed alternative fingerprinting method (Vos *et a*l., 1995), is a Polymerase chain reaction-based typing system technically different from Restriction fragment-based genotyping method, with a high-resolution approach and throughput capacity. This method is sensitive to contamination that may affect its reproducibility, and subsequently its sensitivity and specificity. In addition, this method requires relatively more expensive equipment, a DNA sequencer, which is not available in most research laboratories. The optimal number of scorable bands (50 – 100) can easily be set by selection of the appropriate AFLP primers and restriction enzymes.

2.4.2.6 Fluorescent in situ hybridization

Fluorescent *in situ* hybridization (FISH) uses gene probes with a fluorescent marker, typically targeting the 16S rRNA (Amann, 1995b). Concentrated and fixed cells are permeabilized and mixed with the probe. The DNA, RNA, and oligonucleotide probes can be obtained from GeneBank and GeneDetect where there are databases for the probes that have been identified. Incubation temperature and addition of chemicals can influence the stringency of the match between the gene probe and the target sequence. Since the signal of a single fluorescent molecule within a cell does not allow detection, target sequences with multiple copies in a cell have to be selected (e.g. there are 10^2 – 10^4 copies of 16S rRNA in active cells).

A number of FISH methods for the detection of coliforms and enterococci have been developed (Patel *et al*., 1998), but few fluorescent oligonucleotide probes have been developed for waterborne bacterial pathogens. The rRNAs are the main target of FISH for reasons such as: rRNAs can be found in all living organisms, rRNAs are relatively stable and occur in high copy numbers (usually several thousands per cell); and include both variable and highly conserved sequence domains. Signature sequences unique to a chosen group of microrganisms, ranging from whole phyla to individual species, can be identified by comparative sequence analysis. The public databases now include 16S rRNA sequence for most cultured microbial species, as well as numerous sequences directly retrieved from the environment. FISH involves the detection of morphologically intact cells utilizing labelled probes (Amann *et al*., 1995a).

Probes are pieces of a single stranded DNA, varying in lengths of 15–25 nucleotides, which are complementary to a site within the rRNA of the bacteria researchers wish to examine. Probes are designed using sequence information from the databases and program packages such as ARB database (Amman, 1995b). This procedure includes the following steps: (i) fixation of the specimen on the microscopic slides; (ii) preparation of the sample; (iii) hybridization with the respective probes for detecting the respective target sequences; (iv) washing steps to remove unbound probes; (v) mounting, visualization and documentation of results.

The following chapter focuses on the experimental study conducted in four wastewater treatment plants. It mainly deals with the physico-chemical characteristics of the respective final effluent after wastewater treatment.

CHAPTER 3

PHYSICO-CHEMICAL CHARACTERISTICS OF EFFLUENT DISCHARGES AND THE IMPACT ON THE RECEIVING WATER BODIES: CASE STUDY – BUFFALO CITY AND NKONKOBE MUNICIPALITIES, EASTERN CAPE, SOUTH AFRICA.

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3.1 ABSTRACT

The efficiency of four wastewater treatment plants that serve the Buffalo City (Dimbaza and East London) and Nkonkobe (Alice and Fort Beaufort) Municipal areas in the Eastern Cape Province of South Africa was investigated in terms of nutrient removal and physicochemical characteristics that might lead to the pollution of receiving water bodies. The study was conducted from 6th August 2003 to 24th March 2004 using standard physicochemical methods. The Student's t-test was used to compare the physicochemical parameters at the effluents and receiving water bodies to assess the significant impact of the quality of effluent discharges on the respective receiving water bodies. Statistical evidence showed a relationship between the quality of the final effluent and that of the receiving water body and the relationship was such that the better the quality of the final effluent, the better the quality of the receiving water body. The quality of both the effluents and the receiving water bodies were acceptable with respect to the temperature (mean range: 16.52 - 23.33 °C), pH (mean range: 7.79 - 8.97), chemical oxygen demand (COD) (mean range: 7 - 20 mg/l) and total suspended solids (TSS) (mean range: 161.43 - 215.67 mg/l). However, the nutrients (orthophosphate - mean range: 3.70 - 11.58 mg/l and total nitrogen - mean range: 2.90 - 6.90 mg/l) were eutrophic. The dissolved oxygen (DO) (mean range: 3.26 - 4.57 mg/l) and the biological oxygen demand (BOD) (mean range: 14 - 24 mg/l) did not comply with the EU guidelines for the protection of the

aquatic ecosystems. The effluents discharges from the Dimbaza, East London, Alice and Fort Beaufort wastewater treatment plants were identified as point source pollution into the respective receiving water bodies (Tembisa Dam, the Nahoon and Eastern Beach which are part of the Indian Ocean; the Tyume River and the Kat River).

Key words: Effluent discharge, physicochemical quality, receiving water bodies

3.2 INTRODUCTION

Prosperity for South Africa depends upon the sound management and utilization of many resources, with water playing a pivotal role. Located largely in a semi-arid part of the world, South Africa's water resources are, in global terms, scarce and extremely limited. A key environmental problem facing South Africa is water pollution. This arises from many sources, including municipal and industrial effluents, and runoff of biocides, nutrients and pathogens from agricultural lands, urban areas and informal settlements with poor sanitation (Kotze, 2000).

The data available in the literature, an increasing awareness of the need to control the pollution of South African water resources (DWAF, 1986; SWLR, 1995) and to protect their quality exists (Jagals, 1997; Quilbell *et al*., 1997). In recent years, stricter discharge permits into rivers or other surface water bodies require wastewater treatment plants to produce better quality effluent. This is important in order to minimize environmental pollution and disease transmission.

Sewage discharges are a major component of water pollution, contributing to oxygen demand and nutrient loading of water bodies, promoting toxic algal blooms and leading to a destabilized aquatic ecosystem (WRC, 1995). The presence of dissolved oxygen (DO) in wastewater is desirable because it prevents the formation of noxious odours. Pollution can cause DO to drop below the level necessary for maintaining a healthy biota. Extreme pH levels less than 5 alter the toxicity of other pollutants in rivers and result in a direct health effect such as irritation of the mucous membranes thereby impairing recreational use of such water (DWAF, 1996b). Chemical oxygen demand is defined as a measure of the oxygen equivalent of the organic matter content of a sample that is susceptible to oxidation by a strong chemical oxidant (APHA, 1998). Most organic compounds can be oxidized to 95% - 100% of the theoretical value. It is an important parameter for stream and industrial waste studies and for the control of wastewater treatment plants (APHA, 1998). Phosphate in sewage effluents arises from human wastes and domestic phosphate-based detergents. Phosphates are undesirable anions in receiving waters and act as the most important growth-limiting factor in eutrophication and result in a variety of adverse ecological effects (OECD, 1982). High nitrate levels in waste effluents could also contribute to the nutrient load of the receiving water and so contribute to eutrophication effects (OECD, 1982, Fried, 1991). The potential health risk from nitrate in drinking water is linked to the condition known as methaemoglobinemia otherwise known as blue baby syndrome in bottle-fed infants and pregnant women (Bush and Meyer, 1982, Kelter *et al.,* 1997; Kempster *et al.,* 1997). Whilst this condition occurs very rarely and only with water containing more than 30 mg NO-N/L, it is still a cause of concern.

Realization of the above-mentioned undesirable consequences has led many local and national authorities to set up stringent guidelines for the control of chemicals in surface waters and wastewater effluents. In South Africa, little data on quality of water sources and associated health problems are available, since limited surveys have been conducted. The Eastern Cape Province of South Africa is mostly non-urban, poor and without adequate infrastructure though urban and semi urban sectors can be found. Most people in the rural areas often utilize the contaminated surface water for drinking, recreation and irrigation, which creates a situation that, poses a serious health risk to the people.

In this chapter the impact of the physico-chemical quality of the effluent discharges from Buffalo City and Nkonkobe wastewater treatments on the Eastern Cape water sources was assessed. To achieve this aim, the performance of four wastewater treatment plants in terms of nutrients and physicochemical status of the receiving water bodies after impact was assessed. Our intention was to provide information that could assist water authorities in addressing problems in the management of wastewater treatment in the region.

3.3 MATERIALS AND METHODS

3.3.1 Description of the sampling sites

Four wastewater treatment plants that serve the Buffalo City (Dimbaza and East London) and Nkonkobe (Alice and Fort Beaufort) Municipal areas in the Eastern Cape Province of South Africa were used in the present study. The wastewater treatment plants are located in urban (East Bank Reclamation Works, East London), semi-urban (Dimbaza Sewage Treatment Works) and in rural areas (Alice and Fort Beaufort Sewage Treatment Works). Appendix I illustrates the schematic diagrams of the wastewater treatment plants under the study.

The Alice Sewage Treatment Facility is situated approximately 1 km east of Alice town on a land belonging to the University of Fort Hare. The plant receives domestic sewage, some light industrial wastewater and town and street run-off water. The wastewater treatment system operating at Alice is one of a basic design comprising a screen, grit channel, anaerobic and aeration basins and clarifier (Appendix 1). Incoming effluent enters a solid and grit screening system where it flows to a primary settler where the sludge goes through a maceration process and flows to the activated sludge reactor. There, it enters the secondary clarifier where the sludge is separated from the effluent and disposed into sludge drying beds, while the remainder is returned to the activated sludge reactor. The final effluent is chlorinated, discharged into the Tyume River and a nearby pond for irrigation. The design capacity of the plant is 2 000 m^3/day and it currently operates approximately 1 100 m^3/day or 55% of its design capacity. Daily and hourly fluctuations in the volume of effluent accepted at the plant obviously occur. At about mid-day on 22 May 2002, the inflow was measured as being equivalent to 997 m^3/day. The average daily inflow during 2001 was 1 109 m^3/day. On average, the plant is producing 70 m^3/day of wet sludge.

The Dimbaza sewage treatment works is situated in the Eastern section of the township and it accepts municipal domestic sewage and wastewater containing a heavy industrial contribution. The wastewater treatment system is a basic design. The inlet works comprises of two screens, three grit channels and a flow recorder. The plant has two aeration tanks, each equipped with three vertically mounted mechanical aerators, two anaerobic tanks and two sedimentation tanks (Appendix 2). There is a return activated sludge (RAS) pump station which lifts the recycle sludge from the sedimentation tanks to the aeration tanks. A splitter box controls the flow of the raw sewage and RAS to the aeration tank. The plant has a waste mixed liquor pump station which pumps the waste mixed liquor from the aeration tank to two sludge lagoons. Chlorine contact is carried out by means of a water pressure operated, wall mounted, gas chlorinator in a baffled reinforced concrete contact tank. Thereafter the final effluent is pumped to a pair of final effluent reservoirs and into Tembisa sewerage dams. The plant is designed to treat an average dry weather flow of 7 000 m^3/day and an average wet weather flow of 21 000 m^3/day. The plant accounts for large daily inflow due to the high number of industries located in the area and high population of residents.

The East Bank Reclamation Works is situated in the city of East London, a large and highly populated urban area. It accepts municipal domestic sewage and wastewater containing a heavy industrial contribution. The inlet works receives raw sewage from the city pump station and Ihlanza and comprises of four screens, a grit channel and two flow meters. The plant has two aerobic tanks, each equipped with three vertically mounted mechanical aerators, two anaerobic and two anoxic tanks. The plant has six sedimentation tanks, five of which were functional at the time of the study. RAS is pumped from the bottom of the clarifiers via the screens with raw sewage to the aeration tanks. The plant has two waste mixed liquor valves which pump the waste mixed liquor from the aeration tank. A splitter box controls the flow of waste mixed liquor from the aeration tank. Chlorine contact is carried out by means of a water pressure operated, wall mounted, gas chlorinator in a baffled reinforced concrete contact tank (Appendix 3). The final effluent is discharged into the Indian Ocean between Nahoon and Eastern Beach at Bats cave and into a pond for the irrigation of a nearby golf course. Supernatant liquor from the sedimentation tanks is channelled into a fish pond located within the plant premises. Daily fluctuations in the volume of effluent accepted at the plant obviously occur and it is measured and recorded. On 25 February 2004, the inflow was measured as being equivalent to 17 105m^3 at the City Pump Station, 5 532 m^3 at Ihlanza, a total of 23 367m^3/day flowing into the East Bank Reclamation Works. On 17 March 2004, the inflow was measured as being equivalent to 29 554 m^3 at the City Pump Station, 4 098 m^3

at Ihlanza, a total of 33 652 m^3 flowing into the East Bank Reclamation Works. The average daily inflow during in the month of March 2004 was 31 364m^3/day.

The Fort Beaufort Sewage Works is situated in the peri-urban town of Fort Beaufort. The plant has two distinct sections, that is, the new section (Aerators 1 and 2), which is a compact version of the activated sludge systems and the older (Ditches 1 and 2) section which has an aeration stage and a separate settling tank. The plant receives domestic sewage, some light industrial wastewater and town and street run-off water. Incoming sewage arriving at the night soil facility is screened at the manually raked bar screen and enters the inlet works. The inlet works comprises of a pipeline feeding the raw sewage directly into the aeration tank (Appendix 4). The works executes the processes of aeration, settling, decanting of the settle effluent and retention of the waste sludge in the aeration tank. The settling tank was never functional throughout the time of this study. Stabilised excess sludge is separated and disposed into sludge drying beds, whereas the remaining effluent is returned to the activated sludge reactor. Effluent from the plant goes into settling ponds and chlorination. Chlorination is achieved with chlorine gas in a baffled reinforced concrete contact tank. The final effluent is discharged into the Kat River.

3.3.2 Collection of wastewater samples

Wastewater samples were collected between 6th August 2003 and 24th March 2004 from the raw influent, the final effluent and the RWB of the four plants. Samples were collected weekly in thoroughly cleaned and sterilised 2 L glass bottles according to the standard procedures described in the sampling guide (DWAF, 1992; DWAF *et al.,* 1999). The samples were placed in coolers containing ice-packs and transported to the base laboratory at the University of Fort Hare for analyses within 2 to 4 h after collection.

3.3.3 Physico-chemical analysis

Temperature and pH were determined on-site with a mercury thermometer and a pH meter, model 2000 (Crisson Instruments, S.A., Alella, Barcelona, Spain). The concentrations of orthophosphate as P, total nitrogen (Nitrate + Nitrite as N) and chemical oxygen demand (COD) were determined by the standard photometric method

(DWAF, 1992) using the Spectroquant NOVA 60 photometer (Merck Pty Ltd, Germany). Samples for COD analyses were digested with a Thermo reactor Model TR 300 (Merck Pty Ltd, Germany) and analysed by the Spectroquant NOVA 60 photometer (Merck Pty Ltd, Germany). Biochemical oxygen demand (BOD_5) was determined using the Oxitop WTW BOD meter (Merck Pty Ltd, Germany). The incubation period for BOD determinations was 5 days. Dissolved oxygen (DO) was measured with the Merck DO meter, Model Ox 330 (Merck Pty Ltd, Germany) while total suspended solids (TSS) were estimated according to standard methods (APHA, 1998).

3.3.4 Statistical analysis

Student's t-test was used to compare the physicochemical parameters at the effluent and receiving water body zones. The first test was to test if the homoscedasticity assumption for the t-test was satisfied. Once the assumption was satisfied, the t-test was performed.

3.4 RESULTS AND DISCUSSION

The results of the physicochemical analysis of the evaluated samples are illustrated in Figs 3.1 – 3.3.

3.4.1 Temperature and pH

In general there was a decrease in the mean temperature values from all the influents to the effluents with the exception of the East London wastewater treatment plant, which had similar mean temperature values in both the influent (23.34ºC) and the effluent (23.33ºC) (Fig. 3.1). These variations were however not significant (Equality of Variance: P= 0.6685; t- test: P=0.1830). The mean temperature values in all effluents and receiving water bodies (range: 16.52 - 23.33ºC) were below 25°C, which is the recommended limit for no risk according to the *South African Water Quality Guidelines for Domestic Use* (DWAF, 1993). Based on these guidelines, the temperature of the effluent does not appear to pose any threat to the homeostatic balance of the receiving water bodies.

The mean pH values in the influents, effluents and in the receiving water bodies from the Dimbaza, East London and Fort Beaufort wastewater treatment plants were almost similar (8.0), while in the Alice wastewater treatment plant, there was a decrease in mean pH from the influent (9.1) to the effluent (7.8), which increased to 9.0 in the receiving water bodies (Fig. 3.1). The South African target water quality pH range for domestic use (DWAF, 1996a) is 6.0 to 9.0 and the target water quality range for pH in water for full contact recreational purpose is 6.5 to 8.5. The European Union sets protection limits of 6.0 to 9.0 for fisheries and aquatic life (Chapman, 1996). The pH obtained in all effluents and receiving water bodies fell within these ranges (Fig. 3.1), and this suggests that the impact of final effluents on the receiving water bodies would not adversely affect their use for domestic uses, recreational and the aquatic ecosystem purpose.

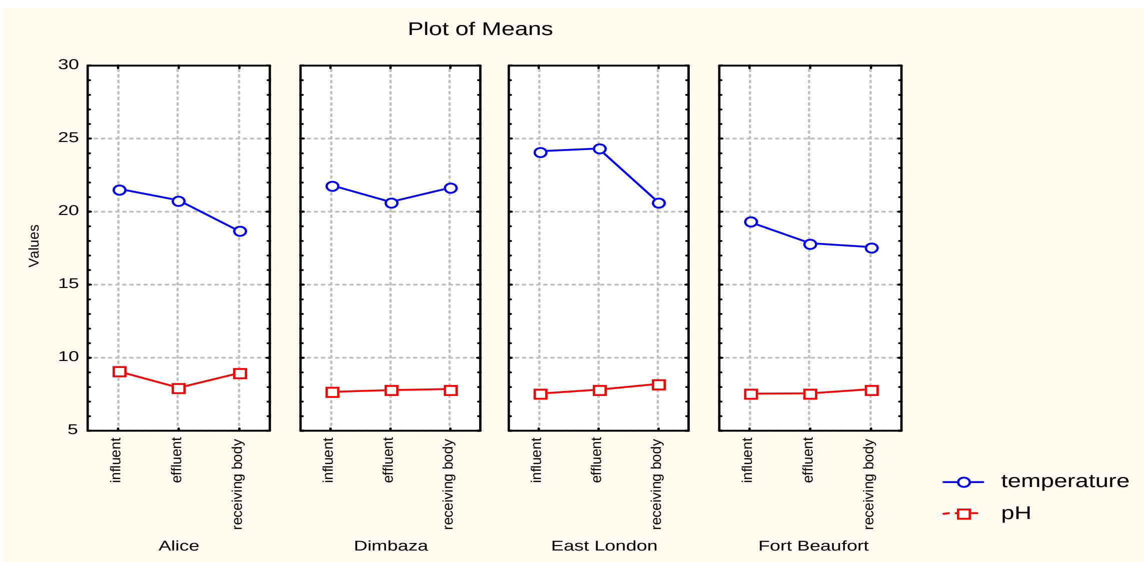


Figure 3.1: Mean values of temperature (º C) and pH from the influents to the effluents and the receiving water bodies of the Alice, Dimbaza, East London and Fort Beaufort wastewater treatment plants.

3.4.2 Nutrient removal

The mean total nitrogen (Nitrate + Nitrite as N) levels showed a gradual decline from the influents to the effluents in each wastewater treatment (Fig. 3.2). In the final effluents, the total nitrogen levels averaged 7.2 mg/l in Alice, 5.8 mg/l in Dimbaza, 4.7 mg/l in East London and 6.7 mg/l in Fort Beaufort. In the receiving water bodies, the total nitrogen levels averaged 6.9 mg/l, 3.8 mg/l, 2.9 mg/l and 4.2 mg/l in Alice, Dimbaza, East London and Fort Beaufort respectively. Although these variations were not significant (Equality of Variances: P= 0.8684; t-test: P= 0.2009), the values clearly show that the total nitrogen levels in effluents influenced those in receiving water bodies. However, the South African guidelines for total nitrogen (Nitrate + Nitrite as N) in drinking water for domestic use is <6.0 mg/l as N (DWAF *et al.*, 1998) and the target water quality range for total nitrogen in water for full contact recreational purpose is 6.0 to10 mg/l as N. The World Health Organization safe limit for nitrate for lifetime use is 10 mg/l as N (WHO, 2004a). The total nitrogen levels obtained during the study period did not exceed the regulatory limits and thus total nitrogen is not considered to pose a problem to communities when the receiving water bodies are used for the domestic and recreational purposes. However, it is important to note that the total nitrogen levels in the final effluents could be a source of eutrophication for the receiving water bodies as the values obtained in all wastewater treatment plants (and especially in the Alice wastewater treatments) exceeded the recommended limits for no risk of 0 to 0.5 mg/l as N (DWAF, 1996d).

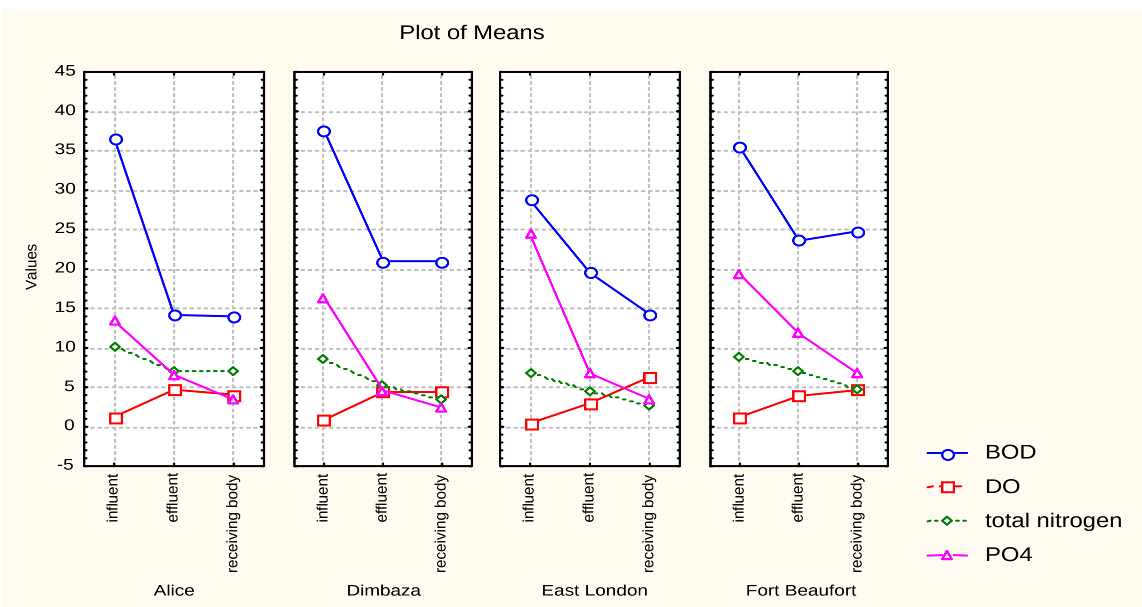


Figure 3.2: Mean values of BOD (mg/l), DO (mg/l), total nitrogen (mg/l) and phosphate (mg/l) from the influents to the effluents and the receiving water bodies of the Alice, Dimbaza, East London and Fort Beaufort wastewater treatment plants.

Although the levels of phosphate in influents varied from one plant to another, a gradual removal of phosphate was noted from the influent to the effluent in each wastewater treatment plant (Fig. 3.2). The mean levels of phosphate in effluents were 6.2 mg/l in Alice, 5.4 mg/l in Dimbaza, 5.9 mg/l in East London and 11.6 mg/l in Fort Beaufort. The high phosphate level recorded in the effluent of Fort Beaufort is expected since there was an absence of an anaerobic zone in the plant impacting on biological phosphorus removal. The levels of phosphate in receiving water bodies varied in accordance with those recorded in effluents (Fig.3.2). In receiving water bodies, the levels of phosphate averaged in the range of 3.1 to 6.8 mg/l (Fig.3.2) and differ significantly ($p<0.05$) compared to the effluents as higher phosphate levels were found in effluent zones than in receiving water bodies. The possible reason could be as a result of dilution effect. The South African guidelines do not specify the target water quality ranges for phosphate in water for domestic use and recreational purpose (DWAF *et al*., 1998). However the level of phosphate in water systems that will reduce the likelihood of algal and other plant growth is 5µg/l (DWAF, 1996c). Other investigators have pointed out that eutrophication-related problems in temperate zones of aquatic systems begin to increase at ambient total P concentrations exceeding 0.035 mg P/l. In warm-water systems, the values range between 0.34 and 0.70 mg P/l (Rast and Thornton, 1996). The associated N concentration would range between 0.34 and 0.70 mg N/l. These represent nutrient threshold levels beyond which there will be a corresponding increase in the risk and intensity of plant-related water quality problems (OECD, 1982). Based on these limits (DWAF, 1996c, Rast and Thornton, 1996), the nutrient levels obtained in the present study are exceeded in both effluents and receiving water bodies. This is due to inadequate removal of nutrients by the Alice, Dimbaza, East London and the Fort Beaufort sewage treatment works. Their respective final effluent discharges are therefore considered as main sources of phosphate in Tyume River, Tembisa Dam, Nahoon and Eastern Beach (which are part of the Indian Ocean) and Kat River respectively.

In water quality studies, nitrogen and phosphorus are the nutrients most commonly identified as pollutants. Nitrogen in the form of ammonia (NH_3) and nitrates (NO_3) and phosphorus are essential nutrients to plant life, but when found in excessive quantities; they can stimulate excessive and undesirable plant growth such as algal blooms. Eutrophication could adversely affect the use of rivers and dams for recreation purposes as the covering of large areas by macrophytes could prevent access to waterways and could cause unsightly and malodorous scum which could lead to the growth of blue-green algae, which could release toxic substances (cyanotoxins) into the water systems. These substances are well known to cause the death of farm livestock (Holdsworth, 1991) and this must be a matter of concern in the Eastern Cape as these receiving water bodies are used for drinking by the farm livestock. Moreover, it is well known that eutrophication could increase the treatment cost of drinking water through filter clogging in water treatment works (Murray *et al.,* 2000). In the case of Alice and Fort Beaufort, drinking water treatment plants abstract their water from Tyume River and Kat River for further treatment before supplying the communities. There is therefore much need to address the problems of nutrient removal in these sewage treatment works in order to avoid the incidences of eutrophication in receiving water bodies and protect water sources. We therefore recommend local and national government to take responsibility for implementing necessary measures to ensuring good health of water resources through effective treatments of wastewater.

3.4.3 Oxygen-demanding substances

The mean levels of oxygen-demanding substances from the influents to the receiving water bodies are shown in Figs. 3.2 and 3.3. In general, a gradual decrease in BOD levels was noted from the influents to the effluents in all sewage treatment works. Almost the mean BOD levels found in the effluents were recorded in the receiving water bodies in Alice, Dimbaza and Fort Beaufort, and averaged in the range of between 14 and 24 mg/l (Fig.3.2). In East London, the mean BOD levels were 19. 50 mg/l and 14.25 mg/l in the final effluent and receiving water bodies respectively and these variations were not significant. The BOD indicates how much oxygen is needed by the water to completely oxidize its organic pollution load. There are no South African guidelines for BOD in the effluent. The EU guidelines stipulate the BOD target limits of 3.0 to 6.0 mg/l for the protection of fisheries and the aquatic life (Chapman, 1996). The BOD levels recorded in all effluents and receiving water bodies are much higher than those indicated in the EU guidelines. Consequently the high levels of BOD in both effluents and receiving water bodies disqualify these water sources for the use of aquatic ecosystems.

In all of the sewage treatment works, an increase of the DO levels was observed from the influents to the effluents and thereafter to the receiving water bodies (especially in East London and Fort Beaufort) (Fig. 3.2). In the effluents, the dissolved oxygen levels averaged in the range of between 3.26 and 4.57 mg/l while in the receiving water bodies a range of between 3.72 and 6.13 mg/l was observed. These variations were however not significant. Dissolved oxygen is an important parameter used for water quality control. The effect of waste discharge on a surface water source is largely determined by the oxygen balance of the system and its presence is essential to maintaining biological life within a system (DFID, 1999). Dissolved oxygen concentrations in unpolluted water normally range between 8 and 10 mg/l (DFID, 1999). Concentrations below 5 mg/l adversely affect aquatic life (DFID, 1999). The concentrations of DO in effluents and receiving water bodies (with the exception of the receiving water bodies in East London and Fort Beaufort) are less than 5 mg/l (Fig. 3.2). Consequently, these water sources would not be suitable for use of aquatic ecosystems.

Remarkable removal of COD was observed in all sewage treatment works (Fig. 3.3). In Alice, Dimbaza and Fort Beaufort, the mean COD values in the effluents ranged between 14 mg/l and 20 mg/l and between 7 and 15 mg/l in the receiving water bodies. These variations were however not significant. The East London plant had a similar mean COD concentration of 8.42 ± 0.29 mg/l in both the effluent and the receiving water body (Fig. 3.3).

The new South African water quality guidelines do not specify the COD concentrations for domestic, recreational, aquatic ecosystems and agricultural purposes. The COD guidelines available are for industrial purpose and the values range between 0 and 10 mg/l (DWAF, 1996c). However the old South African guidelines for COD in effluents that are allowed to be discharged into the surface water sources state a target of 30 mg/l (Government Gazette, 1984). The mean COD in all effluents fell within the acceptable limits (Government Gazette, 1984, DWAF, 1996c). This indicates the efficiency of the wastewater treatment plants in removing the chemical oxygen-demanding substances in the influents.

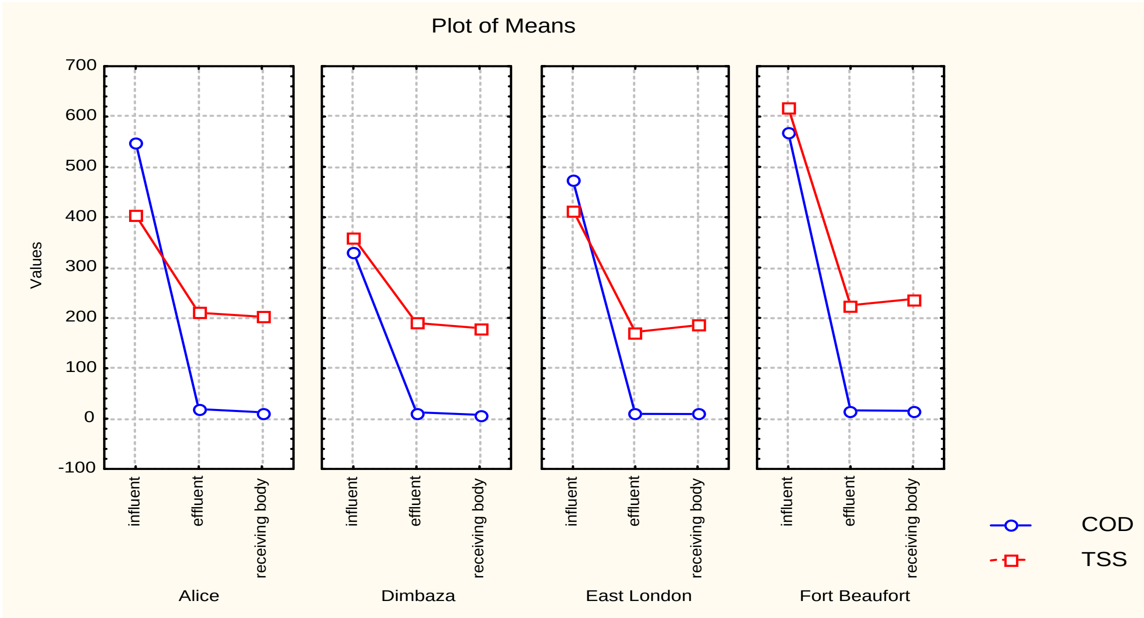


Figure 3.3: Mean values of COD (mg/l) and TSS (mg/l) from the influents to the effluents and the receiving water bodies of the Alice, Dimbaza, East London and Fort Beaufort wastewater treatment plants

3.4.4 Removal of total suspend solids

The total suspended solids (TSS) concentrations gradually decreased from the influents to the final effluents (Fig. 3.3). The mean TSS concentrations in the effluents ranged between 161.43 and 215.67 mg/l. In the receiving water bodies, the values ranged between 171.43 and 200.67 mg/l, but not significant compared to the effluents. The TSS refers to the amount of undissolved solids in the sewage. All the wastewater treatment plants performed well in terms of total suspended solids removal because concentrations in final effluents fell within the allowed limits of 0 to 450 mg/l (DWAF, 1996d). These TSS concentrations automatically influenced the quality of the received water bodies. Excess TSS can block sufficient sunlight from reaching underwater plant life, thus preventing normal growth and productivity (National Sewage Report Card, 1999). This can affect aquatic organisms feeding on these plants. Trace metals and organic contaminants, harmful to human health and the environment, can adhere to TSS and enter receiving water bodies through effluents (Nantel, 1996).

3.5 CONCLUSIONS AND RECOMMENDATIONS

The adequate long-term protection of South Africa's water sources is of vital importance for sustained economic growth and development. Water is scarce in this country and further deterioration of the quality of the already limited sources should not be allowed to occur.

The levels of orthophosphate, biological oxygen demand and dissolved oxygen did not comply with the acceptable limits in both the effluents and the receiving water bodies of the Alice, Dimbaza, East London and Fort Beaufort wastewater treatment plants. Although the total nitrogen is not considered to pose a problem to communities when the receiving water bodies are used for the domestic and recreational purposes, the mean values recorded in final effluents could be a source of eutrophication for the receiving water bodies as they exceeded the recommended limits in all wastewater treatment plants. The final effluents of these wastewater treatment plants are therefore significant sources of pollution into their respective receiving water bodies. A better management of the Alice, Dimbaza, East London and Fort Beaufort is needed in order to improve their performance in terms of orthophosphate, total nitrogen, biological oxygen demand and dissolved oxygen status.

The next chapter dealt with the microbiological quality of the individual wastewater treatment plants, their impact on the receiving water bodies and the health of the surrounding communities.

CHAPTER 4

IMPACT OF THE MICROBIOLOGICAL QUALITY OF EFFLUENT DISCHARGES ON RECEIVING WATER BODIES AND PUBLIC HEALTH: CASE STUDY – BUFFALO CITY AND NKONKOBE MUNICIPALITIES

4.1 ABSTRACT

The performance of four wastewater treatment plants that serve the Buffalo City (Dimbaza, East London) and Nkokonbe (Alice, Fort Beaufort) Municipal areas in the Eastern Cape Province of South Africa were investigated for the efficiency in the removal of microbial contaminants particularly *Salmonella typhimurium*, *Shigella dysenteriae* and *Vibrio cholerae*. Standard methods were applied in all aspects of the analyses for the isolation and detection of these microorganisms and for the identification of the general microbiological quality of the effluent and the receiving water bodies. Polymerase chain reaction (PCR) was used to confirm the presence of the target microorganisms. The risk assessment was conducted based on the outcome of molecular characterization of isolates to study the impact of target microorganisms on the health of the communities. Other potentially dangerous microbial pathogens identified were *Aeromonas hydrophila, Enterobacter cloacae, Escherichia coli, Klebsiella pneumoniae, Klebsiella ornithinolytica, Pasteurella pneumonia, Proteus mirabilis* and *Providencia rettgeri*. The general microbiological quality of the effluents discharged from all the plants did not comply with the limits set by the South African Authorities in respect of pathogens such as *Salmonella typhimurium*, *Shigella dysenteriae* and *Vibrio cholerae*. Statistical evidence showed a relationship between the quality of the final effluent and that of the receiving water body and the relationship was such that the better the quality of the final effluent, the better the quality of the receiving water body. The PCR successfully amplified the *hylA* gene encoding the haemolysin gene and *ctxA* gene encoding the cholera toxin of *Vibrio cholerae* in the effluent and receiving water body samples in Alice, Dimbaza, East London and Fort Beaufort. The presence of *Salmonella enteritidis* was implicated in the receiving water body while *Salmonella typhimurium* was implicated in the effluent and receiving water body samples. Risk assessment was

performed to predict and analyze the health impact of these *Salmonella* species and *Vibrio cholera* that resulted from the effluent discharge into the receiving water sources. The health risks were found to be high for exposure events to a small volume of the receiving water body samples. The effluents discharges from the Dimbaza, East London, Alice and Fort Beaufort wastewater treatment plants were identified as point source pollution into the respective receiving water bodies (Tembisa Dam, the Nahoon and Eastern Beach which are part of the Indian Ocean; the Tyume River and the Kat River).

Key words: Effluent discharge, microbiological quality, microbial risk assessment

4.2 INTRODUCTION

Microbial contamination of water is the largest and most immediate health hazard. Surface water quality is subjected to frequent dramatic changes in microbial quality as a result of the variety of activities on the watershed. These changes are caused by discharges of municipal raw waters or treated effluent at a specific point-source into the receiving water such as streams, rivers, lakes, ponds etc (Geldereich, 1990). Point-source pollution problems not only increases treatment costs considerably, but introduces a wide range of potentially infectious agents to water that may be supplied to many rural and urban communities, thus resulting in incidences of waterborne diseases with far reaching socio-economic implications (Craun, 1991). Wastewater treatment plants discharge significant amounts of pollution-indicator and pathogenic micro-organisms, leading to the deterioration in the quality of water sources (Bahlaoui *et al*., 1997; Simpson and Charles, 2000). The presence of pollution indicator and pathogenic microorganisms in water bodies is of major concern since most countries in Southern Africa in general and South Africa in particular are primarily water-scarce countries with extensive industrialization and an ever-increasing population density, the majority of whom have no access to a reliable water service. Faecal pollution of Southern Africa's water resources is emerging as one of the major water quality problems due to lack of appropriate and properly maintained wastewater treatment works in many parts of the region (Kühn, *et a*l., 2000).

The pathogens, *Salmonella typhimurium, Shigella dysenteriae* and *Vibrio cholerae,* have been known to cause severe diarrhoea in children and adults which can lead to morbidity and mortality. Waterborne diseases constitute a major health problem throughout the world and contribute significantly to loss in productivity and million of lives each year (American Academy of Microbiology, 2002). South Africa has experienced outbreaks of diarrhoea due to *Shigella dysenteriae* and *Vibrio cholerae* that have resulted in 13 and 288 fatalities, respectively in the KwaZulu-Natal and Eastern Cape Provinces (Pegram *et al*., 1998; Department of Provincial and Local Government, 2001). *Shigella dysenteriae* and *Vibrio cholerae* are usually transmitted to humans by ingestion of contaminated water and foods. The genus *Shigella* is composed of four species, *S. dysenteriae*, *S. boydii*, *S. sonnei* and *S. flexneri*. The infective dose of *Shigella* spp. is very low, varying from 10^1–10^4 organisms (Rowe and Gross, 1984). Virulent *Shigella* bacteria cause bacillary dysentery (shigellosis), which may lead to death in some cases if effective intervention strategies are not used. Toxigenic *V. cholera* is responsible for cholera, a highly epidemic diarrhoeal disease which continues to devastate many developing countries. The ingestion of approximately 10^4–10^6 *V. cholerae* O1 organisms is likely to produce clinical cholera (Cash *et al*., 1974). *Salmonella typhimurium*, a facultative intracellular pathogen, is the causative agent of typhoid fever in man and animals. Typhoid fever remains endemic to many parts of South Africa, including Kwazulu-Natal, Northern Transvaal and the Transkei (Coovadia *et al.*, 1992), with a recent outbreak occurring in Delmas, Mpumalanga. In this province, health spokespersons reported that there were 380 cases of diarrhoea, 30 suspected cases of typhoid fever and nine confirmed cases (Mail and Guardian, September 2005). The outbreak originated in the town's water supply, suspected to have been contaminated with human faeces. In the vast majority of cases, human beings acquire *Salmonella* by the ingestion of contaminated food and water. At least 10^9 cells of *S. typhimurium* must be ingested to cause symptoms of toxic infection (Hook, 1985).

Current practices to predict a possible risk of infection related to the microbiological quality of water include environmental health practitioners and water quality managers generally testing water for the presence of indicator microorganisms. If present, a negative health effect can be expected with increasing risk as organisms numbers increase. This approach can be referred to as an observed-adverse-effect-level approach (OAELA) based on the occurrence of microbiological indicator organisms instead of actual pathogens. It does not provide a quantitative value for the microbiological waterborne health hazards that threaten water users it can, at best, indicate the risk of infection by diarrhoea-causing pathogens potentially occurring in the same water should a person ingest it. Quantitative health risk assessment is an emerging tool in the field of microbial food and water safety (ILSI, 1996). In its simplest form it consists of four main steps, namely: hazard identification, dose-response assessment, exposure assessment and risk characterization.

Hazard identification is a step where potential contaminants of concern and the effects on human health are identified. The likelihood that the exposure to a pathogenic microorganism under specific exposure conditions poses a threat to human health was assessed. The hazard characterization step of risk assessment is the one most amenable to a common approach (Genthe and Seager, 1996; Fewtrell and Bartram, 2001).

Dose-response assessment involves the collection of data on the relationship between the dose of microbial contaminants (i.e. the amount of the target pathogenic microorganisms taken into the body through skin contact, ingestion or drinking) and the incidence of an adverse health effect in the exposed population. This relationship is characterized by the infectivity and there are three ways to characterize infectivity: (i) The ID_{50} or the infective dose is the dose that causes infection in 50% of the persons exposed to the pathogen; (ii) The Pinf is the probability of infection following the exposure to a single organism; (iii) The most complete form for characterizing infectivity is with dose/response curves, giving the probability of infection as a function of the dose. The criteria used for infectivity differ from one study to another thereby providing a quantitative basis for assessing health risks (Genthe and Seager, 1996; Fewtrell and Bartram, 2001).

Exposure assessment establishes the pathways along which the microbial contaminants may be released. The intensity, frequency and duration of human exposure to each of the targeted pathogenic microorganism in potentially exposed populations can be measured or estimated. As a default number, two litre/person/day is used to estimate drinking water exposure, (Macler and Regli, 1993) although this may be conservative (Roseberry and Burmaster, 1992). During contact recreational exposure, 100 mL/day has often been assumed as an exposure measure but actual data to validate this number are lacking (Haas, 1983). The purpose of an exposure assessment is to determine the microbial doses typically ingested by the direct user of water (or food). In the case of water microbiology, this may necessitate the estimation of raw water microorganism levels followed by estimation of the likely changes in microbial concentrations with treatment, storage and distribution to the end-user (Regli *et al*., 1991). A second issue arising in exposure assessment is the amount of ingested material per 'exposure'.

The final step, risk characterization, combines the information concerning the hazard identification, dose-response and exposure assessment and uses it to characterize and describe the extent of the overall individual or population risk.

The aim of the study was to identify the origin and clinical relevance of microbiological contaminants responsible for the pollution of water sources and waterborne diseases. The study was also aimed at the impact on public health in general and health risk posed to the Eastern Cape Province rural communities in particular as many of the inhabitants of the rural and peri-urban areas in the Eastern Cape Province depend largely on surface water sources for their daily water needs. To achieve this aim, the study evaluated the performance of the individual wastewater treatment plants for the removal of *Salmonella typhimurium, Shigella dysenteriae* and *Vibrio cholerae* in order to establish the relationship between the microbial quality of the final effluent and that of the receiving water body in relation to the standards for domestic, irrigation and recreational water use as set down by *South African Water Quality Guidelines* (DWAF, 1996a, b, d). The study assessed the risk of infection due to the target pathogens that resulted from the effluent discharge into the receiving water sources. This study was planned to generate valuable information that could be used by water engineers, wastewater treatment plant managers, microbiologists and especially the Department of Water Affairs and Forestry and the Department of Health in order to develop and review effective policy for the discharge of effluents into water sources.

4.3 MATERIALS AND METHODS

4.3.1 Study site and sampling

Four wastewater treatment plants that serve the Buffalo City (Dimbaza and East London) and Nkonkobe (Alice and Fort Beaufort) Municipal areas in the Eastern Cape Province of South Africa were investigated in the present study. The wastewater treatment plants are located in urban (East Bank Reclamation Works, East London), semi-urban (Dimbaza Sewage Treatment Works) and in rural areas (Alice and Fort Beaufort Sewage Treatment Works). A full description of the individual wastewater treatment plants is given in the preceding chapter (Section 3.3.1).

Two litre glass bottles with screw cap lids were used for sample collection. The bottles were cleaned with detergent and rinsed with distilled water three times; 2 mL of Sodium Thiosulfate (ca. 17.5 mg/L) was added before autoclaving the bottles. At each wastewater plant, samples were aseptically collected from three points, namely, the influent (raw wastewater), the final effluent and the receiving water body. After collection, the bottles were placed in cooler boxes with ice-packs while being transported to the laboratory. The analyses were performed under aseptic conditions within 2 to 4 h after sample collection. The samplings were conducted once a week from the individual plant for the period starting from the 6th of August 2003 to 24th of March 2004.

4.3.2 Culture-based isolation and biochemical tests for the identification of *Salmonella typhimurium, Shigella dysenteriae* and *Vibrio cholerae*

Standard procedures were used to isolate *Salmonella typhimurium*, *Shigella dysenteriae* and *Vibrio cholerae* (APHA, 1998; SABS, 2001). To isolate *Salmonella typhimurium* and *Shigella dysenteriae*, 200 mL of wastewater samples were centrifuged in Beckman model TJ-6 Centrifuge at 1520 g for 15 minutes. The supernatant was decanted leaving 2 mL of it and the pellet. The pellet was resuspended in the supernatant and 2 mL of the suspension was added to tubes containing 8 mL of Tetrathionate Broth (TTB) (Merck). The suspension was incubated at 35ºC ± 2ºC overnight. Thereafter, *Salmonella typhimurium* and *Shigella dysenteriae* were enumerated by spread plate procedure on Xylose-Lysine-Desoxycholate agar (XLD) (Merck) for all the samples and incubated overnight at 35ºC± 2ºC (APHA, 1998). The agar plates were prepared according to the manufacturer's instruction.

To isolate *Vibrio cholerae*, 1 L wastewater sample collected in 1 L sterile glass bottle containing a sterile Moore gauze swab made with absorbent cotton was incubated at room temperature for 48 h. Thereafter the swab was aseptically transferred into a sterile plastic container. Prior to and after use, both the weights of the sterile swab (Natuuril, Smith and Nephew) and the plastic container were determined. The difference between the weights was used for the enrichment step. Enrichment was carried out in a double-strength alkaline peptone broth (pH 8.5) at a 1:1 (w/v) ratio for 6 to 8 h at 35 ± 2ºC (SABS, 2001). *Vibrio cholerae* was next enumerated by the spread plate procedure on Vibrio diagnostic agar (Biolab) and the agar plates were incubated aerobically at 35 ± 2ºC for 16 to 24 h. All these tests for the above mentioned microorganisms were performed in triplicates. The presence of the bacteria was based on the colour of the colonies corresponding to presumptive bacterial strain as indicated in Table 4.1.

Table 4.1. Summary of the Various Presumptive Bacterial Colonies Isolated from Different Culture Media (Merck, Catalogue No. 1.05287.0500, 2003; Merck Catalogue No. 1.10263.0500, 2003).

Culture Medium	Presumptive Bacterial Strain	Colour of the Colony
XLD agar	*E. coli*	Yellow
	Enterobacter/Klebsiella	Yellow
	Proteus	Red to yellow, most strains have black centres
	Salmonella typhimurium	Red-yellow with black centre
	Shigella dysenteriae	Red
	Pseudomonas	Red
VDA agar	*V. cholerae*, *V. cholerae* type El Tor	Yellow
	V. parahaemolyticus	Blue-green
	V. alginolyticus	Yellow
	Pseudomonas, *Aeromonas* and others	Blue
	Enterobacteriaceae and others	Translucent

The individual bacterial colonies from different wastewater samples were randomly selected from different plates and transferred onto the corresponding medium by the streak plate technique and incubated at 35 ± 2ºC for 24 h. The colonies were further purified by the same methods at least three times using nutrient agar (Biolab) before Gram staining (Pelczar *et al*., 1999) was done. Oxidase tests were conducted on those colonies that were Gram-negative. The 20E API kit (Omnimed) was used for the oxidase-negative colonies and the strips were incubated at 35 ± 2ºC for 24 h. The strips were analyzed and the strains identified using API LAB PLUS computer software (BioMérieux, Marcy l'Etoile, France).

4.3.3. PCR based methods for the confirmation of *Salmonella typhimurium, Shigella dysenteriae* and *Vibrio cholerae*

Polymerase Chain Reaction (PCR) is a primer-mediated enzymatic amplification of specifically cloned or genomic DNA sequences. It has become a routine procedure in every molecular biology laboratory for manipulating and identifying genetic material. It was used to confirm the targeted pathogenic microorganisms obtained during this study.

4.3.3.1 Bacterial strains

Salmonella typhimurium strain 14028, *Shigella dysenteriae* strain 29027 and *Vibrio cholerae* strain 9458 were obtained from American Type Culture Collection (ATCC), USA. These strains were reconfirmed using standard procedures (APHA, 1998; SABS, 2001) and were used as reference (positive control) strains. *Salmonella typhimurium* and *Shigella dysenteriae* strains were cultured on Xylose-Lysine-Desoxycholate agar (Merck) plates and incubated at 35ºC ± 2ºC aerobically for 24 h. *Vibrio cholerae* strain was cultured on Vibrio diagnostic agar (Biolab) plates and incubated aerobically at 35°C± 2ºC for 18 to 24 h. After incubation, the bacteria were maintained on Nutrient agar (Merck) slants and stored at 4ºC.

4.3.3.2 Preparation of lysates for PCR

A direct lysis method was used for the isolation of DNA from the bacteria (Theron *et al*., 2000). Bacterial colonies were suspended in 200 µl of sterile Molecular grade water and the bacteria were lysed by heating for 10 min at 100°C in a Dri-Block DB.2A (Techne). Cell debris present after processing was removed by centrifugation at 10 000 g for 2 min using a MiniSpin microcentrifuge (Eppendorf). The lysate supernatant was collected and 10 µl containing bacterial genomic DNA were used as the template in the PCR analysis.

4.3.3.3 Oligonucleotide primers

Oligonucleotide primers given in Table 4.2 were used for the amplification of *Salmonella* invasive gene *invA* (Hong *et al*., 2003; Malorny *et al*., 2003), *sefA* gene found in *Salmonella enteritidis* (Doran *et al*., 1996; Oliveira *et al*., 2002), *fliC* gene of *Salmonella typhimurium* encoding flagellin H1 (Soumet *et al*., 1999), *virA* gene of virulent *Shigella* species (Villalobo and Torres, 1998), *ctxA* gene of *Vibrio cholerae* encoding the cholera toxin (Lipp *et al*., 2003; Kapley and Purohit, 2001) and *hylA* encoding the haemolysin gene (Singh *et al*., 2001) as these genes have been reported to be specific for the above-mentioned bacteria.

4.3.3.4 PCR amplification and electrophoretic detection of amplicons

The reaction mixtures (50 µl) used in the PCR analysis contained 1 U Super Therm GOLD Buffer, 1.5 mM $MgCl_2$, each deoxynucleoside triphosphate (Promega) at a concentration of 0.25 mM, either 100 pmoL of each of the *Salmonella typhimurium* or *Shigella dysenteriae* primers or 50 pmoL of each *Vibrio cholera* primers and 1 U of Super Therm *Taq* polymerase (Southern Cross Biotechnology). The sample volume was 10 µl. The reaction tubes were placed in an Eppendorf model AG 22331 thermal cycler. The PCR amplification for *Salmonella* spp was performed as described by Hong and co-workers (2003). The following conditions were used for *invA* gene: heat denaturation at 94ºC for 1 min followed by 30 cycles of heat denaturation at 94ºC for 30 sec, primer annealing at 55ºC for 30 sec and DNA extension at 72ºC for 20 sec. This was followed by incubation at 72ºC for 4 min and cooling at 4ºC. The PCR amplification for *Salmonella* spp was performed as described by Malorny and co-workers (2003). The following conditions were used for *invA* gene: initial denaturation at 95ºC for 1 min followed by 35 cycles of denaturation at 95ºC for 30 sec, annealing at 64ºC for 30 sec and extension at 72ºC for 30 sec. This was followed by incubation at 72ºC for 4 min and cooling at 4ºC. The PCR amplification for *Salmonella enteritidis* was carried out as described by Doran and co-workers (1996) and Oliveira and co-workers (2002) with modification. The following conditions were used for *sefA* gene: heat denaturation at 94ºC for 5 min followed by 35 cycles of denaturation at 94ºC for 30 sec, annealing at 63ºC for 30 sec and extension at 72ºC for 30 sec. This was followed by incubation at 72ºC for 7 min and cooling at 4ºC. The PCR amplification for *Salmonella typhimurium* was performed in accordance with the description of Soumet and co-workers (1999). The following conditions were used for *fliC* gene: heat denaturation at 94ºC for 5 min followed by 35 cycles of denaturation at 94ºC for 1 sec, annealing at 55ºC for 1 sec and extension at 72ºC for 21 sec with a final extension at 72ºC for 7 min and cooling at 4ºC.

The following conditions were used for *Shigella* spp for *virA* gene: heat denaturation at 94°C for 45 sec followed by 35 cycles of denaturation at 94°C for 45 sec, annealing at 65°C for 30 sec and extension at 72°C for 30 sec. After the last cycle, the samples were kept at 72ºC for 30 seconds to complete synthesis of all strands. The PCR amplification for *Shigella* spp was performed as described by Villalobo and Torres (1998).

The *ctxA* gene of *Vibrio cholerae* was amplified in accordance with the descriptions of Lipp and co-workers (2003); Kapley and Purohit (2001) and Singh and co-workers (2001). The amplification conditions are as follows: heat denaturation at 94ºC for 1 min, followed by 35 cycles of denaturation at 94ºC for 45 sec, annealing at 60ºC for 45 sec, extension at 72ºC for 1 min and final extension at 72ºC for 5 min (Lipp *et al*., 2003); heat denaturation at 95ºC for 2 min, followed by 30 cycles of denaturation at 95ºC for 30 sec, annealing at 54ºC for 30 sec, extension at 72ºC for 15 sec and final extension at 72ºC for 15 sec (Kapley and Purohit, 2001); heat denaturation at 94ºC for 2 min, followed by 30 cycles of denaturation at 94ºC for 1 min, annealing at 60ºC for 1 min, extension at 72ºC for 1 min and final extension at 72ºC for 10 min (Singh *et al*., 2001). Control reaction mixtures containing sterile molecular biology grade water and all other reagents were amplified along with the test samples throughout the amplification reaction. Positive controls consisting of DNA of the reference bacterial strains were included. The amplicons were resolved on 2 % (w/v) agarose gel (ABgene, UK) in 1 U TAE (40 mM Tris-HCl (BDH, England), 20 mM Na.acetate (Merck), 1 mM EDTA (Merck), pH 8.5) and visualized and photographed under the BioDoc-It transilluminator

System after staining with 0.5 μg of ethidium bromide (BDH, England) per mL. A 100 bp DNA ladder (Promega) was included on each gel as a molecular size standard.

4.3.4. Health risk assessment

The methodology recommended by the United States Environmental Protection Agency (US EPA, 1989) was used for the health risk assessment during the study period. Although this protocol was originally intended to accommodate only carcinogen assessment, current trends favour the application of similar procedures to the assessment of microbiological hazards (US EPA, 1989; Asano and Sakaji 1990). Exposure assessment comprised of the following volumes:

- 0.1 mL and 1 mL accidentally ingested during recreational and bathing activities as well as during irrigation.
- 100 mL ingested via drinking water**.**
- Yearly exposure risk was calculated by multiplying the risk for a single exposure per day by the number of exposures in a single year, assuming exposure of 350 days in a single year (Pepper *et al*., 1996). The average as well as the 95th percentile probability of infection was calculated.

Dose-response assessment. The β-distribution model was used to calculate the probability of infection (*Pi*) after single exposure based on dose-response parameters (α, β) for *Salmonella* and *Vibrio cholerae* (Rose and Gerba, 1990). The model was one identified in literature as the most appropriate model of pathogen dose-response relationships on the basis of results reported (Rose and Gerba, 1990). This model is expressed as follows:

$$Pi = 1 - (1 + N / \beta) \wedge {}^{(-\alpha)}$$

Where *Pi* = probability (risk) of infection

N = dose or exposure (number of *Salmonella* and *Vibrio*)

α, β = values defined by the dose-response curves specific to individual organisms

(α = 0.33 – *Salmonella* and 0.097 – *Vibrio cholerae*)

(β = 139.9 – *Salmonella* and 13.02 – *Vibrio cholerae*)

Table 4.2: Primers used for the identification of bacterial isolates obtained from the final effluent and receiving water body.

Bacterium	Gene target	Amplicon size (bp)	Primer	Sequence (5' → 3')	Reference
Salmonella spp	*invA*	408	*invA-1*	CGCTCTTTCGTCTGGCATTATC	Hong *et al*., 2003
			invA-2	CCGCCAATAAAGTTCACAAAG	
Salmonella spp	*invA*	284	*invA-3*	GTGAAATTATTCCCAGCGGGTACTG	Malorny *et al*., 2003
			invA-4	TCATCGCACCGTCAAAGGAACC	
Salmonella enteritidis	*sefA*	488	*A058*	GATACTGCTGAACGTAGAAGG	Doran *et al*., 1996;
			A01	GCGTAAATCAGCATCTGCAGTAGC	Oliveira *et al*., 2002
Salmonella typhimurium	*fliC*	559	*Fli15*	CGGTGTTGCCCAGGTTGGTAAT	Soumet *et al*., 1999
			Tym	ACTCTTGCTGGCGGTGCGACTT	
Shigella spp	*virA*	215	*virA-1*	CTGCATTCTGGCAATCTCTTCACAT	Villalobo and Torres, 1998
			virA-2	TGATGAGCTAACTTCGTAAGCCCTCC	
Vibrio cholerae	*ctxA*	647	ctxA-1	CAACAGAATAGACTCAAGAA	Lipp *et al*., 2003
			ctxA-2	TATCTTCTGATACTTTTCTAC	
Vibrio cholerae	*ctxA*	564	*ctxA-5*	CGGGCAGATTCTAGACCTCCTG	Lipp *et al*., 2003
			ctxA-6	CGATGATCTTGGAGCATTCCCAC	
Vibrio cholerae	*ctxA*	302	ctxA-7	CTCAGACGGGATTTGTTAGGCACG	Kapley and Purohit, 2001
			ctxA-8	TCTATCTCTGTAGCCCCTATT	
Vibrio cholerae	*hylA*	738	*hylA-1*	GGCAAACAGCGAAACAAATACC	Singh *et al*., 2001
			hylA-2	CTCAGCGGGCTAATACGGTTTA	

4.4 RESULTS AND DISCUSSION

South Africa is a water-scarce country and the demands on this resource are growing as the country expands and the population increases. Urgent steps must be taken to protect the quality of water resource for the country to develop economically and meet the wide-ranging needs for water. In this part of the study, the efficiency of wastewater treatment plants located in rural (Alice), peri-urban (Dimbaza and Fort Beaufort) and urban (East London) areas of the Eastern Cape were assessed for their ability to remove *Salmonella typhimurium*, *Shigella dysenteriae* and *Vibrio cholera*. As the disinfection of wastewater provides the first line of defence for drinking water from water sources, regular monitoring of the free chlorine residual concentration was performed in the final effluent during the study period. A risk assessment was conducted to predict and analyse the public health impact of the above target pathogens once found in the effluent discharge and the respective water sources.

4.4.1 Concentration of chlorine residual in the final effluent

Table 4.3 illustrates the free chlorine residual concentrations in the final effluents of the wastewater treatment plants during the study period. Chlorine residual concentration ranged between 0.05 and 3.50 mg/l throughout the sampling period, with overdosing observed during the months of August 2003 in Dimbaza and September 2003 in Fort Beaufort (Table 4.3). A regular acceptable concentration of free chlorine residual was noted in the East London plant while low concentrations were noted in Alice plant during the study period.

Table 4.3. Concentrations of free chlorine residual (mg/L) in the final effluents from during the sampling period (ranges and means)

Wastewater treatment plant	**Chlorine residual (mg/L)**	
	Ranges	Means
Dimbaza	0.53 – 3.50	1.67
East London	0.19 – 0.67	0.52
Alice	0.14 – 0.66	0.29
Fort Beaufort	0.05 – 1.40	0.49

Samples collected between the 6 August 2003 and 24 March 2004

According to White (1992) the most prevalent practice of disinfection is free chlorine (HOCl + OCl-) which dissociates into molecular hypochlorite (HOCl) and the hypochlorite ion (OCl-). This is also the practice in South Africa as was confirmed by a survey recently conducted by Momba, Thompson and Obi (2006a). Although the *South African Water Quality Guidelines* (DWAF, 1996b) do not specify any standard for the concentration of free chlorine residual in treated effluent, this study considered the concentration reported by Voysey and co-workers (1998). According to these authors, the residual free chlorine in the final stages of sewage treatment should be 0.1 mg/L (Voysey *et al*., 1998). Based on this concentration, the free chlorine residual in the effluents indicated the availability of free chlorine residual in all the wastewater treatment plants. The exception in free chlorine residual concentration of 0.05 mg/L was noted at the Fort Beaufort wastewater treatment plant (Table 4.3). Although the results of this study revealed that the free chlorine residual concentration fell within 0.1 mg/L, the occurrence of coliform bacteria could still be detected in the final effluent. The concentration could have had minimal effect in the removal or inhibition of bacterial growth in the final effluent (Fig. 4.3; Table 4.4). This study agrees with previous investigators who observed the potential of bacterial growth in treated water with a chlorine residual concentration of up to 1 mg/L (Olivieri *et al*., 1985; LeChevallier *et al*., 1987; Momba *et al*., 1998). The results of this study suggested the use of a very efficient disinfectant for the treatment of the effluent before being discharged into the receiving water bodies.

4.4.2 Microbiological characteristics of the wastewater samples

To assess the effectiveness of wastewater treatment plants, the presence of target pathogens was determined in the wastewater samples and in the receiving water bodies. Culturing methods involving the isolation of *Salmonella typhimurium, Shigella dysenteriae* and *Vibrio cholerae* and biochemical tests for the identification of these microorganisms preceded the PCR methods. It is important to note that the detection of presumptive *Salmonella typhimurium*, *Shigella dysenteriae* and *Vibrio cholerae* in the influent and effluent zones and in the respective water sources by culturing methods do not definitely indicate the presence of these bacterial species in water samples as these methods are often associated with selectivity, sensitivity and specificity problems (Momba *et al*., 2006b), and therefore they may lead to the isolation of other microorganisms.

Xylose-lysine-desoxycholate (XLD) agar, which is the medium of choice for the isolation of *Salmonella typhimurium* and *Shigella dysenteriae* is both a selective and differential medium (Merck, Catalogue Number. 1.05287.0500, 2003). It utilises Sodium desoxycholate as the selective agent and is inhibitory to gram-positive microorganisms. Xylose is incorporated into the medium since it is practically fermented by all enteric bacteria except *Shigella* spp and this property enables the differentiation of other microorganisms from *Shigella* species. Lysine is included to enable *Salmonella* spp to be differentiated from non-pathogens since without lysine; *Salmonella* spp rapidly would ferment the xylose and be indistinguishable from non-pathogenic species. An H_2S indicator system consisting of sodium thiosulfate and ferric ammonium citrate is included for the visualisation of the Hydrogen sulfide produced, resulting in the formation of colonies with black centers. The non-pathogenic H_2S-producers do not decarboxylate Lysine; therefore the acid reaction produced prevents the blackening of the colonies (Merck, Catalogue Number. 1.05287.0500, 2003).

The concentration technique used in the present investigation for the isolation of presumptive *Salmonella typhimurium* and *Shigella dysenteriae* was based on the isolation and selection of all colonies, which appeared red to yellow with black centre and red respectively on XLD agar. However, these red to yellow with black centre colonies might be different strains of *Proteus* (*Proteus mirabilis*) while the red colonies might be *Escherichia coli*, *Enterobacter cloacae*, *Klebsiella pneumoniae* and *Pseudomonas* spp (Merck, Catalogue Number. 1.05287.0500, 2003). The standard procedure used for the isolation of presumptive *Vibrio cholera* was by enrichment in a broth (Alkaline Peptone Water) and recovered on a selective medium, (VDA), where colonies appeared as yellow and smooth colonies (South African Bureau of Standards, 2001). However, these yellow colonies might not only be different strains of *V. cholerae* but also *V. aginolyticus* (Merck, Catalogue Number. 1.10263.0500, 2003). Consequently, the high numbers of presumptive *Salmonella typhimurium, Shigella dysenteriae* and *Vibrio cholerae*, as indicated in Figure 4.1, could be linked to a combination of these target pathogens with other microorganisms (Table 4.1), which might not be necessary strains capable of causing infections and diseases in humans.

Based on the data obtained from the culturing media, there was generally a gradual removal of presumptive bacteria from the influent zones to the effluent zones of the various wastewater treatment plants (Figure 4.1). Although, there were variations with regards to both the patterns and the efficiency of each plant for the removal of the target pathogens, about 71% of the total influent samples contained presumptive *Salmonella typhimurium*, while only 50% and 33.5% of the effluent and receiving water body samples were observed to contain presumptive *Salmonella typhimurium* (Figure 4.1). Similar observations were made for presumptive *Shigella dysenteriae* and *Vibrio cholerae* pathogens with decreasing incidences of the pathogens from influents to the receiving water bodies (Figure 4.1). The presence of these presumptive pathogens in the enriched cultures is indicative of the presence of at least one cell per 100 ml of the wastewater samples. Hence, the microbial qualities of the effluents in all locations exceeded the maximum safety limit for effluent discharge by the South African General and Special Standards of nil faecal coliforms/100mL (National Water Act No 45, 1999). The microbiological quality of the effluent generated and in turn discharged into the receiving water body was poor, unsafe for recreational or irrigational use and not acceptable for human consumption.

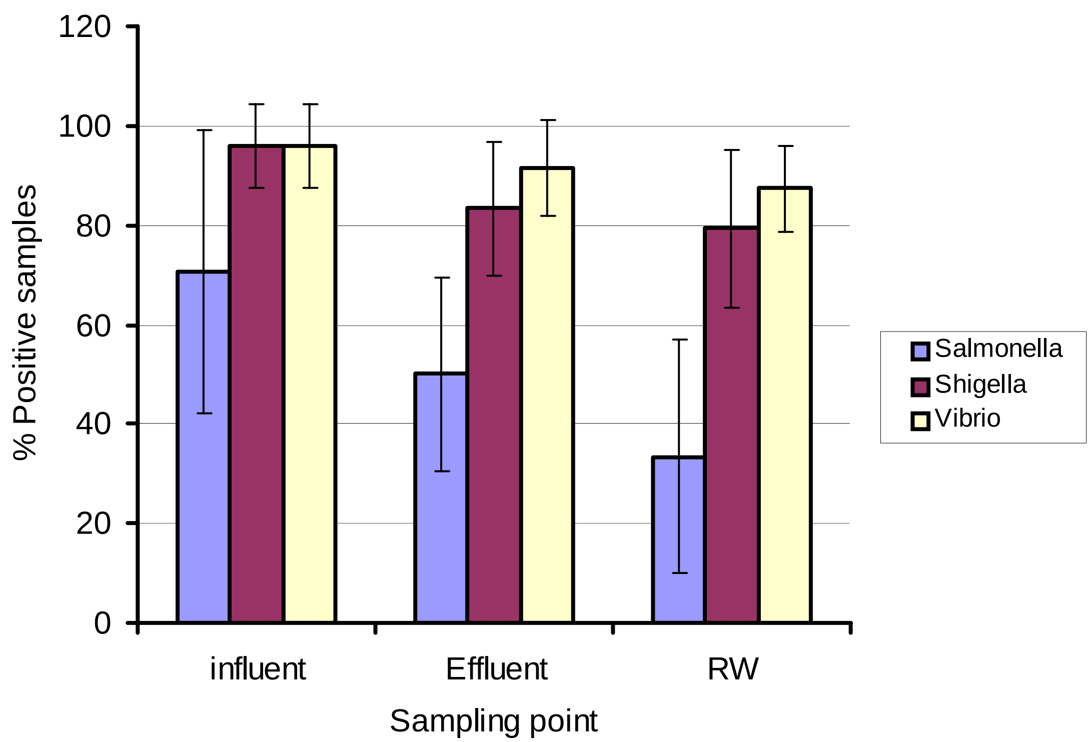


Figure 4.1. Cumulative proportions of the wastewater samples containing different presumptive bacterial pathogens.

To identify the target pathogens and differentiate them from other microorganisms, biochemical tests were applied on 179 isolates randomly selected from the influent (71), effluent (57) and receiving water body (51) samples of all the plants. These tests revealed a total of 21 culturable species. The number of species identified from specific treatment stages in all the plants ranged between 6 and 11 (Table 4.4), but their distribution does not appear to follow any regular pattern.

Table 4.3. List of bacteria species isolated from the treatment plants located at Alice, Dimbaza (DIM), East London (E/London) and Fort Beaufort (F/Beaufort) using the API 20E system.

Bacterial isolates	**OCCURRENCE**											
	ALICE			**DIM**			**E/LONDON**			**F/BEAUFORT**		
	In	Eff	Rw	In	Eff	Rw	In	Eff	Rw	In	Eff	Rw
Aeromonas hydrophilia	√	√	√	√	√	√	√		√	√		
Aeromonas salmonicida							√	√				
Enterobater aerogenes	√	√		√	√		√					
Enterobater cloacae	√	√	√	√		√	√	√	√	√	√	√
Escherichia coli	√	√	√							√	√	
Klebsiella pneumoniae	√	√	√	√	√	√	√	√	√			√
Klebsiella ozoenae				√	√							
Klebsiella oxytoca							√	√				
Klebsiella ornithinolytica							√	√	√			
Morganella morganii	√	√	√	√						√	√	
Pasteurella pneumoniae										√	√	√
Proteus mirabilis	√	√		√	√	√	√	√	√	√	√	√
Providencia rettgeri	√	√		√	√	√	√	√		√	√	√
Pseudomonas fluorescens			√			√	√		√			
Salmonella spp.	√			√			√	√	√	√	√	√
Serratia liquifaciens	√			√								
Serratia odorifera	√	√										
Serratia plymithica									√			
Shewan putrifaciens										√		
Kluyvera spp.						√						
Vibrio parahaemolyticus							√					√
TOTAL	**11**	**9**	**6**	**10**	**6**	**7**	**12**	**8**	**8**	**9**	**7**	**7**

Key: In: Influent (21), Eff: Effluent (21), Rw: Receiving water body (21).

Among the potential pathogens isolated were: *Aeromonas hydrophila*, *Enterobacter cloacae*, *Escherichia coli*, *Klebsiella pneumoniae*, *Klebsiella ornithinolytica*, *Pasteurella* *pneumonia, Proteus mirabilis, Providencia rettgeri* and *Salmonella* spp. As illustrated in Table 4.4, some of the pathogenic microorganisms found in the influent of the wastewater treatment plants were retained in the respective final effluent. These pathogens can have an impact on the microbiological quality of the receiving water bodies.

The presence of *Salmonella* spp was noted in the final effluent and the receiving water bodies of the East London and Fort Beaufort wastewater treatment plants (Table 4.4). There was also a possibility of *Vibrio* spp (*Vibrio parahaemolyticus*) in the East London influent samples and in the Fort Beaufort receiving water body samples. The API 20E system indicated no *Shigella* spp in any of the water samples collected from all the wastewater treatment plants and the respective receiving water bodies (Table 4.4).

Nevertheless, the presence of potential pathogens such as *Aeromonas hydrophila, Escherichia coli* and *Klebsiella pneumoniae* in the effluents and receiving water bodies is a cause for concern as most people in the rural Eastern Cape region use these surface waters for drinking, recreational and irrigation purposes. Previous reports have shown that the impact of waterborne diseases in the rural Eastern Cape Province of South Africa is significant as a result of drinking water sources with poor microbiological quality (Watson *et al*., 1985; Momba *et al*., 2006a). It has been reported that *Aeromonas hydrophila* strains are capable of causing gastrointestinal illness in humans who may acquire infections through open wounds or by ingestion of sufficient number of bacteria in food and water. This pathogen has been implicated as a causative agent of septicemia, ocular and respiratory tract infections, pneumonia and urinary tract infections (Watson *et al*., 1985; Material Safety Data Sheet, 2004). The most common clinical infections that are associated with these bacteria are diarrhoea (Agbonlahor, 1983; Bhat *et al*., 1984). Pathogenic *E. coli* causes urinary tract infections (UTI), neonatal meningitis and intestinal diseases (gastroenteritis) in humans (Todar, 2002). *Klebsiella pneumoniae* is an important pathogen that causes urinary tract infections (UTI), pneumonia and intra-abdominal infections in hospitalized immunocompromised patients with severe underlying diseases (Podschum and Ullmann, 1998; Tumbarello *et al*., 2006). The bacteria are implicated in nosocomical bloodstream infections (BSIs) (Podschum and Ullmann, 1998; Tumbarello *et al*., 2006).

Other pathogenic bacteria whose occurrence could be noted in the final effluent were: *Enterobacter aerogenes* and *Enterobacter cloacae*. These bacterial species are by far the most frequently encountered human pathogens among the genus *Enterobacter* (Sanders and Sanders, 1997). Over the past decades, members of the *Enterobacter cloacae* complex have emerged as important nosocomical pathogens ranging among the five bacteria most frequently recovered from intensive care patients (Sanders and Sanders, 1997). *Proteus mirabilis* is a well-known cause of clinically significant infection and several outbreaks of nosocomical infection while few reports of serious nosocomical infection due to *Proteus morganii* (*Morganella morganii*) have been made (Williams *et al*., 1983). Although *Serratia marcescens* has been thought to be the only pathogenic species of *Serratia* (Basilio, 2004), reports of disease resulting from infection with *Serratia odorifera* have also been on the increase and this bacterium is fast establishing itself as an emerging human pathogen (Cook and Lopez, 1998; Basilio, 2004).

The preponderance of *Aeromonas hydrophila, Enterobacter cloacae, Klebsiella pneumoniae, Proteus mirabilis* and *Providencia rettgeri* in the final effluents is an indication of the inefficiencies of the wastewater treatment plants for the removal of these potential pathogens and a consequence of inadequate disinfection practices and inadequate maintenance of the infrastructure in the Eastern Cape Province as suggested elsewhere (Pearson and Idema, 1998; Momba and Mfenyana, 2005). The effluents discharged from the Dimbaza, East London, Alice and Fort Beaufort wastewater treatment plants were therefore identified as pollution point sources into the respective receiving water bodies (Tembisa, The Nahoon and Eastern Beach which are part of the Indian Ocean, The Tyume and The Kat River). This is consistent with the finding of previous investigators (Momba and Mfenyana, 2005) who pointed out that the faecal contamination of drinking water supplied by untreated or inadequately treated sewage effluents entering rivers and dams that serve as the source of municipal water supplies create conditions for the rapid spread of pathogens.

In order to ensure the safety of the final effluents and the receiving water bodies that are commonly used by the community of the Eastern Cape for multiple purposes (drinking, washing, cooking, recreational and irrigation), a subsequent PCR analysis of the *Salmonella* spp, *Shigella* spp. and *Vibrio cholerae* was important; since this method relies on the *in vitro* amplification of a DNA fragment and offers a higher level of specificity of strain detection (Rompré *et al*., 2002; Momba *et al*., 2006b)

The *invA* gene is essential for virulence in *Salmonella* spp. and is thought to trigger the internalization required for invasion of deeper tissues (Khan *et al*., 2000). The PCR produced a 408 bp amplified fragment from the *invA*-1; *invA*-2 primer sets in 2 isolates from the final effluent and 5 isolates from the receiving water body. This amplified fragment was obtained by the reference strain *Salmonella enteritidis* giving the same banding pattern. The PCR amplified a 284 bp amplified fragment from the *invA*-3; *invA*-4 primer sets in 4 isolates from the final effluent while no amplified product of the expected size was found in the isolates from the receiving water body. Only 1 of the isolate from the receiving water body produced a 488 bp amplified fragment with the *sefA* primer set for *Salmonella enteritidis* (Fig. 4.5). No amplified product of the expected size was found in the final effluent isolates. Four of the final effluent and 2 of the receiving water body isolates produced a 559 bp amplified fragment of the gene with the *fliC* primer set for *Salmonella typhimurium* (Fig. 4.4).

The PCR analysis indicated negative results for the *virA* gene in all the presumptive *Shigella dysenteriae* isolated from the effluent and receiving water bodies. The PCR technique in this study targeted the plasmid virulence (*VirA*) gene specific for *Shigella* spp.

Using the oligonucleotide primer pairs (*hylA*-1; *hylA*-2), 11 isolates from the final effluent and 7 of the isolates from the receiving water body gave amplified gene products corresponding to 738 bp which is the expected size of the *hylA* gene (Fig. 4.6; 4.7). This amplified fragment was reproduced by the reference strain *Vibrio cholerae*. A 564 bp amplified fragment with the primer sets (*ctxA*-5; *ctxA*-6) was produced by 4 of the *Vibrio cholerae* isolates from the final effluent and 5 of the isolates from the receiving water body. An explanation for the extra bands (Fig. 4.4) in three lanes is that the primers are hybridized to secondary sites of template and as a result of miss priming (Titus, 1991).

Using the oligonucleotide primer pairs (*ctxA*-1; *ctxA*-2) (Lipp *et a*l., 2003) and (*ctxA*-7; *ctxA*-8) (Kapley and Purohit, 2001), no amplified products were detected in the final effluent and the receiving water body. The 647 bp (*ctxA*-1; *ctxA*-2) and 302 bp (*ctxA*-7; *ctxA*-8) amplified fragments were reproduced by the reference strain *Vibrio cholerae*. The PCR analysis of the isolates showed that 27% of the effluent and 63% of the receiving water body samples proved positive for *Salmonella* spp. Thirty six percent of the effluent samples were found to be positive for *Salmonella typhimurium* while none of it was positive for *Salmonella enteritidis*. Thirteen percent of the receiving water body samples tested gave positive results for *Salmonella enteritidis* and 25% gave positive results for *Salmonella typhimurium* (Table 4.5).

The PCR results revealed that 18% of the final effluent and 24% of the receiving water body samples tested positive for toxigenic *Vibrio cholerae*. The results of the PCR analysis which targeted the haemolysin gene of *Vibrio cholerae* showed positive results in

50% of the effluent and 33% of receiving water body samples. The overall results indicated outside contamination of the receiving water bodies as more target pathogens were reflecting in the receiving water bodies compared to the prevalence rate in the final effluents (Table 4.5).

Table 4.5 Number of effluent and receiving water body samples contaminated by *Salmonella typhimurium, Shigella dysenteriae* and *Vibrio cholera* during the study period (August-November 2003, February-March 2004).

Cultural methods			
Target organisms		Water source	
		Final effluent	Receiving water body
Salmonella typhimurium		11 (100%)	8 (100%)
Shigella dysenteriae		18 (100%)	18 (100%)
Vibrio cholerae		22 (100%)	21 (100%)
Total isolates		51 (100%)	47 (100%)
PCR methods			
Target organisms	Target gene	Final effluent	Receiving water body
Salmonella spp	*invA*		
	invA-1; *invA*-2	2 (18%)	5 (63%)
	invA-3; *invA*-4	4 (36%)	0
Salmonella enteritidis	*sefA*	0	1 (13%)
Salmonella typhimurium	*fliC*	4 (36%)	2 (25%)
Shigella dysenteriae	*virA*	0	0
Vibrio cholerae	*ctxA*		
	ctxA-1; *ctxA*-2	0	0
	ctxA-7; *ctxA*-8	0	0
	ctxA-5; *ctxA*-6	4 (18%)	5 (24%)
Vibrio cholerae	*hylA*	11 (50%)	7 (33%)
Total isolates		25 (32%)	20 (32%)

559bp

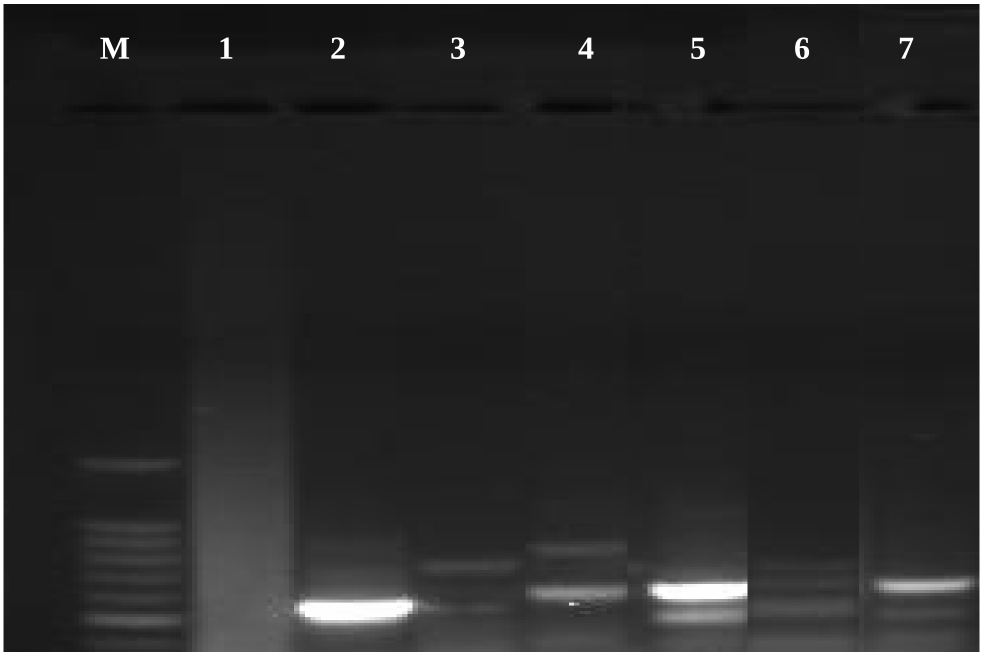


Figure 4.2.: **The amplification product from the oligonucleotide primer pair for the *fliC* gene in final effluent samples. Lane M: 100 bp DNA ladder molecular weight marker; Lane 1 PCR negative control; Lane 2 PCR positive control (*Salmonella typhimurium*); Lane 3 Sample from Alice; Lane 4 Sample from Alice; Lane 5 Sample from Dimbaza; Lane 6 Sample from East London; Lane 7 Sample from Fort Beaufort.**

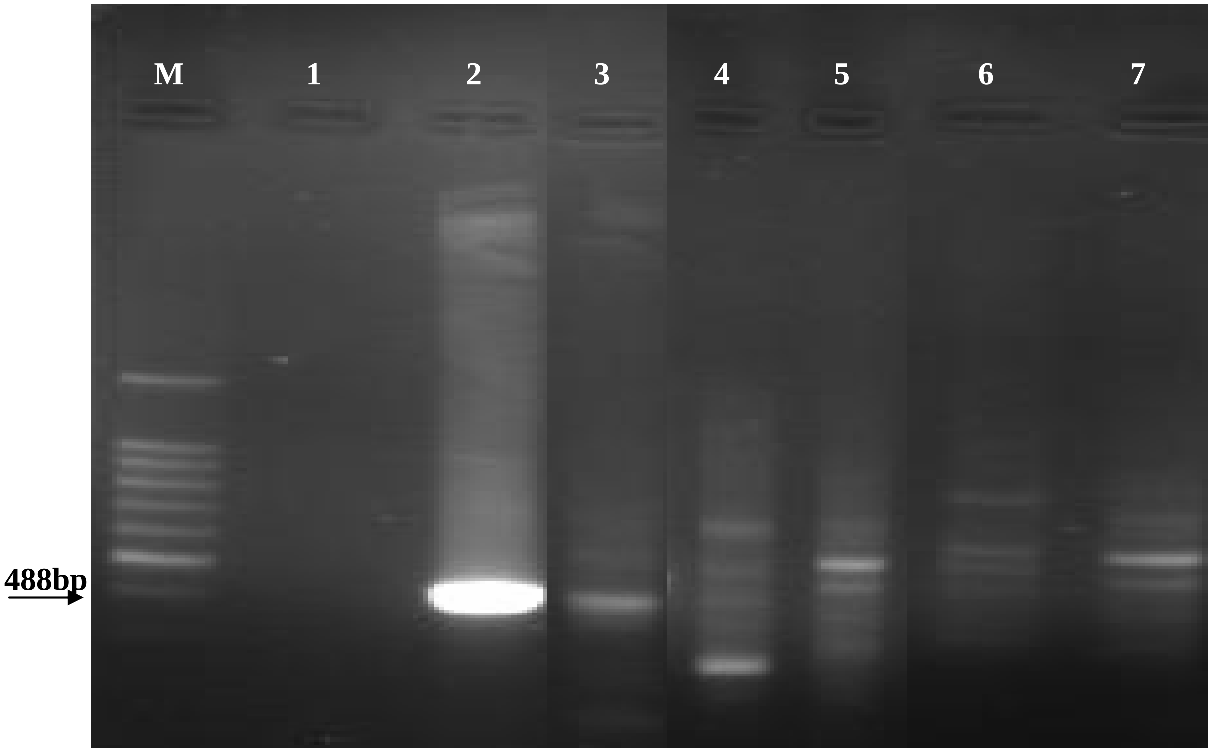


Figure 4.3.: **The amplification from the oligonucleotide primer pair for the *sefA* gene in the receiving water body samples. Lane M: 100 bp DNA ladder molecular weight marker; Lane 1 PCR negative control; Lane 2 PCR positive control (*Salmonella enteritidis*); Lane 3 Sample from Alice; Lane 4 Sample from Dimbaza; Lane 5 Sample from East London; Lane 6 Sample from Alice; Lane 7 Sample from Dimbaza.**

738bp

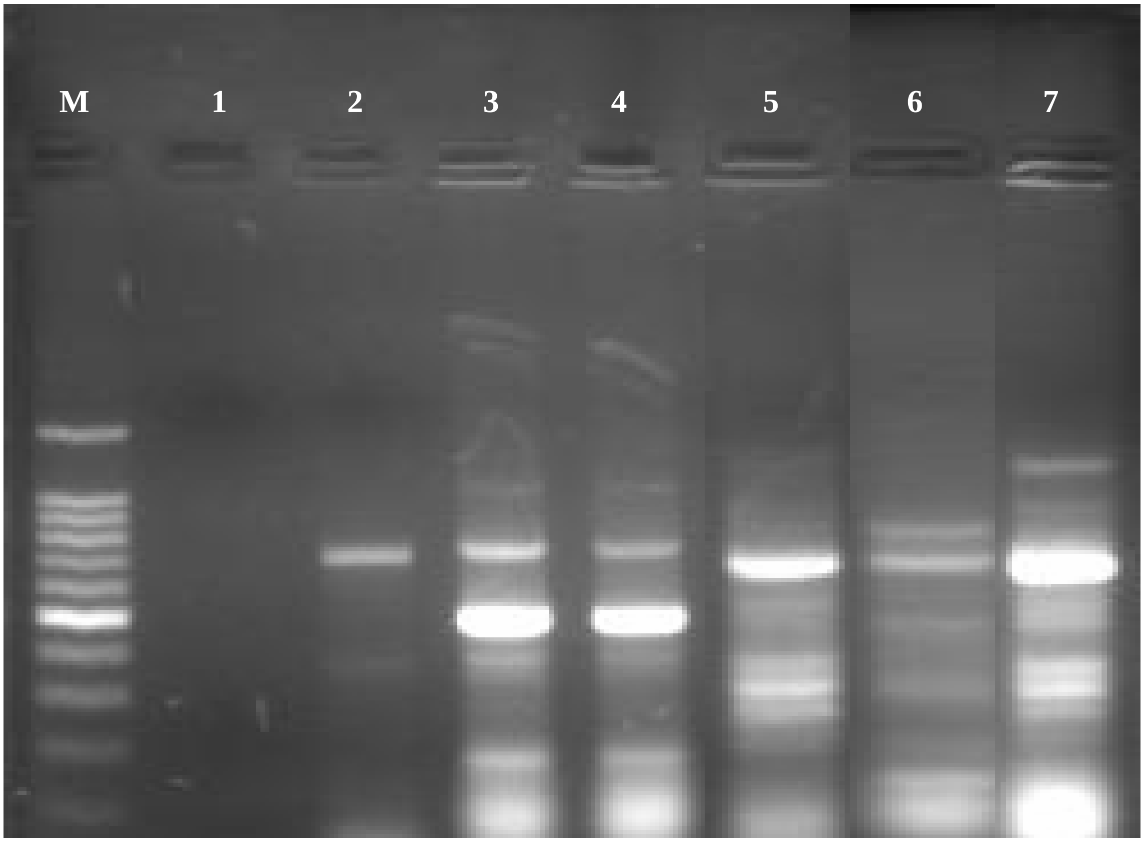
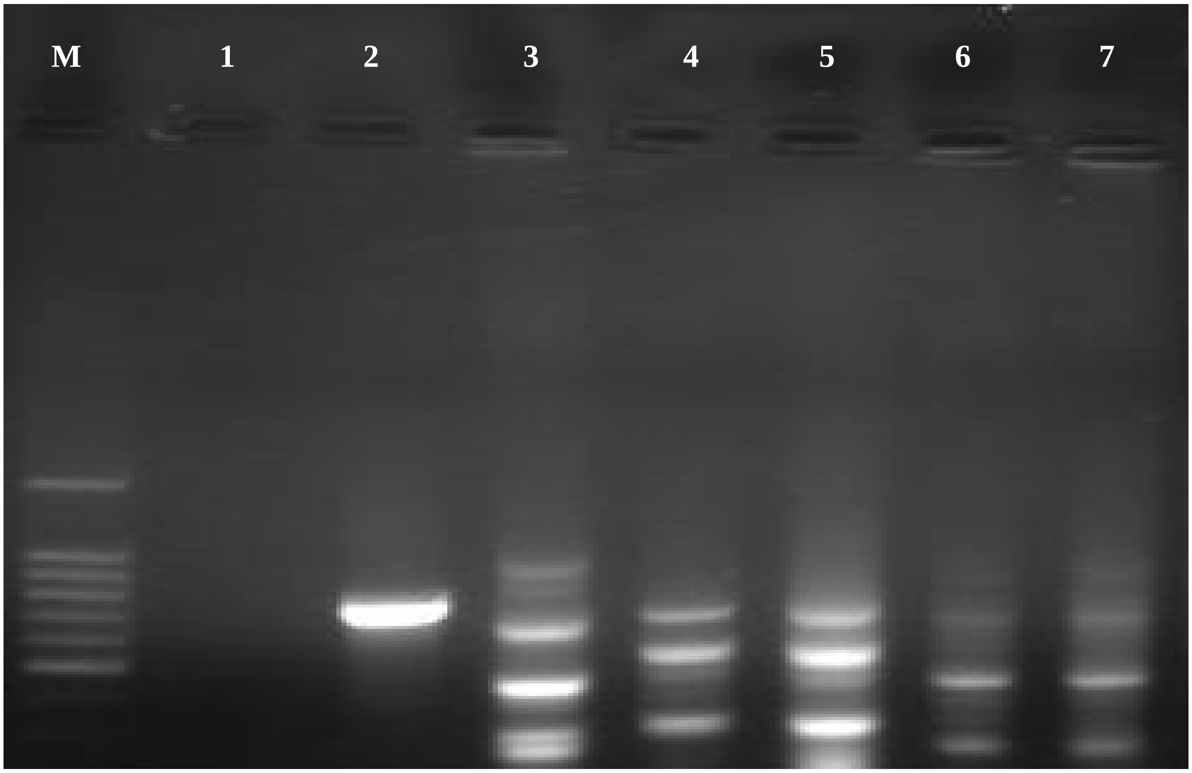


Figure 4.4: **The amplification product of the oligonucleotide primer pair for the *hylA* gene in final effluent samples. Lane M: 100 bp DNA ladder molecular weight marker; Lane 1 PCR negative control; Lane 2 PCR positive control (*Vibrio cholera*); Lane 3 Sample from Alice; Lane 4 Sample from Alice; Lane 5 Sample from Alice; Lane 6 Sample from Fort Beaufort; Lane 7 Sample from Dimbaza.**



738bp

Figure 4.5: The amplification product of the oligonucleotide primer pair for the *hylA* gene in River water samples. Lane M: 100 bp DNA ladder molecular weight marker; Lane 1 PCR negative control; Lane 2 PCR positive control (*Vibrio cholera*); Lane 3 Sample from Alice; Lane 4 Sample from Alice; Lane 5 Sample from Alice; Lane 6 Sample from Alice; Lane 7 Sample from Alice.

In the present study we used the PCR method to amplify the invasive (*invA*) gene for *Salmonella spp*, *sefA* gene for *Salmonella enteritidis* and *fliC* for *Salmonella typhimurium*, plasmid virulence gene (*virA*) for *Shigella* spp, cholera toxin (*ctxA*) gene and haemolysin (*hylA*) gene for *Vibrio cholera*. The PCR results on presumptive *Shigella dysenteriae* DNA showed the absence of *virA* gene in all the wastewater samples. The results obtained by culturing methods using selective media indicated that 100% of the samples were contaminated with presumptive *Salmonella typhimurium, Shigella dysenteriae* and *Vibrio cholerae* during the study period (Table 4.5). Several factors may have contributed to the apparently high *Salmonella typhimurium, Shigella dysenteriae* and *Vibrio cholerae* frequency using cultural methods and their low frequency recovery or absence using the PCR method. This might either have been due to the culture media or to species-specific genes that were targeted. Using the PCR method, this study revealed that *Salmonella enteritidis* was implicated in the receiving water body while *Salmonella typhimurium* and *Vibrio cholerae* was more implicated in the final effluent and receiving water body samples in Alice, East London and Fort Beaufort samples.

4.4.3 Risk assessment

The risk assessment was conducted based on the outcome of the molecular characterisation of isolates. Figures 4.6–4.11 showed the values of the average probability of infection and the 95th percentiles of the probability of infection for *Salmonella* and *Vibrio cholerae* due to accidental ingestion of 0.1 mL, 1 mL and 100 mL volumes of the individual receiving water body samples. On close observation, the trend of the average probability of infection and the 95th percentile probability of *Salmonella* and *Vibrio cholerae* infections was such that there was higher risk associated with the ingestion of a higher volume of the receiving water body samples in all the sites.

The average probability of *Salmonella* infection due to accidental ingestion of 0.1 mL volume of the receiving water body sample was less than 0.05 in all the sites while the highest average probability (0.10) of *Vibrio cholerae* infection was recorded in Dimbaza followed by Alice (Fig. 4.8). The highest average probability (0.10) of *Salmonella* infection due to accidental ingestion of 1 mL volume of receiving water body sample was recorded in Alice followed by Fort Beaufort while the highest average probability (0.20) of *Vibrio cholerae* infection was recorded in Dimbaza followed closely by Alice (Fig. 4.9). The highest average probability (0.40) of *Salmonella* infection due to accidental ingestion of 100 mL volume of the receiving water body sample was recorded in Fort Beaufort and Alice while the highest average probability (0.50) of *Vibrio cholerae* infection was recorded in Alice and Dimbaza (Fig. 4.10).

The 95th percentile probability of *Salmonella* infection due to accidental ingestion of 0.1 mL of the receiving water body sample was highest in Alice (0.03) followed by Fort Beaufort (0.02) while the highest 95th percentile probability of *Vibrio cholerae* infection was recorded in Dimbaza (0.20) followed by Fort Beaufort (0.10) (Fig. 4.11). The 95th percentile probability of *Salmonella* infection due to accidental ingestion of 1 mL of the receiving water body sample was highest in Alice (0.20) followed by Fort Beaufort (0.15) while the highest 95th percentile probability of *Vibrio cholerae* infection was recorded in Dimbaza (0.33) followed by Fort Beaufort (0.26) (Fig. 4.12). The 95th percentile probability of *Salmonella* infection due to accidental ingestion of 100 mL of the receiving water body sample was highest in Alice (0.80) followed by Fort Beaufort (0.70) while for *Vibrio cholerae* infection, the highest was recorded in Dimbaza (0.60) while the same value of 0.50 was recorded in Alice, Fort Beaufort and East London (Fig. 4.13).

Current practices to predict a possible risk of infection related to the microbiological quality of water include environmental health practitioners and water quality managers generally testing for the presence of indicator microorganisms such as those considered in the study. If present, a negative health effect can be expected with increasing risk expressed as organisms increase. This approach can be referred to as an observed-adverse-effect-level approach (OAELA) based on the occurrence of microbiological organisms. The study indicated the possible risk of infection (OAELA) based on the occurrence of *Salmonella* spp. and *Vibrio cholerae* in the receiving water body samples.

The 95th percentile of microbial infection is the predominant multi-stage model used to give a conservative estimate of the dose of infection by pathogenic microorganism in a sample. A high value of 95th percentile probability of *Salmonella* infection due to accidental ingestion of 0.1 mL, 1 mL and 100 mL of the receiving water body sample was constantly recorded in Alice. This was constantly observed for values of the 95th percentile probability of *Vibrio cholerae* infection due to accidental ingestion of 0.1 mL, 1 mL and 100 mL of the receiving water body sample in Dimbaza. The 95th percentiles of *Salmonella* and *Vibrio cholerae* infection were above the US EPA, 1994 acceptable risk limit of 0.01% which indicates no probability of infection (US EPA, 1994). There are no acceptable risks for South Africa. The US EPA has recommended risks acceptable for the USA but whether these are applicable to South Africa is uncertain. However, the dose-response information (models and parameters) applied in this study were from international literature and may not have reflected the true possibility of infection in South Africa.

It is most important from the society's perspective to control microbial exposures from surface water bodies that receive wastewater effluent. Wastewater treatment does not normally include any complete barriers and the treatment processes are not optimized for pathogen removal, although each process generally inactivates or removes a part of the pathogens. Control points for all of the hazardous exposures could ensure that each treatment step maintains measurable process parameters within operational limits and does not exceed established critical limits. The results showed that health effects are potentially associated with significantly higher occurrence of the target pathogens detected during the water quality monitoring period. There is therefore much need to address wastewater treatment problems in these communities as the effluents or the receiving water bodies are used for various purposes.

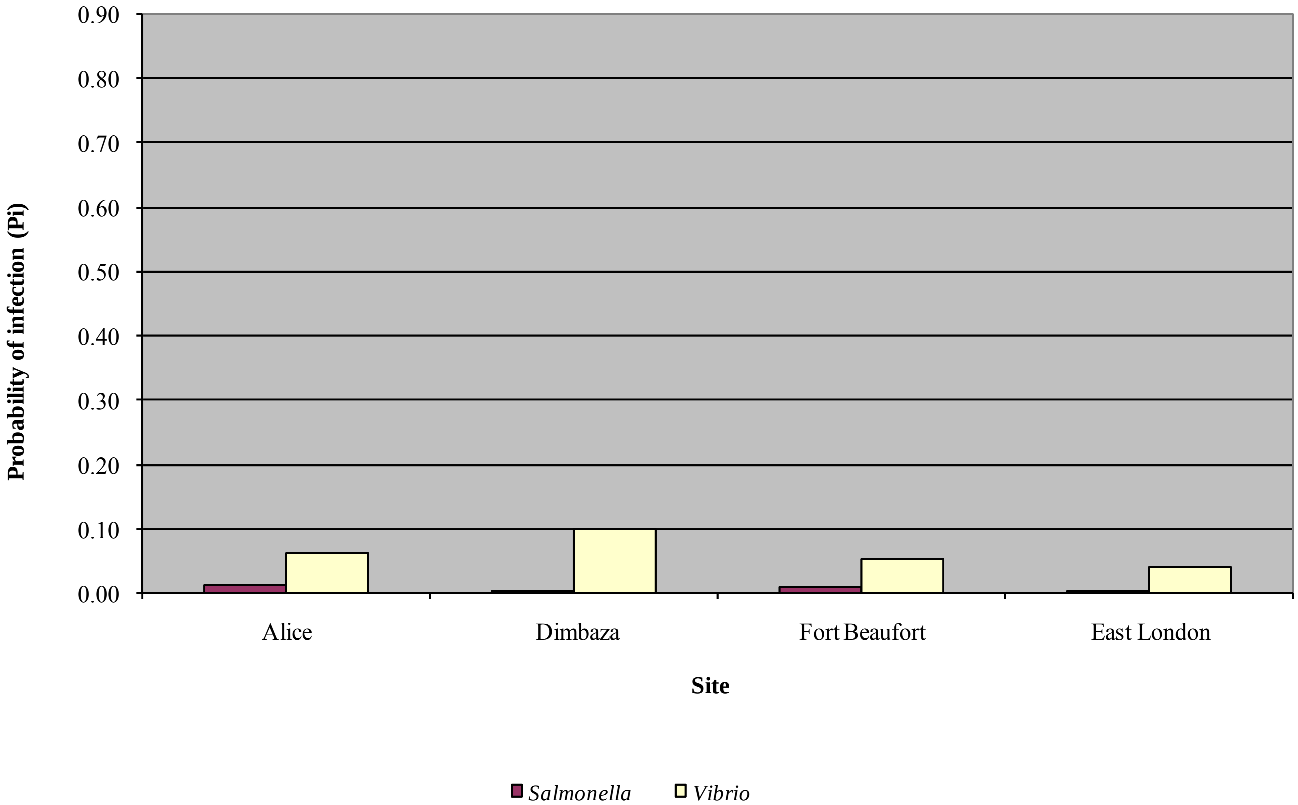


Figure 4.6. Average Pi due to accidental ingestion of 0.1 mL of water.

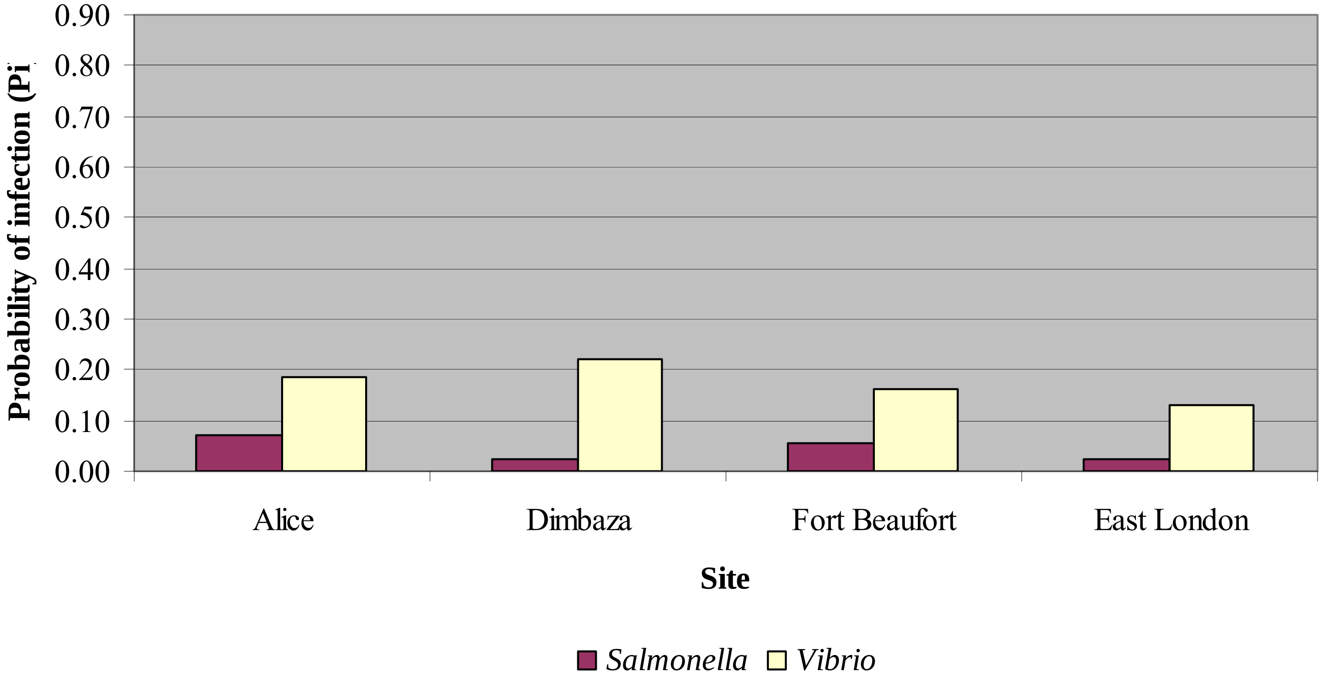


Figure 4.7. Average Pi due to accidental ingestion of 1 mL of water.

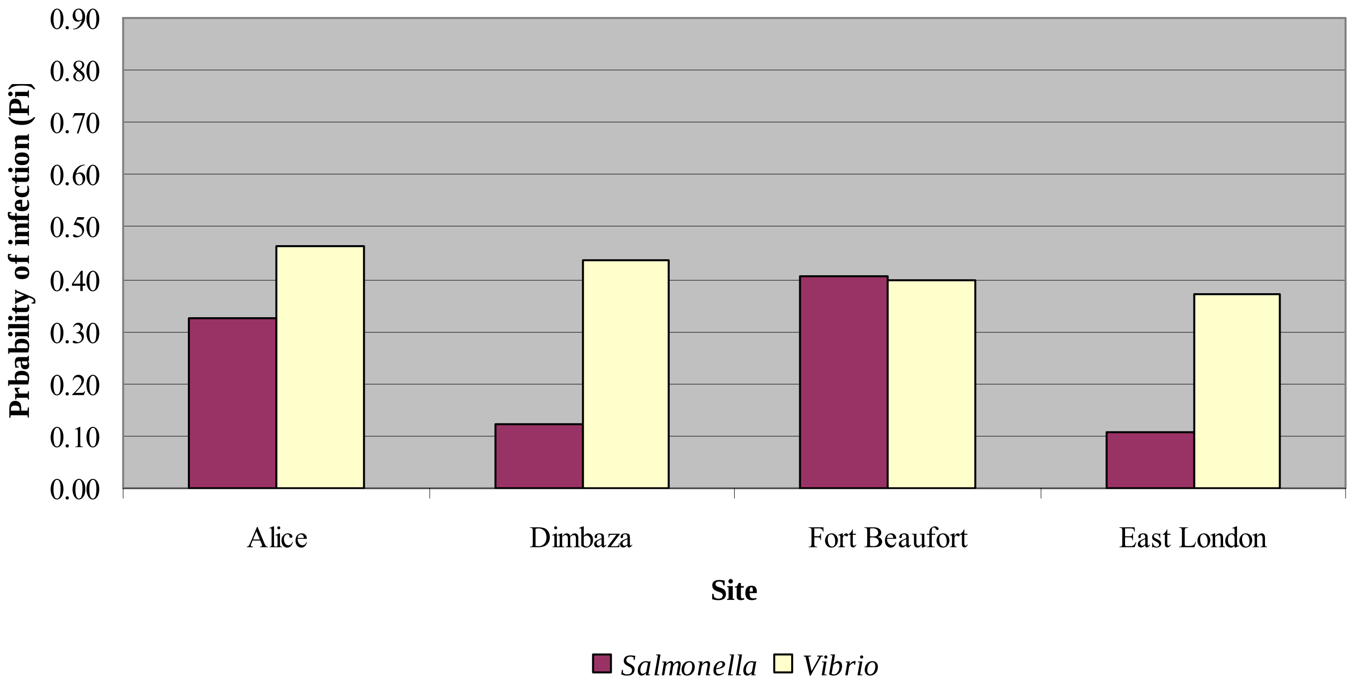


Figure 4.8. Average Pi due to accidental ingestion of 100 mL of water.

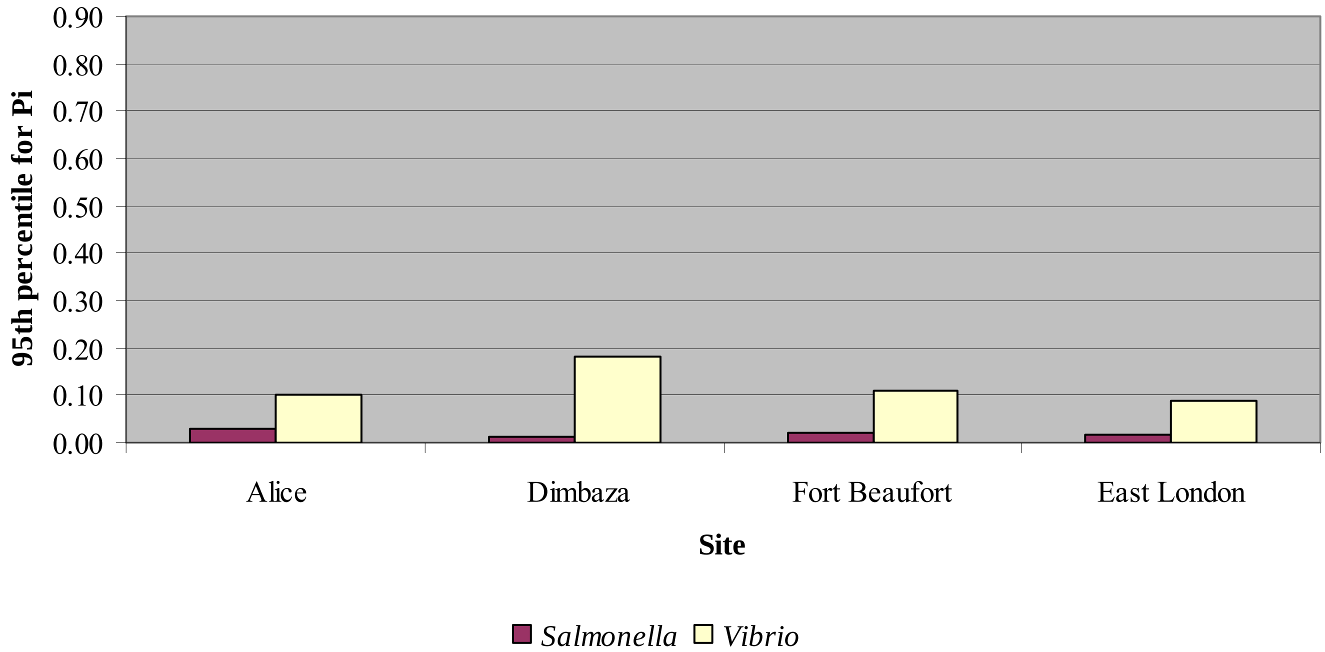


Figure 4.9. The 95th percentile Pi due to accidental ingestion of 0.1 mL of water

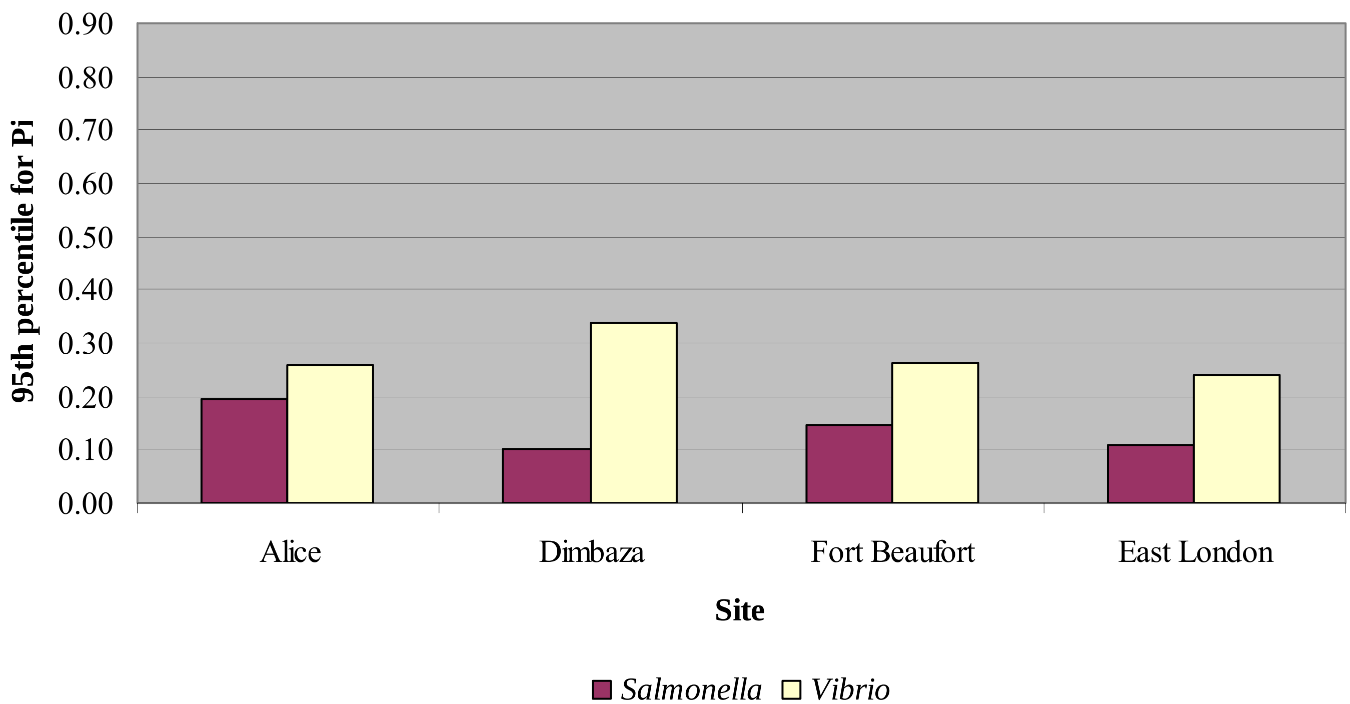


Figure 4.10. The 95th percentile Pi due to accidental ingestion of 1 mL of water.

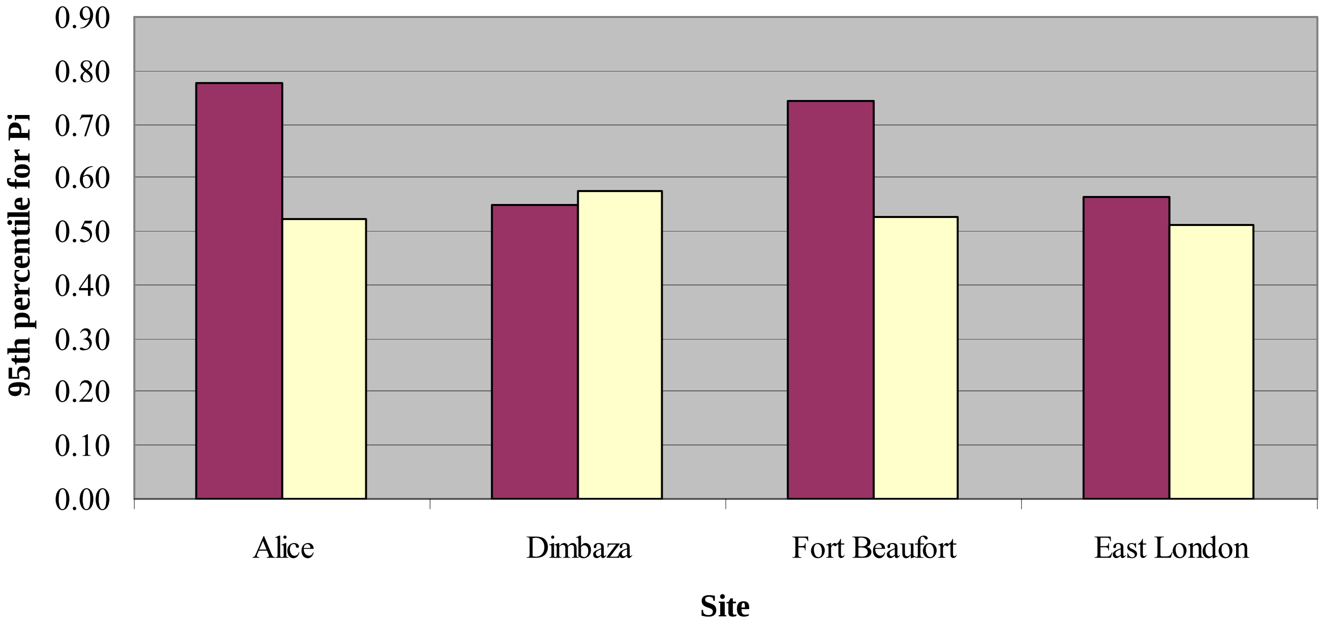


Figure 4.11. The 95th percentile Pi due to accidental ingestion of 100 mL of water.

The contamination of water sources with potentially hazardous microbial organisms such as *Salmonella typhimurium* and *Vibrio cholerae* as well as increased awareness of waterborne diseases in the public has resulted in the need for more investigation into the adequate purification of wastewaters prior to discharge into watercourses. Inadequate treatment of effluents might have a link with cholera or diarrhoeal infections in the rural communities of South Africa. In 2001/02, a cholera epidemic started in October 2001 in Kwazulu-Natal and between January and March 2002; the disease was reported in other provinces (National Department of Health, 2003). A total of 17 890 cases of cholera were reported compared to the 106 389 cases reported during the previous epidemic (National Department of Health, 2003). Most of the cases and deaths were in KwaZulu-Natal and the Eastern Cape (National Department of Health, 2003). The Eastern Cape was the second most affected province, where the epidemic started in the Oliver Tambo District, and lasted for six months (National Department of Health, 2003). The epidemic was mainly attributed to unsafe water as a result of untreated wastewater that was emptied into the Umtata River. On the 14th of August 2004 a local newspaper, Daily Dispatch, reported that the Buffalo City Municipality pumped sewage out to the sea from the East Bank wastewater treatment works (Appendix 3) due to a faulty valve in the second of three pumps (Daily Dispatch, 2004). This led to the closure of the Nahoon, Eastern and Orient beaches (Daily Dispatch, 2004). On 15 January 2005, it was reported that an outbreak of the Hepatitis A virus (HAV) in Buffalo Flats was caused by the pumping of untreated sewage into the Buffalo River by the Buffalo City municipality (Daily Dispatch, 2005). It had already claimed the life of a young Buffalo Flats boy and was believed to have infected more than 80 children in the area (Daily Dispatch, 2005). On 19 April 2005, it was reported that surfers and swimmers were turned away from the Nahoon and Eastern beaches because these water sources were polluted by a sewage spill from the East Bank wastewater treatment works (Daily Dispatch, 2005). An electrical fault at the treatment works tripped pumps and led to sewage spills (Daily Dispatch, 2005). The pumping of raw sewage into the river and other surface water sources in the Eastern Cape Province is very dangerous as the sewage may carry pathogenic bacteria (Daily Dispatch, 2005). The reports showed that high incidence of cholera were as a result of the indiscriminate discharge of untreated wastewater and that many of the wastewater treatment plants in the Eastern Cape Province lack proper maintenance (Daily Dispatch, 2005). Therefore urgent attention is needed to curb the menace of waterborne diseases in the province.

4.5 CONCLUSIONS AND RECOMMENDATIONS

The diversity of pathogenic strains in wastewater samples could be expected to be extensive and investigative studies on this diversity could seem trivial. However, the possibility of inefficiency of wastewater treatment plants for the removal of these dangerous pathogens was the reason why this task was undertaken during the study. Monitoring of influents for pathogens that occur has been demonstrated to be an excellent epidemiological tool for determining what diseases may be prevalent in the community at any moment (Moore, 1948), and this kind of investigative work can aid health authorities in risk assessment studies on predicting potential future disease outbreaks in the different regions of the province.

All aspects of the control of microbial quality in water and wastewater, general environmental health protection is critical. This will have impacts on the quality of water used for drinking, wastewater reused and on water used for recreation and domestic chores. The promotion of sanitation, proper siting of wastewater disposal facilities in relation to drinking water sources, fish ponds and natural water courses and good management of wastes and hygiene will lead to reduced hazards. This study suggested that discharges should be monitored in the Eastern Cape Province since this is a major determinant of water quality. A monitoring programme should be implemented for all the point source discharges. While monitoring has been extremely useful in controlling waterborne disease outbreaks, it is possible that insufficient protection is provided against levels of relatively low-grade gastrointestinal illness and other complaints associated with waterborne pathogens.

Although the treatment plants succeeded in removing some presumptive pathogens from the influents, effluent discharges were only occasionally devoid of the organisms, thus constituting a potential threat of incidences of infectious diseases. In many cases in developing countries, a high level of reliability of water supply schemes, particularly the treatment process, is the exception rather than the rule and various factors such as cost, operator training and problems with maintenance of infrastructure could contribute to these problems (Pearson and Idema, 1998). The current disinfection practices and guidelines in terms of chlorine residuals were found not to be sufficient for the removal of *Salmonella* spp and *Vibrio cholerae* since these pathogens could still be detected in the final effluent. The chlorine residuals concentration could have had minimal effect in the removal or inhibition of bacterial growth in the final effluent. The inefficiency of all four wastewater treatment plants for the removal of bacteria from their final effluents had a negative effect on the quality of receiving water bodies, although there might be other sources of faecal pollution. A case is made for more stringent surveillance of the performances of wastewater treatment facilities in the Eastern Cape Province of South Africa, in order to ensure compliance with stipulated standards.

CHAPTER 5

GENERAL CONCLUSIONS AND RECOMMENDATIONS

5.1 CONCLUSION

Access to a clean, pathogen free water supply is a major priority of any community if it is to remain disease-free. In rural communities of South Africa, untreated or inadequately treated water is still drawn from rivers, ponds and streams as part of their daily lifestyle. Various water-related infectious diseases and diarrhoea are often contracted and in some cases causing the death of immuno-compromised individuals (WHO, 2003). In today's highly urbanized society, the best way to achieve this objective is to recycle water by treating used or wastewater through a treatment plant system (Wilsenach, 2006). The inefficiency of wastewater plants in removing pathogenic microorganisms from their final effluents can therefore be a leading cause of the spread of waterborne diseases in the communities that they serve, which is the reason why it is crucial for the final effluents to be free of hazardous chemicals and pathogenic organisms (UNEP, 1997). Different wastewater treatment plants use various ways to treat their influents but biological methods, the activated sludge method in particular, have been identified as the most economically viable and are renowned for their high effluent qualities (Merz, 2000).

The current study showed that the quality of both the effluents and the receiving water bodies were acceptable with respect to the temperature, pH, chemical oxygen demand (COD) and total suspended solids (TSS). However, the nutrients (orthophosphate and total nitrogen) were eutrophic. The dissolved oxygen (DO) and the biological oxygen demand (BOD) did not comply with the European Union (EU) guidelines for the protection of the aquatic ecosystems (Chapman, 1996). This led to the conclusion that the effluents discharges from the Dimbaza, East London, Alice and Fort Beaufort wastewater treatment plants were a point source pollution into the respective receiving water bodies (Tembisa Dam, the Nahoon and Eastern Beach which are part of the Indian Ocean; the Tyume River and the Kat River). Statistical evidence showed a relationship between the quality of the final effluent and that of the receiving water body and the relationship was such that the better the quality of the final effluent, the better the quality of the receiving water body.

The free chlorine residual concentrations in all the plants fell within 0.1 mg/L which was recommended by Voysey and co-workers (1998). Although this limit was met, the occurrence of pathogenic and potential pathogenic microorganisms could still be detected in the final effluent. It therefore meant that the chlorine concentrations detected did not have any effect on the removal or inhibition of bacterial growth in the final effluent. Many of the pathogenic microorganisms found in the influents of all the four wastewater treatment plants, were retained in the respective final effluents. The bacteria isolated from the final effluents and the receiving water bodies of the various wastewater treatment plants have the potential to cause severe waterborne diseases. Among the potential bacteria identified were *Aeromonas hydrophilia, Enterobater cloacae, Escherichia coli, Klebsiella pneumoniae, Klebsiella ornithinolytica, Pasteurella pneumoniae, Proteus mirabilis, Providencia rettgeri* and *Salmonella spp.* In general the results suggested that the performance of the Dimbaza, East London, Alice and Fort Beaufort wastewater treatment plants for the removal of pathogenic bacteria were inadequate and therefore identified as pollution point sources into the respective receiving water bodies. The presence of *Salmonella* spp was noted in the final effluent and the receiving water bodies of the East London and Fort Beaufort wastewater treatment plant. The Dimbaza wastewater treatment plant was found the most efficient as it produced the final effluent with the least pathogenic or potential pathogenic bacteria.

The PCR successfully amplified the *ctxA* gene of *Vibrio cholerae* encoding the cholera toxin and *hylA* gene encoding the haemolysin gene in the effluent and receiving water body samples. The presence of *Salmonella enteritidis* was implicated in the receiving water body sample while *Salmonella typhimurium* was implicated in the final effluent and receiving water body samples. The presence of *Vibrio cholerae* was implicated in final effluent and receiving water body samples in Alice, Dimbaza, East London and Fort Beaufort. In terms of the presence of *Vibrio cholerae* there was no difference between the quality of the effluent in East London and in Alice. This led to the conclusion that although urban wastewater treatment plants may be better equipped than those in rural areas, rural wastewater treatment plants can achieve relatively good microbiological qualities of effluents by taking good care of the resources available.

At this stage there is no indication of endeavours in South Africa to initiate a process leading to the definition of an acceptable risk of infection for treated wastewater in the country. Since this is a long and complex task, it will probably take sometime before an officially acceptable risk regime is available. This will provide an additional measuring tool for risk assessment based on quality of effluents.

5.2 RECOMMENDATIONS

Based on the results of the present study, the following recommendations could be suggested:

- **Monitoring:** It is recommended that wastewater effluents and water sources particularly those used by the Nkonkobe and Buffalo City communities should be routinely monitored to ensure that strict adherence to effluent discharge standards is met. The important parameters to be monitored include: total and faecal coliforms (Pathogenic *Escherichia coli*, *Salmonella* spp, *Shigella* spp, *Vibrio cholerae, Campylobacter jejuni, Campylobacter coli, Yersinia enterolytica*) and physico-chemical (BOD, COD, Total Nitrogen (Nitrates, Nitrites) and chlorine residuals. The surveillance of the performance of wastewater treatment facilities would in turn reduce the presence of pathogenic bacteria and negative impact of nutrient concentration on the receiving water body and the surrounding community who are supplied with such water.

- **Management:** Better management of the wastewater treatment plants is needed in order to improve the performance in terms of orthophosphate, total nitrogen, biological oxygen demand and dissolved oxygen status for sustainable use of water from the receiving water bodies by downstream users. One of the issues that have to be considered in the management of the plants is the proper maintenance of the equipments. This is as a result of the irregularities observed in East London and Fort Beaufort during the study period when some portion of the treatment system was under repairs. Technical personnel should be placed on stand-by to check on the equipment and a back-up plan should be put in place to ensure the continuous and smooth running of the system so that the discharge generated might not have any impact on the microbiological quality of effluent and the receiving water body.

- **Improvement of the pre-treatment stages:** The results of this study suggested the improvement of the physical processes in the wastewater treatment system prior to disinfection and if this fails, then the chlorine dose should be increased or the use of another efficient disinfectant for the treatment of the effluent before returning it into the receiving water body. The monitoring of the disinfectant residuals, measurement of daily inflow rates and record keeping is also recommended for all the wastewater treatment plants.

- **Education:** Educating the plant staff about wastewater treatment, the limits that have to be met and setting performance standards is recommended. Informing and educating the rural populace about pathogenic bacteria is also recommended.

- **Maintenance:** It is recommended that the municipality in concern should ensure the protection of all the plant staff and that the premises of the wastewater treatment plant be properly maintained.

The findings of this study reveal that there is a lot of work ahead in terms of improving the sanitation practices in the Province. It is for this reason that the investigators in this work have proposed to liaise with the staff in all the four wastewater treatment plants with the purpose of transferring skills that will aid improved running of the facilities, maintaining and thereby uplifting the standard of living in the Eastern Cape community as well as protecting it against waterborne diseases.

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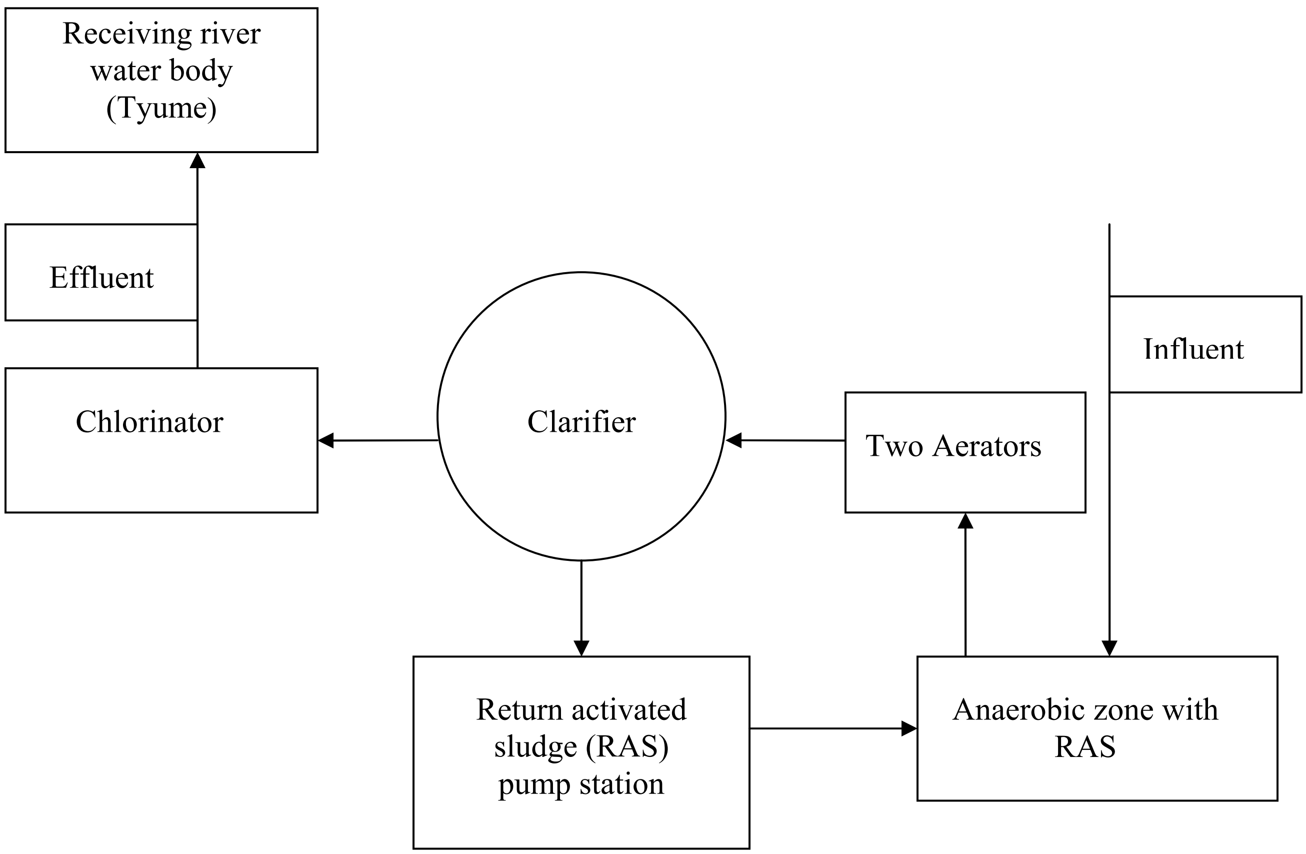
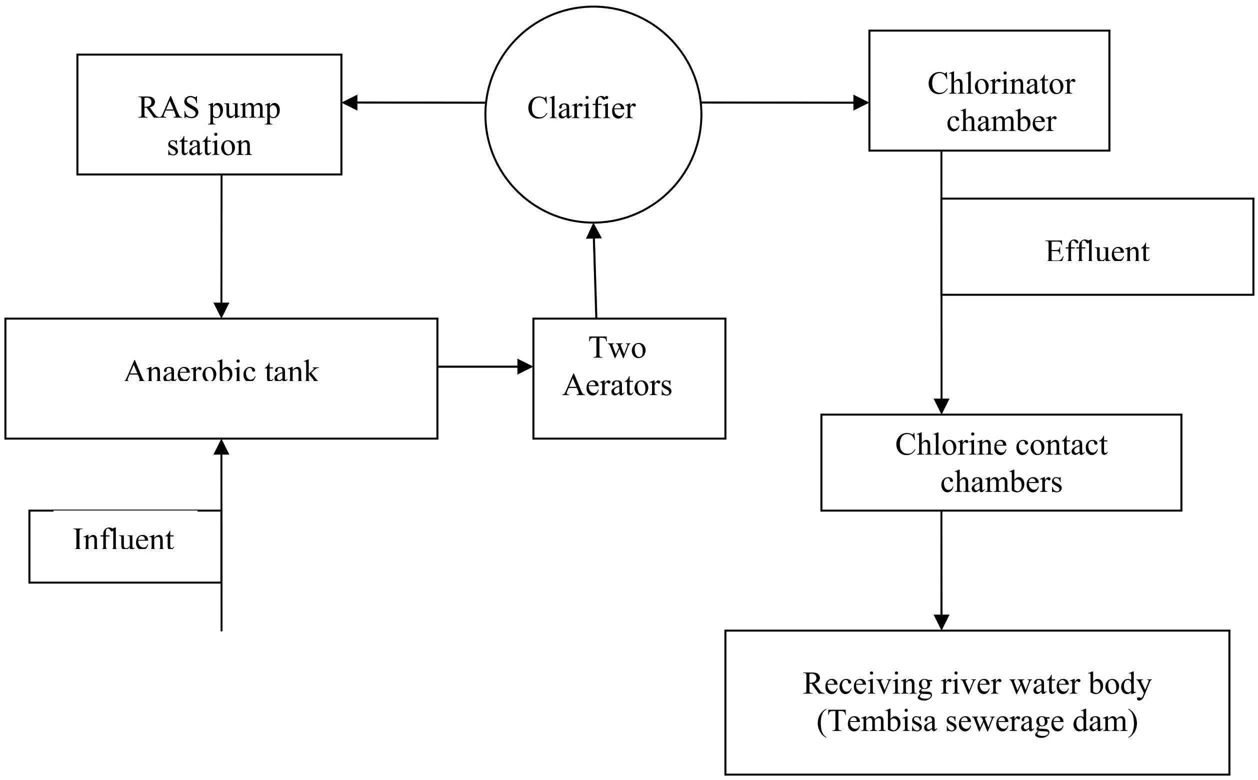
APPENDICES

APPENDIX 1



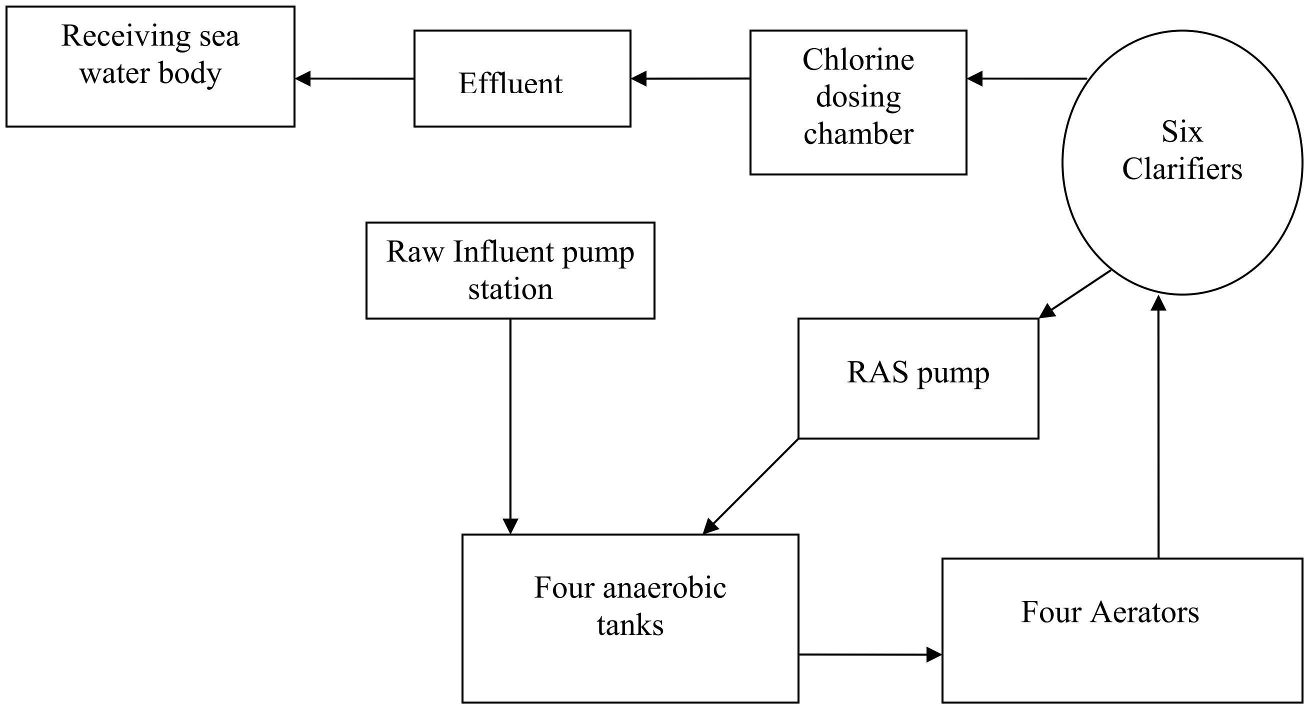
The Alice wastewater treatment plant diagram

APPENDIX 2



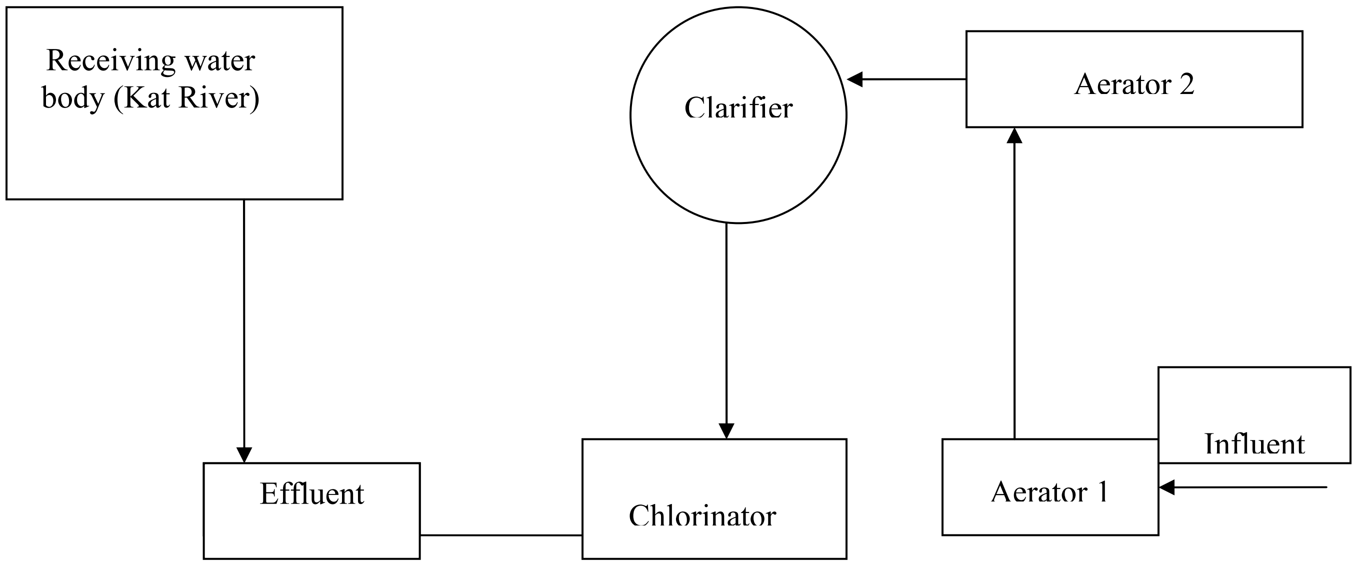
The Dimbaza wastewater treatment plant diagram

APPENDIX 3



The East London wastewater treatment plant diagram

APPENDIX 4



The Fort Beaufort wastewater treatment plant diagram