ASSESSMENT OF THE INCIDENCE OF FAECAL INDICATOR BACTERIA AND HUMAN ENTERIC VIRUSES IN SOME RIVERS AND DAMS IN THE AMATHOLE DISTRICT MUNICIPALITY OF THE EASTERN CAPE PROVINCE OF SOUTH AFRICA

Report to the
Water Research Commission

by

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EXECUTIVE SUMMARY

BACKGROUND TO THE STUDY AND STATEMENT OF PROBLEM

Regional disparities in access to piped water are sizeable in South Africa. When comparing the percentage of the population covered by the service, the lowest rates of access to pipe-borne water are observed in the rural areas of the Eastern Cape Province. More than 40% of the South African population live in rural areas (2008 numbers) and often have to depend on other sources of water, including river water, for their water needs. Tyume River and Buffalo River are located in the Amathole District Municipality, within the Eastern Cape Province of South Africa. Both rivers have dams that serve as source waters for several potable water-production plants in the areas. The rivers also serve as important freshwater resources to their host rural communities as they are used for domestic, agricultural, recreational and other purposes.

South Africa's surface-water bodies are very vulnerable to pollution, with decomposable organic matter and pathogenic agents, as well as the use of raw/treated wastewater for irrigation, constituting serious public health risks. Pathogenic agents (including bacteria, protozoa, helminth eggs, fungi and viruses) can render water contaminated and non-potable, and could result in the transmission of water-related diseases to end-users for domestic purposes, swimmers, agricultural workers and consumers of crops irrigated with polluted waters.

Globally, human enteric viruses are responsible for a large proportion (30% to 90%) of gastroenteritis cases and these viruses have been detected in water sources worldwide. There are reports of similar studies that have been carried out in a few of the South African provinces including Gauteng, Western Cape and Limpopo Provinces. However, no record exists of similar investigations in the Eastern Cape Province.

This study was motivated by the absence of reports on the virological quality of water bodies in the Eastern Cape Province, and the paucity of information on the microbiological quality of the rivers selected for this study. The aims of the study are articulated below.

PROJECT AIMS

The aims of this project were as follows:

1. To do an overview of the network of rivers and dams in the Amatole District Municipality, and to carry out a reconnaissance visit in order to select the rivers and dams to be studied and to determine sampling sites, as well as to develop analysis protocols.
2. To assess the prevalence and distribution of human viral pathogens in the selected rivers and dams in the study area.
3. To assess the occurrence and distribution of faecal indicator bacteria in the selected rivers and dams in the study area.
4. To determine the physicochemical qualities of the selected rivers and dams in the study area.
5. To correlate the occurrence of viral and bacterial pathogens with the physicochemical qualities of the various freshwater bodies.

6. To assess the fitness-for-use of the water for the intended use for recreational, domestic, and agricultural purposes, as well as to determine the risk of infection and burden of disease associated with consumption of the water.

METHODOLOGY

Water samples from the rivers were collected monthly, over a 12-month period starting from August 2010 and ending in July 2011, and transported in cooler boxes to the Applied and Environmental Microbiology Research Group (AEMREG) Laboratory at the University of Fort Hare, Alice for analyses within 6 h of collection. Electrical conductivity (EC), total dissolved solids (TDS), temperature, pH and dissolved oxygen (DO) of water samples were determined *in situ* using a multi-parameter ion-specific meter. Concentrations of orthophosphate and total nitrogen (nitrate + nitrite) were determined by standard photometric methods. Total coliforms (TC), faecal coliforms (FC) and enterococci were determined by membrane filtration and direct plating methods. Viruses in water samples were concentrated using the adsorption-elution method, followed by extraction of viral nucleic acids and purification done using commercially available kits. The concentrations of human enteric viruses in the river-water samples were estimated using quantitative PCR. RNA viruses were quantified in a two-step protocol where RNA was first transcribed into cDNA in a separate reverse-transcription step. Adenovirus species and serotypes were simultaneously detected using serotype-specific multiplex PCR. Norovirus genogroups GI and GII were detected by semi-nested PCR. The risk of infection associated with recreational and domestic use of the water was also estimated.

SUMMARY OF MAJOR FINDINGS

- BOD levels fell within the stipulated BOD guideline of 10 mg/ℓ for surface waters where full contact use is allowed and ≤ 30 mg/ℓ where public access is prohibited, restricted, or infrequent.

- For the Tyume River, DO concentrations generally ranged between 7.47 mg/ℓ and 10.42 mg/ℓ, while in the Buffalo River, the range was 6.88 mg/ℓ to 11.14 mg/ℓ. In unpolluted surface waters, dissolved oxygen concentrations are usually close to saturation. Typical saturation concentrations at sea level and at TDS values of below 3 000 mg/ℓ, are: 12.77 mg/ℓ at 5°C; 10.08 mg/ℓ at 15°C; 9.09 mg/ℓ at 20°C. The target water quality requirement which will protect all life stages of most Southern African aquatic biota endemic to, or adapted to, aerobic warm-water habitats for DO is 80% to 120% saturation. Taking into effect the observed temperature ranges in both water bodies for the duration of the study, the observed DO concentrations were within the target water quality requirements.

- The temperature regime for the Tyume River ranged between 6°C and 28°C and between 13.7°C and 27.9°C for the Buffalo River. For most sampling sites, the temperature regimes were within the acceptable limit of no risk (≤ 25°C) for domestic water uses in South Africa. While it is not possible to define a single cut-off point below which water temperatures are dangerous for recreational activities, unintentional exposure to cold water at temperatures of below 16°C can result in a debilitating shock response and hypothermia. However, this will vary according to the specific circumstances and physical condition of the person involved and the duration of their exposure.

- Electrical conductivity for the Tyume River generally ranged between 47 µS/cm and 408 µS/cm. In the Buffalo River, the lowest mean conductance values were consistently obtained at the Parkside

sampling site within the study period, except in June 2011 when the highest mean conductance (6 947 µS/cm) was obtained at Parkside. It should be pointed out that while electrical conductivity far exceeded the South African target water quality electrical conductivity guideline of 700 µS/cm at Parkside (Buffalo River) in winter, it generally fell within this value at all the other sites in both rivers. It was observed that, in both rivers, electrical conductivity increased as the rivers flowed through settlements.

- The pH of the Tyume River in the period beginning September 2010 through to January 2011 was consistently below pH 9, but from February 2011 to June 2011 the pH significantly increased to between pH 10 and pH 11 at most sampling sites. In the Buffalo River, mean monthly pH values ranged between pH 6.62 and pH 10.73. Unpolluted waters normally show a pH of about pH 6.5 and pH 8.5. Most of the pH values observed in this study lie between pH 8.5 and pH 10.8 levels. The upper level guideline for domestic use is pH 9.0.

- The turbidity of Tyume River water ranged between 6 NTU and 281 NTU while that of the Buffalo River ranged between 1.71 NTU and 132.7 NTU. For both rivers, turbidity exceeded the target water quality range (0 NTU to 1 NTU) of no risk for domestic water uses in South Africa.

- Monthly TDS values and the monthly EC values showed direct proportionality. For the Buffalo River, the highest TDS levels were obtained at the Parkside sampling site (range: 3 473 mg/ℓ to 23 350 mg/ℓ). TDS concentrations at all sites in both rivers except for Parkside (in Buffalo River) fell within the acceptable guideline of 0 mg/ℓ to 450 mg/ℓ of TDS for domestic use.

- *S*alinity of the Buffalo River showed a very wide range of values, from a mean value of 0.02 recorded at Maden Dam to 33.78 at Parkside. Salinity varied with sampling sites, increasing as the water flowed from its source in the Amathole Mountain range down to the East London river mouth. While salinity at five of the six sampling sites (Maden Dam, Rooikrantz Dam, King William's Town, Eluxolzweni and Bridle Drift Dam) fell within the freshwater range (< 0.5), salinity levels at Parkside were significantly higher and fell within the range for salt water.

- Chemical oxygen demand (COD) of Tyume River water was not determined. The COD of Buffalo River water samples generally ranged between 3.5 mg/ℓ and 45.9 mg/ℓ and increased down the river course in the following order: Maden Dam (23.19 mg/ℓ), Rooikrantz Dam (13.62 mg/ℓ), King William's Town (23.19 mg/ℓ), Eluxolzweni (24.98 mg/ℓ), Bridle Drift Dam (30.8 mg/ℓ) and Parkside (34.83 mg/ℓ).

- Nutrient profiles were as follows: nitrate (0.18 mg/ℓ to 4.21 mg/ℓ and 1 mg/ℓ to 4.5 mg/ℓ for Tyume River and Buffalo River, respectively); nitrite (0.02 mg/ℓ to 2.35 mg/ℓ and 0.02 mg/ℓ to 0.21 mg/ℓ for Tyume River and Buffalo River, respectively); and orthophosphate (0.06 mg/ℓ to 2.72 mg/ℓ and 0.01 mg/ℓ to 1.72 mg/ℓ for Tyume River and Buffalo River, respectively).

- The bacteriological qualities of the water in both rivers were poor, exceeding the guideline of 200 CFU/100 mℓ and 33 CFU/100 mℓ for faecal coliforms and enterococci, respectively, for recreational use.

- Faecal coliform counts also exceeded the 1 000 CFU/100 mℓ guideline for water used in fresh produce irrigation.

- Generally, higher counts of total coliforms, faecal coliforms and enterococci were recorded at the sampling sites located at the lower reaches of both rivers compared to the upper reaches.

- The prevalence and concentrations of adenovirus were as follows: 31% positive samples in concentrations ranging between 1.0 genome copies/ℓ and 8.49×10^4 genome copies/ℓ for the Tyume River and 35% positive samples in concentrations ranging between 1 genome copies/ℓ and 470 genome copies/ℓ for the Buffalo River.

- Tyume River samples were positive for species C adenovirus serotypes 1, 2, 6 and 7, and species F adenovirus serotype 41, while Buffalo River samples were positive for species B adenovirus serotype 21 and species F adenovirus serotypes 40 and 41.

- The prevalence of norovirus was 4% and 3% in Tyume River and Buffalo River samples, respectively.

- Rotavirus was detected in 4% of Tyume River samples in concentrations ranging between 9 genome copies/ℓ and 5.64×10^3 genome copies/ℓ while 14% of Buffalo River samples were positive in concentrations ranging between 3.0 genome copies/ℓ and 2.1×10^2 genome copies/ℓ.

- Hepatitis A virus was detected in 13% of Tyume River samples in concentrations ranging between1.67×10^3 and 1.64×10^4 genome copies/ℓ while in the Buffalo River the detection rate was 43% in concentrations ranging from 7 genome copies/ℓ and 1.4×10^5 genome copies/ℓ.

- Enteroviruses were not detected in Tyume River samples but 9.7% (7/72) of Buffalo River samples were found to be positive.

- Enteric viruses were inversely correlated with temperature.

- Both hepatitis A virus and adenovirus present significantly higher risk of infection compared to rotavirus in the case of ingestion of 10 mℓ (representing domestic use) or 100 mℓ (representing recreational use) of water from both rivers.

- Enteroviruses did not present any significant risk of infection in either of the rivers.

SUMMARY OF CONCLUSIONS REACHED

- The Tyume River, unlike the Buffalo River, is relatively clean with respect to physicochemical parameters.
- The bacteriological water quality of both rivers is poor.
- Most of the microbiological contamination observed in this study (especially Faecal Indicator Bacteria - FIBs) can be blamed on inadequate sanitary infrastructure in both catchments as we observed that open defecation is commonplace in these catchments, which also serve as recipients of effluent discharges from wastewater-treatment facilities and from other unidentified chemical industries.

- Enteric viruses were detected along courses of the rivers in a sporadic pattern, generally not related to natural hydrological cycles and so we conclude that the presence of enteric viruses in the rivers is suggestive of the dynamics in the host populations.
- Even though the proportion of infective viruses was estimated in this study, the fact remains that there is considerable risk of infection posed by the use of raw surface water for either domestic or recreational use.
- This study confirms the lack of correlation between faecal indicator bacteria and enteric virus occurrence in environmental waters, showing that assaying for enteric viruses in environmental waters remains the best method for determining the health risks associated with the use of faecally contaminated water.

RECOMMENDATIONS FOR FUTURE INTERVENTIONS

- Provision of adequate sanitation infrastructure, e.g. the introduction of the public toilet concept; and upgrading of the existing sanitary facilities to cater for the expanding population may go a long way in reducing microbiological contamination of our rivers.
- Educational campaigns to improve health literacy and to create an awareness of the dangers when using raw-water sources may also need to be conducted in communities who directly rely on surface waters for domestic uses. These campaigns should be aimed at reducing the risks of contracting waterborne illnesses.
- Recreational water managers may take steps to identify periods when water quality is poor and issue advisory notices warning the public of increased risk. Actions to protect public health may include permanently discouraging recreational activity in contaminated water, for example by fencing or signposting.

RECOMMENDATIONS FOR FUTURE RESEARCH

- It is recommended that future projects of this kind incorporate identification and enumeration of protozoan parasites.
- Faecal contamination of surface waters has been affirmed (presence of FIB); there is need for future research to focus on the assessment of these surface waters for the presence of bacterial pathogens. Microbial source tracking (MST) may also help to ascertain the origin of this faecal pollution, which data may be useful in pollution-mitigation measures.
- The presence of enteric viruses in surface waters located in Amathole District Municipality has been affirmed. Future research work in this field may include cell culture to verify/confirm the proportion of infectious viruses to total virus particles in environmental water samples.
- Since this project appears to be the first of its kind in the Eastern Cape Province and coupled with the interesting revelations from the study, there is a need to extend this investigation to determine the nature and extent of pollution in other rivers and recreational water bodies in the Province.
- Questionnaire surveys may also be conducted in communities within river catchments in the Eastern Cape Province so that risk-assessment profiling is aligned to water-use patterns specific for communities in those catchments.

CAPACITY-BUILDING

Real-time PCR appears to be a very viable and most sensitive option in assaying for viruses in environmental samples, compared to conventional PCR. As we have perfected the use of this facility at the University of Fort Hare, there is a need to enhance capacity in the use of this technology in microbial analyses in the Province. The following students are currently enrolled to study this particular field:

- BSc. Honours Microbiology:
  Mr Xolani Mndende (2010).
  Ms Onele Gcilitshana (2011)
  Ms Andisiwe Stuurman (2011)
- MSc. Microbiology:
  Ms Ntokozo N Mazibuko (2012)
  Mr Siyabulela S Gusha (2012)
- PhD. Microbiology:
  Ms S Koba (Inview)
  Mr T Sibanda (Inview)
  Mr VN Chigor (Inview)

KNOWLEDGE DISSEMINATION

Published paper (s)

No papers published to date

Paper (s) submitted for publication

No papers submitted to date

Conference presentation(s)

Timothy Sibanda, Siziwe Koba and Anthony I Okoh (2011) Assessment of the impact of human settlement on the microbiological and physicochemical qualities of Tyume River in Amathole District, Eastern Cape Province. SASM 2011 Congress, Cape Town from 6 to 9 November 2011.

Vincent Nnamdigadi Chigor and Anthony I Okoh (2011) Semi-nested reverse transcriptase-polymerase chain reaction (RT-PCR) detection of hepatitis A virus in Buffalo River, Eastern Cape Province, South Africa. SASM 2011 Congress, Cape Town from 6 to 9 November 2011.

Timothy Sibanda, Andisiwe Stuurman, Siziwe Koba and Anthony I Okoh (2012) Assessment of the bacteriological and physicochemical qualities of Tyume River in Amathole District, Eastern Cape Province. Water Institute of Southern Africa (WISA) Biennial Conference and Exhibition 2012, CTICC, Cape Town, South Africa, 6-10 May 2012.

Vincent N Chigor, Onele Gcilitshana and Anthony I Okoh (2012) Studies on the water quality of the Buffalo River catchment in the Eastern Cape Province of South Africa. Water Institute of Southern Africa (WISA) Biennial Conference and Exhibition 2012, CTICC, Cape Town, South Africa, 6-10 May 2012.

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The project team wishes to thank the following people for their contributions to the project.

Reference Group	Affiliation
Dr Kevin Murray	Water Research Commission (Chairperson)
Mrs Annatjie Moolman	Water Research Commission (Previous Chairperson)
Ms Eiman Karar	Water Research Commission (Previous Chairperson)
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Ms Noluvuko Mabusela	Amatola Water
Prof WOK Grabow	Private Consultant
Dr TG Downing	Nelson Mandela Metropolitan University

CONTENTS

LIST OF FIGURES

LIST OF TABLES

ACRONYMS

APHA	American Public Health Association
ATCC	American type culture collection
BOD	Biochemical oxygen demand
COD	Chemical oxygen demand
DEAT	Department of Environment Affairs and Tourism
DO	Dissolved oxygen
DWAF	Department of Water Affairs and Forestry
EC	Electrical conductivity
EnV	Enterovirus
EtBr	Ethidium bromide
FIB	Faecal indicator bacteria
HAdV	Human adenovirus
HAV	Hepatitis A Virus
HEV	Hepatitis E Virus
NoV	Norovirus
PCR	Polymerase chain reaction
NTU	Nephelometric turbidity units
qPCR	Quantitative real-time PCR
RHP	River Health Programme
RoV	Rotavirus
RT-PCR	Reverse transcription polymerase chain reaction
TDS	Total dissolved solids
WHO	World Health Organisation
WRC	Water Research Commission

CHAPTER 1: BACKGROUND

1.1 INTRODUCTION

Human enteric viruses are responsible for a large proportion (30% to 90%) of gastroenteritis cases (Van Heerden et al., 2005b) and these viruses have been detected in water sources worldwide (Pina et al., 2001; Pusch et al., 2005; De Paula et al., 2007; Kiulia et al., 2010). A literature search revealed that aquatic virology research in South Africa was pioneered by one of the seminal names in that field, WOK Grabow and his colleagues in the Gauteng Province (Grabow et al., 1983; Grabow et al., 1996; Grabow, 2001; Taylor et al., 2001; Van Heerden et al., 2003; Grabow, 2007; Venter et al., 2007). There are reports of similar studies in a few other provinces including the Western Cape and Limpopo Provinces (Van Heerden et al., 2005a; Van Zyl et al., 2006), but no record exists of similar investigations in the Eastern Cape Province.

Recent studies have shown that bacteria cannot be used as indicators of viral particles in water (Jurzik et al., 2010; Okoh et al., 2010); hence, surveillance of source waters for viral pathogens is necessary to protect public health. The cell-culture propagation procedure is still the best method to enumerate viruses and to demonstrate their infectivity. However, this procedure is unsuitable for the detection of hepatitis A virus and other enteric viruses, as appropriate cell cultures are not available or their growth is limited (Schvoerer et al., 2000). Molecular methods have been successfully applied in assessment of environmental samples, allowing a rapid and specific detection of human enteric viruses (De Paula et al., 2007; Costafreda et al., 2006; Bosch et al., 2008), and these methods form the backbone of the virological assessment component of this study.

This study was motivated by the absence of reports on the virological quality of water sources in the Eastern Cape Province, and the paucity of information on the microbiological quality of the rivers selected for this study. The aims of the study are articulated below.

1.2 PROJECT AIMS

The aims of this project were as follows:

1. To do an overview of the network of rivers and dams in the Amathole District Municipality, and to carry out a reconnaissance visit in order to select the rivers and dams to be studied and to determine the sampling sites, as well as to develop analysis protocols.
2. To assess the prevalence and distribution of human viral pathogens in the selected rivers and dams in the study area.
3. To assess the occurrence and distribution of faecal indicator bacteria in the selected rivers and dams in the study area.
4. To determine the physicochemical qualities of the selected rivers and dams in the study area.

5. To correlate the occurrence of viral and bacterial pathogens with the physicochemical qualities of the different freshwater bodies.
6. To assess the fitness-for-use of the water for the intended use for recreational, domestic, and agricultural purposes, as well as to determine the risk of infection and burden of disease associated with consumption of the water.

1.3 CHANGES TO ORIGINAL WORKPLAN

The original aims of the study were to:

1. Do an overview of the network of rivers and dams in the Amathole District Municipality, and to carry out a reconnaissance visit in order to select the rivers and dams to be studied in typical urban and rural communities of the municipality; to determine the sampling sites; and to develop analysis protocols.
2. Assess the prevalence and distribution of human viral pathogens in the selected rivers and dams in the study area.
3. Assess the occurrence and distribution of coliphages and faecal indicator bacteria in the selected rivers and dams in the study area.
4. Determine the physicochemical qualities of the selected rivers and dams in the study area.
5. Correlate the occurrence of viral and bacterial pathogens with the physicochemical qualities of the different freshwater bodies.
6. Assess the fitness-for-use of the water for the intended use for recreational, domestic, and agricultural purposes, and the implications of the water quality for water regulatory agencies, especially from the perspective of predictive models for the control of disease epidemics.

The Reference Group recommended a revision of the original aims along with the incorporation of the real-time PCR technique being more sensitive to the conventional PCR that was initially proposed for the detection of viruses. This real-time PCR has the added advantage of being able to quantify viral genomes in water. Also, due to the delayed release of funds which led to some logistics problems, coupled with the impossibility of preservation of samples for coliphage analyses, the project Reference Group suggested that the coliphage component of this study be dropped so as not to jeopardise the overall progress of the project.

Though the Reference Group had recommended the inclusion of hepatitis E virus (HEV) as an emerging pathogen in addition to other target viruses, HEV detection was dropped because the positive control virus which is needed for the qPCR assay could not be obtained from the ATCC or any other source. Also, though the Reference Group had recommended the inclusion of Microbial Source Tracking (MST), this technique had to be dropped for the reason that, although we acknowledge that viruses found in the environment could (with the exclusion of hepatitis A virus) be from a wide array of hosts, the TaqMan® assays that were employed in this study were for the detection of human enteric viruses including adenovirus, rotavirus and enterovirus. From the design of this work therefore, only clinically important enteric viruses had to be targeted. The following primer sets, available in the literature, were used: Jothikumar et al. (2009) for the detection of rotavirus; Costafreda et al. (2006) for the detection of hepatitis A virus; Jothikumar et al. (2005)

for adenovirus detection; and Gregory et al. (2006) for the detection of enteroviruses. To prove the specificity of the primer sets for the detection of human viral particles, bovine enterovirus type 1 (ATTC VR-248) was included as a control in the qPCR assay for enteroviruses and no cross-reactivity was observed. Another motivation for dropping MST was the limitation we encountered in procuring virus control strains and hosts required for MST analyses. Although we also proposed microbial source tracking using the multiple antibiotic resistance (MAR) approach, there is no existing library for such in this study area. For these reasons, along with the fact that both HEV and MST analyses are not in the original aims of the project, these approaches were eventually dropped from this study.

In addition, the Reference Group agreed that the study and sampling sites as proposed were way too many, and would involve time-consuming sampling and analyses which might require considerably more funding than had been anticipated by the proposer. Hence, the Reference Group recommended that they be significantly narrowed down. Motivations were made in support of Buffalo and Tyume River catchments in our revised study plan and these were accepted for the study. The Reference Group also considered one of the other original aims of the study, namely to formulate a predictive model, and noted that time and human resources would not allow it to be done and as such it should also be dropped. Motivation was made to replace this aim with an assessment of the fitness-for-use of the water for the intended use for recreational, domestic, and agricultural purposes, as well as to determine the risk of infection and burden of disease associated with consumption of the water, and this was accepted.

CHAPTER 2: LITERATURE REVIEW

Providing access to potable water and sanitation is an essential element for the effective functioning of human settlements, and is integral to human health and well-being. The contamination of water, therefore, remains a problem of global concern contributing to high morbidity and mortality from waterborne and food-borne diseases, such as typhoid fever, cholera and other diarrhoeal diseases (Pruss et al., 2002; WHO, 2009). A report from the American Academy of Microbiology on the global burden of gastrointestinal diseases (Payment and Riley, 2002) revealed that an estimated 6.6 billion cases of gastrointestinal illness occur annually. The World Health Organization (WHO) declared that diarrhoeal diseases alone contribute to an estimated 4.1% of the total DALY (disability-adjusted life years) global burden of disease and is responsible for the deaths of 1.8 million people every year. It was estimated that 88% of that burden is attributable to unsafe water supply, sanitation and hygiene and it is mostly concentrated in children in developing countries. A significant proportion of diseases could be prevented especially in developing countries through better access to safe water supply, adequate sanitation facilities and better hygiene practices (Bosch et al., 2008).

Although the recently published World Health Statistics 2009 report shows that 93% of South Africans (as opposed to 100% in Mauritius and Western Europe, and 99% in the United States of America) have access to improved drinking-water sources, only 59% have access to improved sanitation (WHO, 2009). Lack of safe water and poor sanitation are important risk factors for mortality and morbidity, including diarrhoeal diseases, especially in the developing world (Pruss et al., 2002; WHO, 2009). A recent report by the WHO/UNICEF Joint Monitoring Programme for Water Supply and Sanitation shows that 884 million people in the world still do not receive their drinking-water from improved sources; almost all of them in developing regions. Sub-Saharan Africa accounts for over a third of that number. It further revealed that 7 out of 10 people without improved sanitation live in rural areas, and that worldwide, 37% of people not using improved sources of drinking water live in sub-Saharan Africa (WHO/UNICEF, 2010).

According to the South African Department of Environmental Affairs and Tourism (DEAT), the lowest percentages of South African households with access to piped water occur in rural areas of the Eastern Cape and KwaZulu-Natal (DEAT, 2010). Consequently, the health risk is higher in rural areas than in urban areas (Obi et al., 2006), due to the relatively poorer qualities of water in the former. The resulting impact on primary health is significant with diarrhoea being responsible for some 25% of all deaths in the 1-to-5-year age group and an annual estimated 43 000 deaths and 3 million incidences of illness (DEAT, 2010). According to 2008 statistics, more than 40% of the South African population live in rural areas (DWA, 2010).

2.1 VULNERABILITY OF SURFACE WATER

Surface waters, including dams, rivers and streams, constitute an important source of water for drinking, domestic, agricultural, recreational and other purposes. In the Eastern Cape Province, beaches and rivers are, additionally, unique economic resources in the Province. However, they are frequently contaminated

with faecal materials. Non-point sources of such contamination include domestic and wild animal defecation, malfunctioning sewage and septic systems, stormwater drainage and urban runoffs. Point sources include industrial effluents and municipal wastewater treatment plants (Shuval, 1990; Kistemann et al., 2002; Okoh et al., 2007; Igbinosa and Okoh, 2009; Lata et al., 2009).

The vulnerability of surface-water bodies to pollution in South Africa (as in other developing countries) is high, with decomposable organic matter and pathogenic agents as well as the use of raw/treated wastewater for irrigation constituting serious public health risks (Shuval, 1990; Chalmers et al., 2000; Solomon et al., 2002; Obi et al., 2004; Okoh et al., 2007; Igbinosa and Okoh, 2009; Chigor et al., 2010). Pathogenic agents (including bacteria, protozoa, helminth eggs, viruses and fungi) can render water contaminated and non-potable, and could result in the transmission of water-related diseases to swimmers, agricultural workers and consumers of crops irrigated with polluted waters (Shuval, 1990; Mohanty et al., 2002). Typical examples of bacterial waterborne pathogens include *Escherichia coli*, *Salmonella, Shigella* and *Vibrio* species. Also, *Giardia* and *Cryptosporidium* are protozoan pathogens that are found in aquatic environments, especially in freshwaters. Both parasites usually form a protective oocyst inside the intestines of the host animal which also allows them to survive in the environment until ingested by a host (KDHA, 1997). Examples of illnesses caused by other protozoan parasites include amoebiasis, cyclosporiasis and microsporidiosis caused by *Entamoeba histolytica, Cyclospora cayetanensis* and the microsporidia, respectively. Pathogenic helminths include *Diphyllobothrium* genus*, Dipylidium caninum, Echinococcus* genus*, Hymenolepis diminuta, Taenia* species*, Necator americanus* (hookworm) and *Dracunculus medinensis*.

The use of polluted surface water for domestic, agricultural and recreational purposes by large populations in developing nations is a major cause of diarrhoeal-disease-related mortality, and in rural areas devoid of electricity and potable water, the impact is more profound. This is heightened in the Eastern Cape Province by the high number of people whose immune systems are compromised by HIV/AIDS (Bourne and Coetzee, 1996; Obi et al., 2006). Available statistics (based on Census 2001 information) shows that of the 7.3 million Eastern Cape population, only 13.6% have access to piped water either in their dwelling places or within 200 m of their dwelling places (Municipal Demarcation Board, 2009). Diarrhoea is responsible for some 25% of all deaths in the 1-to-5-year age group and an annual estimated 43 000 deaths and 3 million incidences of illness in South Africa (DEAT, 2010).

2.2 PHYSICOCHEMICAL QUALITIES OF SURFACE WATER

Though microbiological parameters are usually the most important in determining the safety of potable water, it is necessary to measure several different physical and chemical properties in order to understand the true nature and pollution level of various water samples. Tebbut (1992) listed many physicochemical characteristics to be analysed for different waters (river water, drinking water, raw sewage and sewage effluent) including: pH, temperature, odour, radioactivity, electrical conductivity, total dissolved solids, turbidity, chloride, phosphate, nitrate-nitrogen and biochemical oxygen demand. Sudden changes in any of these parameters may be indicative of changing conditions in the water (Zamxaka et al., 2004).

Hence, in order to have maximum benefit from our rivers and dams, knowledge of their physicochemical characteristics becomes imperative (Kramer and Botterweg, 1991).

2.3 VIRUSES AS INDICATORS IN MICROBIAL WATER-QUALITY ASSESSMENT

Indicator microorganisms (total and faecal coliforms, *Escherichia coli*, *Enterococcus* spp., *Clostridium perfringens, Bifidobacterium*) have been used extensively for many years as indicators for determining the sanitary quality of surface, recreational, and shellfish-growing waters and are used to predict the presence of and the potential risk associated with pathogenic microbes (*Standard Methods*, 2005; Byamukama et al., 2000; Grabow, 2001; Scott et al., 2002; Jiang et al., 2007; Bosch et al., 2008; Abdelzaher et al., 2010). Numerous epidemiological studies of waterborne illnesses in developed countries indicate that the common aetiological agents are more likely to be viruses and parasitic protozoa than bacteria (Levy et al., 1998). Viral pathogens have been shown to be responsible for a significant proportion of the cases of acute gastroenteritis, particularly sporadic cases in children and outbreaks in all age groups (Fritzinger et al., 2011). The presence of even a few viral particles in a large volume of drinking water and/or in-contact recreational water poses a threat to public health (WHO, 1995). However, given that traditional bacterial indicators have been shown to be non-specific for viral pathogens, direct surveillance of these pathogens may be needed to better protect public health (Bosch, 1998; Grabow, 2001; Desmarais et al., 2002; Bosch et al., 2008).

In his review of human enteric viruses in water, Albert Bosch (1998) listed the following as requirements that a good indicator should fulfil:

- It should be associated with the source of the pathogen and should be absent in unpolluted areas
- It should occur in greater numbers than the pathogen
- It should not multiply outside the host
- It should be at least equally resistant to natural and artificial inactivation as the viral pathogen
- It should be detectable by means of easy, rapid and inexpensive procedures
- It should not be pathogenic

Bosch then concluded that, obviously, the ‘ideal’ indication is provided by the viral pathogen itself (Bosch, 1998).

2.4 DETECTION OF VIRUSES IN WATER

Standard methods for the detection of infectious viruses in water require the use of susceptible cell lines within which the viruses can propagate and produce cytopathic effects (CPE) observable under a light microscope (Dahling, 1991). In what was then the most comprehensive investigation of viruses in a natural water environment carried out in South Africa, Grabow et al. (1996) studied the quality of diffuse-source effluents from informal settlements with restricted sanitary services, namely Botshabelo near Bloemfontein, and Stanza Bopape Village in Mamelodi, Pretoria. They reported the isolation, by conventional cell-culture propagation, of viruses (mostly coxsackie B viruses) from 70% of 210 samples downstream of Stanza Bopape Village and from 95% of Mamelodi sewage samples. However, in the same report, their study of

Modder River and diffuse effluents from informal settlements with restricted sanitary services in parts of Bloemfontein and Pretoria resulted in no viruses detected in representative samples from effluents and river water. The Modder River feeds the Mockes Dam, a raw water source for the City of Bloemfontein and various other communities. However, cell culture can be labour-intensive and host cells for many viruses such as the noroviruses have not been identified (Griffin et al., 2003), and so they cannot be grown in conventional cell culture (Rodriguez et al., 2009).

Progress has been made through development of useful molecular techniques for rapid detection and quantitation of viruses. Polymerase chain reaction (PCR)-based methods have been successfully used to monitor water and food products for viral contamination (Choi and Jiang, 2005; Fong and Lipp, 2005). During PCR, a fragment of the viral genome is amplified using specific primers. Reverse transcription PCR (RT-PCR) is applied for the detection of RNA viruses. Here, reverse transcription of the viral RNA to a copy DNA strand (cDNA) is necessary prior to the PCR. During reverse transcription, a primer is necessary for the reverse transcriptase (RNA-dependent DNA polymerase) to initiate the synthesis of a cDNA from the RNA. Three types of primers are commonly used: random primers, polythymine primers, and specific primers. Random primers are short single-stranded DNA fragments with all possible combinations of bases. They will work as short non-specific primers, and by using them, the RT reaction will non-specifically produce cDNAs from the RNA present in the assay mixture (Rodriguez et al., 2009). Real-time quantitative PCR (qPCR) is a type of PCR used to semi-quantitatively determine the amount of original target present in the sample. Real-time PCR is an excellent tool for environmental virology and has been used successfully to determine the concentrations of viral genomes in the environment (Gersberg et al., 2006; He and Jiang, 2005). Multiplex PCR, which utilises multiple primer sets within a single PCR, can be used to simultaneously detect different groups of viruses (Brittain-Long et al., 2008). Molecular detection of viral pathogens is rapid and highly specific and sensitive.

2.5 TARGET ENTERIC VIRUSES IN THIS STUDY

Over 140 types of pathogenic viruses are excreted in human and animal wastes and are potentially found in both surface water and groundwater sources (Fong and Lipp, 2005). Human enteric viruses are the major cause of water-related diseases and have been estimated to cause about 30% to 90% of gastroenteritis cases worldwide (Bosch et al., 2008). Although enteric virus infections are associated primarily with diarrhoea and gastroenteritis in humans, they may also cause respiratory infections, conjunctivitis, hepatitis, and diseases that have high mortality rates, such as aseptic meningitis, encephalitis, and paralysis in immunocompromised individuals (Fong and Lipp, 2005). In addition, some enteric viruses have been linked to chronic diseases such as myocarditis and insulin-dependent diabetes. Enteric virus infections in animals such as cattle and swine are normally asymptomatic but can lead to abortion, neurological disorders, and mortality (Fong and Lipp, 2005).

Enteric viruses represent diverse and commonly studied groups of viruses belonging to the families Picornaviridae (polioviruses, enteroviruses, coxsackieviruses, hepatitis A virus (HAV) and echoviruses), Adenoviridae (adenoviruses), Caliciviridae (noroviruses and sapoviruses), Astroviridae (astroviruses) and Reoviridae (rotaviruses). This group of viruses is considered to be emerging waterborne pathogens based

on their cellular and molecular structures that make them resistant to current water-treatment processes (Rigotto et al., 2009). These viruses can be classified into two types: those that multiply in the intestinal epithelium and cause gastroenteritis, such as rotaviruses, and those that first multiply in the intestine, and then spread to extra-intestinal target organs where they cause diverse diseases such as meningitis, encephalitis, myocarditis and diabetes, e.g. poliovirus, the agent of paralytic poliomyelitis (Colbere-Garapin et al., 2007).

Enteroviruses (EnVs) include poliovirus, coxsackieviruses, echoviruses and the numbered enteroviruses (Katayama et al., 2002). They belong to the viral family Picornaviridae and are small (20 nm to 30 nm) non-enveloped, single-stranded RNA viruses with an icosahedral capsid structure ranging from 20 nm to 30 nm in diameter. About 70% (62 serotypes) of non-poliovirus enteroviruses have been associated with human infections, and 30% have been associated with animal infections (Fong and Lipp, 2005). EnVs are associated with a broad spectrum of clinical features including acute respiratory illness, aseptic meningitis, meningoencephalitis, myocarditis, hand, foot and mouth disease, neonatal multi-organ failure and acute flaccid paralysis (Caro et al., 2001) and, evidence is growing that EnVs may cause common chronic diseases, including dilated cardiomyopathy, insulin-dependent diabetes mellitus and chronic fatigue syndrome (Muir et al., 1998).

Hepatitis A is endemic in South Africa (Venter et al., 2007). The etiologic agent of the disease - Hepatitis A Virus (HAV) is a 27 to 32-nm non-enveloped, small, single-stranded RNA virus belonging to the family Picornaviridae. It is the only member of the *Hepatovirus* genus. Waterborne outbreaks of hepatitis A disease have been reported worldwide (Tallon et al., 2008; Griffin et al., 1999; Pina et al., 2001; Taylor et al., 2001; Grabow et al., 2001; Gersberg et al., 2006) and associated with contaminated water supply in various countries. Among the agents of gastrointestinal infections that are carried by water, HAV can be considered important because of its prevalence (Fernandez-Molina et al., 2004).

The genus norovirus (NoV) is in the family Caliciviridae (non-enveloped, single-stranded RNA viruses, 27 nm to 32 nm in diameter) and has been found in humans, pigs, cattle, sheep and mice (Hardy, 2005; Wolf et al., 2010). Currently, NoVs have been classified into 5 genogroups (GI through GV) of which the human noroviruses belong to Genogroups I, II, and IV, which are further divided into many genotypes (NoV GI Genotypes 1 to 14 [GI.1-14] and GII.1-17) (Ando et al., 2000; Iwai et al., 2009). Human NoV (HuNoV) is the most common etiological agent for gastroenteritis outbreaks as well as the leading cause of non-bacterial gastroenteritis in children and has a significant public-health impact globally (Hot et al., 2003; Pang et al., 2005; Siebenga et al., 2009; Gentry et al., 2009). HuNoV (GI and GII) have been detected in both freshwaters and estuarine waters worldwide. Aw and Gin (2010) worked in the Far East (Singapore) and reported the detection both NoV genogroups (GI and GII) in 100% of the sewage and secondary effluents. Lee and Kim (2008), reported on the genetic diversity of HuNoV detected in river water in Korea. Numerous studies have also detected NoVs in environmental waters in Europe (Lodder and Husman, 2005; Pusch et al., 2005; La Rosa et al., 2007; Lysen et al., 2009), in the United States of America (Gentry et al., 2009) and in South America (Victoria et al., 2010a). A recent report on the detection of enteric viruses in selected urban and rural river water and sewage in Kenya (Kiulia et al., 2010) revealed that NoV GI and GII were detected in 9 (90%) of samples collected from urban rivers and streams. Also, from 12 samples collected from a rural

river, they detected NoV GI in 1 (8.3%) and GII in 3 (25%). Though the first documented NoV outbreaks in South Africa were described as early as 1993, the current NoV prevalence and circulating genotypes are unknown, and there is also a lack of NoV outbreak reporting systems (Mans et al., 2010). Today, despite recent outbreaks, there appears to be no such report on the occurrence of NoVs in South African water environments.

Rotaviruses (RoVs) are non-enveloped, double-stranded RNA viruses approximately 70 nm in diameter, belonging to the family Reoviridae. The RoV particle consists of 11 segments of double-stranded RNA (dsRNA) genome enclosed in a double-shelled capsid. The outer shell is composed of a major glycoprotein with a molecular weight of 34 000 (vp7) and a minor, trypsin-sensitive protein with a molecular weight of 84 000 (vp4) (Gouvea et al., 1990). These viruses have been divided into six serological groups, three of which (Groups A, B and C) infect humans (Meleg et al., 2008). Group A rotaviruses are predominant and result in severe diarrhoeal diseases in infants and young children. Group A rotaviruses can be classified on the basis of their viral protein 4-associated P type and viral protein 7 (VP7)-associated G type, of which G types G1, G2, G3, G4, G8, and G9 are most commonly detected (Van Zyl et al., 2006). Group B rotaviruses cause adult diarrhoea and are reportedly geographically confined, having been first identified in a large waterborne epidemic in China (Castello et al., 2006). Group C rotaviruses on the other hand, are an emerging cause of gastroenteritis in children over 2 years old and in adults which have now been identified as causative agents of gastroenteritis in both sporadic cases and outbreaks worldwide (Rahman et al., 2005; Meleg et al., 2008). Human rotaviruses are remarkably stable in environmental water (Pancorbo et al., 1987; Ward et al., 1986), and resistant to physicochemical treatment processes in sewage treatment plants, which facilitates their transmission (Rao et al., 1988).

Among human viral pathogens, adenovirus is the only DNA virus in the enteric virus family, hence, the most thermostable virus, and can survive for prolonged periods in environmental waters. Adenoviruses are members of the Adenoviridae family. Members of this family include 70 nm to 100 nm non-enveloped icosahedral viruses. At present, there are 51 serotypes of adenoviruses; about 30% of these are pathogenic in humans, most causing upper respiratory tract infections. The serotypes are classified into six species, designated species A to F (He and Jiang, 2005; Fong and Lipp, 2005). Species F contains two fastidious enteric serotypes, 40 and 41, which are among the leading causes of childhood diarrhoea, although older children and adults may also be infected (Logan et al., 2006). Human adenoviruses (HAdV) are a major cause of clinical infections including gastroenteritis, conjunctivitis and respiratory diseases (Van Heerden et al., 2003) and are the second most important viral pathogens of infantile gastroenteritis after rotavirus (Fong et al., 2009). The role water plays in the epidemiology of HAdV, as well as the potential health risks constituted by these viruses in water environments, are widely recognised (Enriquez et al., 1995; Puig et al., 1994).

2.6 SURFACE-WATER QUALITY ASSESSMENTS IN SOUTH AFRICA

The objective of the National Water Act, 1998 (Act No. 36 of 1998) is to ensure that South Africa's water resources are protected, used, developed, conserved, managed and controlled in a sustainable and equitable manner, for the benefit of all persons (DWA, 2010). The pollution levels and microbiological

contamination of some South African surface waters and wastewater-treatment plants have been studied (Jagals, 1997; Jagals et al., 2000; Muller et al., 2001; Obi et al., 2002; Obi et al., 2004; Igbinosa and Okoh, 2009; Paulse et al., 2009; Omar and Barnard, 2010). Obi et al. (2002) assessed the microbial quality of several untreated surface-water sources (including Levubu River, Vuwani, Mutale, Ngwedi, Tshinane, Makonde, Mutshindudi and Mudaswali Rivers.), used by rural communities in the Venda region of South Africa, to determine their safety for human consumption and to highlight the possible occurrence of waterborne diseases. They concluded that those untreated water sources pose a serious threat to the health of the consumers. In another study, Diergaardt et al. (2004) reported the occurrence of *Campylobacter* spp. in drinking-water and environmental water sources (4 groundwater, 11 surface-water and 4 raw sewage sources) and stressed the need to develop a more efficient method for the isolation of all pathogenic *Campylobacter* and *Arcobacter* species from environmental waters.

In a recent study, Igbinosa and Okoh (2009) assessed the qualities of the treated final effluents of a wastewater treatment plant located in a rural community of the Eastern Cape Province of South Africa over a period of 12 months. Their study generally revealed unacceptably poor effluent qualities. In a similar report, Odjadjare and Okoh (2010) assessed the prevalence of free-living and plankton-associated *Listeria* species in the final effluents of a South African wastewater treatment facility and its receiving catchment between August 2007 and July 2008 and concluded that final effluents of wastewater treatment plants are potential sources of pathogens in water sources in South Africa. Yet surface waters remain key sources of water for drinking, agricultural and recreational purposes. A recent survey involving 181 water-treatment plants across 7 (out of 9) provinces of South Africa, namely Mpumalanga, Limpopo, North West, Free State, KwaZulu-Natal, Eastern Cape and Western Cape Provinces, was undertaken to identify the challenges facing small water-treatment plants (SWTPs) in South Africa. The SWTPs abstracted their raw water from either surface water or groundwater or a combination of both, with greater preponderance for surface-water sources (over 86%) (Momba et al., 2009). According to the report, only 40% of the plants met the recommended target range of 0.3 mg/ℓ to 0.6 mg/ℓ free chlorine residual concentrations at the point of use.

These published data sets have generally investigated only a limited number of locations. Furthermore only a few studies included viruses as targets. Human enteric viruses have been associated with waterborne disease outbreaks and are detected in water sources globally (Bosch, 1998; Ramachandran et al., 1998; Adah et al., 2001; Fong and Lipp, 2005; Lodder and Husman, 2005; Rahman et al., 2005; Castello et al., 2006; Hewitt et al., 2007; Aw et al., 2009; Cunliffe et al., 2009). Iwai et al. (2009) reported that during the winter seasons of 2006 to 2008, a large number of sporadic gastroenteritis outbreaks and many outbreaks caused by norovirus (NoV) GII occurred among inhabitants in Toyama, Japan. They observed that NoV strains of the same genotypes in both raw sewage and human specimens belonged to the same cluster by phylogenetic analysis and had almost identical nucleotide sequences among each genotype. Their data suggest that NoVs detected in raw sewage reflect the viruses circulating in the community.

In South Africa, Grabow (1996) reported the isolation of coxsackie B virus, reovirus, adenovirus, poliovirus and echovirus from water sources in South Africa. In another study, Van Zyl et al. (2006) reported the detection of Group A rotaviruses in 11.8% of partially treated and 1.7% of finally treated drinking water samples and in 14% of irrigation water samples and 1.7% of corresponding raw vegetable samples. Type-

specific reverse transcriptase-PCR and sequence analysis revealed the presence of multiple types (G1, G2, G8, and G9) in irrigation water and single types (G1 or G3) in raw water and treated drinking water. The similarity of environmental types to those in patients with clinical rotavirus infections confirms the value of wastewater screening as a tool for assessing RoVs circulating in communities, with the benefit of detecting types that cause both clinical and subclinical infections**.** Another study reported the detection of poliovirus vaccine strains in sewage and river water in South Africa (Pavlov, 2006).

In their report on molecular characterisation of astroviruses by reverse transcriptase PCR and sequence analysis, Nadan et al. (2003) compared clinical and environmental isolates from South Africa. Phylogenetic analysis demonstrated that human astrovirus serotypes, (HAstV)-1, -3, -5, and -8, were found in human stool and sewage samples, clustered together, indicating that these viruses are closely related. The concurrent presence of identical HAstV strains in wastewater samples and in hospitalised patients suggests that AstVs present in the environment pose a potential risk to communities using faecally contaminated water for recreational and domestic purposes. In an earlier study in which the investigators reported the occurrence of hepatitis A and astroviruses in selected river and dam waters here in South Africa, a seasonal pattern was observed for HAV but not for HAstV. They detected infectious viruses in dam-water samples where microbiological indicators of faecal pollution were absent or within acceptable limits and warned that the presence of these viruses in the dam and river water could pose a potential health risk for people using these waters for domestic or recreational purposes (Taylor et al., 2001). This risk has not abated. Today, hepatitis A is endemic in South Africa. Venter and his colleagues published the report of a study that aimed to assess the potential risk of infection constituted by HAV to persons using surface dam and river water for domestic and recreational purposes (Venter et al., 2007). It estimated the potential risk using a deterministic exponential risk assessment model with mean values and conservative assumptions. Hepatitis A virus was detected in 17.5% of river-water samples and 14.9% of dam-water samples tested and the number of indicator organisms in these sources exceeded drinking and recreational water quality guidelines set by the United States Environmental Protection Agency (US EPA), indicating possible health risks to recreational water users. In a similar report, Van Heerden et al. (2005b; c) assessed the risk of infection of human adenoviruses (HAd) detected in a survey of swimming pool water from two indoor pools and one outdoor pool over a period of 1 year and concluded that the risk of HAd infections calculated for the swimming pool water under investigation exceeded acceptable risk limits.

CHAPTER 3: DESCRIPTION OF STUDY AREA

3.1 INTRODUCTION

The study areas include Tyume River and Buffalo River catchments both located in the Eastern Cape Province. Detailed descriptions of these study areas are as articulated below.

3.2 TYUME RIVER CATCHMENT

The Tyume River is located in the Nkonkobe local municipality, under the Amathole District Municipality, in the Eastern Cape Province, South Africa. It flows from the upper part of the Amathole Mountains in Hogsback, passing through the lower coastal escarpment down to Alice through several rural settlements and finally joins the Keiskamma River at Manqulweni community. Close proximity of the river to its host communities makes it ideal for utilisation for domestic activities where piped potable water is not available. The Tyume River also feeds the Binfield Park Dam which serves as source of raw water for several water treatment plants in the area where water is treated and reticulated to Alice and surrounding rural settlements. The sampling sites for the Tyume River catchments include Hala, Khayalethu, Sinakanaka, Alice, Drayini and Manqulweni communities. Figure 3.1 shows the Tyume River catchment map.

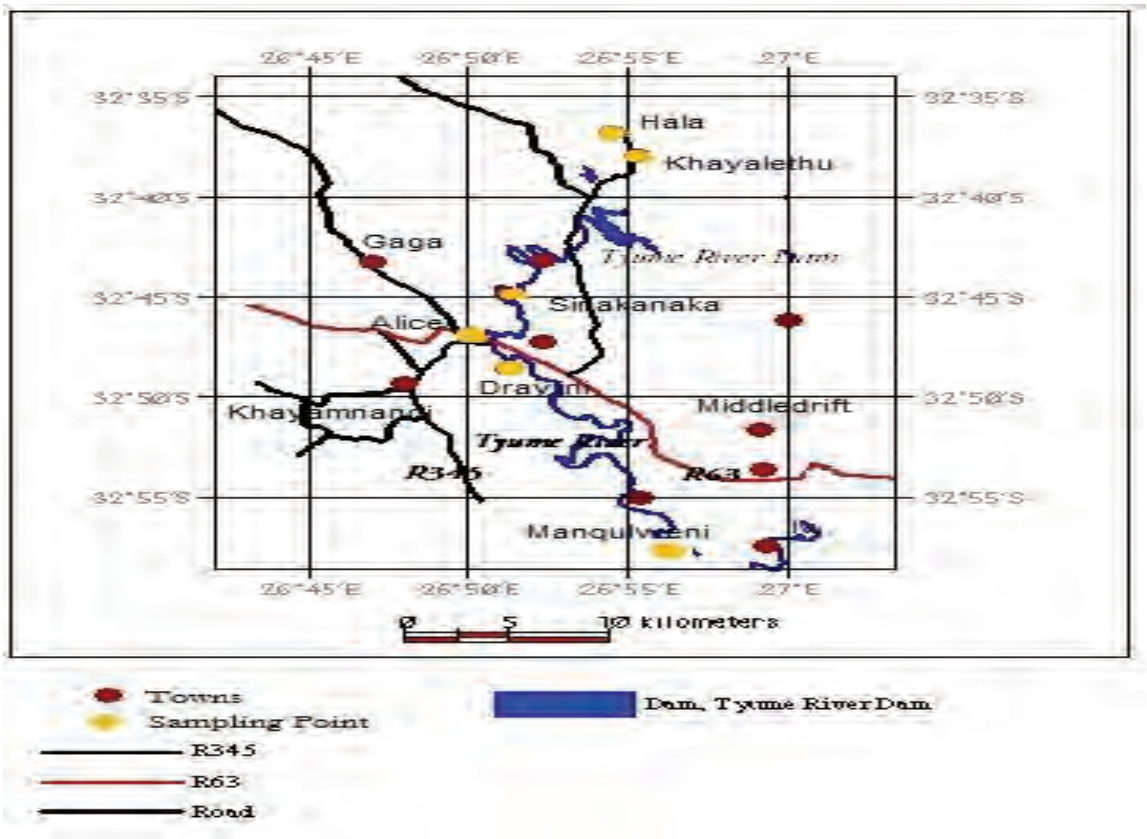

Figure 3.1: Map showing Tyume River catchment

Hala

Hala (Figure 3.2) is a community immediately downstream from the source of the Tyume River in Hogsback. With the river source at Hogsback considered 'pristine' and inaccessible, a sampling point in this community located at the geographical coordinates 32°36'39"S and 26°54'34"E was chosen as the first sampling site.

Figure 3.2: Map showing the Hala site catchment

Khayalethu

Khayalethu (Figure 3.3) is located at the geographical coordinates 32°38'22"S and 26°56'10"E in this major rural community, upstream from the Binfield Park Dam. The inhabitants of this settlement use the river water for irrigation, recreation, stock watering and domestic purposes.

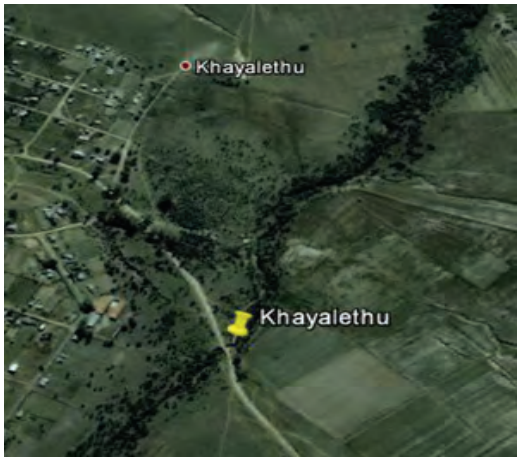

Figure 3.3: Map showing the Khayalethu site catchment

Sinakanaka

Sinakanaka (Figure 3.4) is a rural town on the banks of the Tyume River further downstream from Khayalethu, and comprises several densely populated settlements. The sampling site in this community is located at the geographical coordinates 32°45'37"S and 26°51'27"E. The Tyume River is very important to the inhabitants (humans and animals) of this town as it is used for drinking, fishing, irrigation, recreation and other domestic purposes.

Figure 3.4: Map showing the Sinakanaka site catchment

Alice

Alice (Figure 3.5) has several suburbs which include a golf course to the north-west; Happy Rest to the west; and Gaga, Gqumashe and Ntselamantsi to the north. Adding to the population of Alice is the student population at the University of Fort Hare to the east, which alone has a population of over 6 000.

Figure 3.5: Map showing the Alice site catchment

The sampling site is located near a bridge on the R63 near the University of Fort Hare at geographical coordinates 32°47'17"S and 26°50'31"E. The river is extensively used for irrigation, fishing and domestic purposes, as well as a source of drinking water for livestock.

Drayini

Drayini (Figure 3.6) is a rural town further downstream from Alice on the banks of the Tyume River. The sampling site at geographical coordinates 32°48'37"S and 26°52'20"E is located in Drayini just past Fort Hare farmlands and Alice. Its water appears highly turbid with green aquatic plants covering its surface. The river serves as drinking-water source for domestic animals.

Figure 3.6: Map showing the Drayini site catchment

Manqulweni

Manqulweni (Figure 3.7) is located further downstream from Drayini on the Tyume River at the confluence of the Keiskamma River and the Tyume River at geographical coordinates 32°54'50"S and 026°56'13"E.

Figure 3.7: Map showing the Manqulweni site catchment

3.3 BUFFALO RIVER CATCHMENT

The Buffalo River is located in the Eastern Cape Province of South Africa. It has its source in the Amathola Mountain range and empties into the Indian Ocean at East London. Along the Buffalo River there are four dams supplying water to the urban areas of King William's Town, Zwelitsha, Mdantsane and East London. In the catchments, blockages in the sewerage systems, inadequate treatment capacity and poor management result in the discharge of partially treated and untreated sewage into the river and dams. Industrial effluents are either inadequately treated or not treated at all (RHP, 2004). Six sites along the route of the Buffalo River were selected as sampling points for this study and these include Maden Dam, Rooikrantz Dam, King William's Town, Eluxozweni, Bridle Drift Dam and Parkside (East London). Figure 3.8 shows the Buffalo River catchment map.

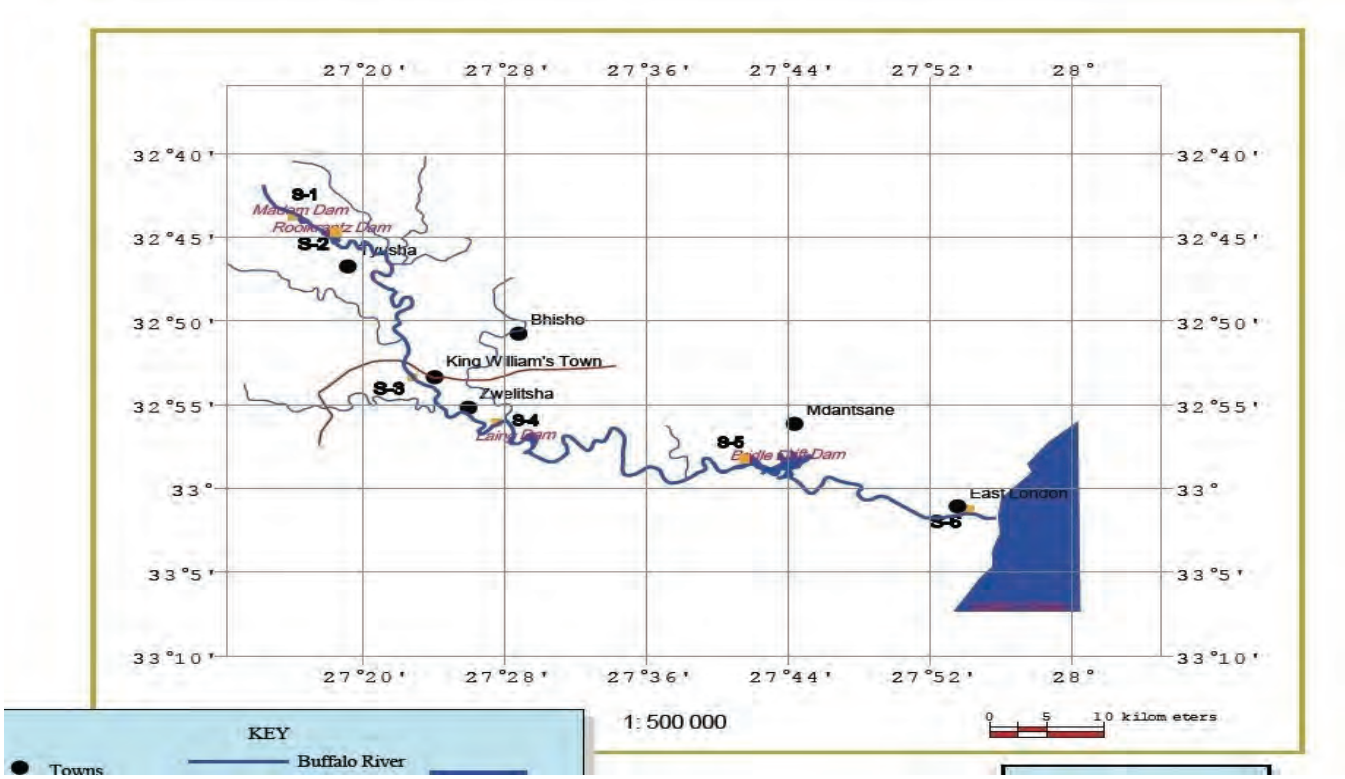

Figure 3.8: Map showing the Buffalo River catchment

Maden Dam

The Maden Dam (Figure 3.9) is a very small dam located at geographical coordinates 32º44'22"S and 27º17'54"E. It is the closest and most accessible point downstream from the source of the Buffalo River. The water is visibly clear and the dam is used for recreational fishing.

Figure 3.9: Map showing the Maden Dam site catchment

Rooikrantz Dam

The Rooikrantz Dam (Figure 3.10) is located further downstream from the Maden Dam on the Buffalo River at geographical coordinates 32º45'19"S and 27º19'35"E. It has a capacity of 5 x 10^6 m^3 and is unprotected (unfenced). Besides being a source of raw water for numerous water-treatment plants, this dam also serves

the inhabitants of the host settlements such as Tyusha and Zele for drinking, bathing, fishing, irrigation and recreation.

Figure 3.10: Map showing the Rooikrantz Dam site catchment

King William's Town

King William's Town (Figure 3.11) is one of the major towns in the Amathole District Municipality along the course of the Buffalo River with a population of over 250 000 inhabitants according to Statistics South Africa. The town has several suburbs which include New Rest, Kaffrarian Heights, Daleview, Club View, and Central. The sampling site in King William's Town is located at geographical coordinates 32º53'23"S; 27º23'17"E. The river at this location is visibly dirty and unprotected and a municipal drainage filter located on the river's banks discharges its contents into the river.

Figure 3.11: Map showing the King William's Town site catchment

Eluxolzweni

This Eluxolzweni site (Figure 3.12) is located at the geographical coordinates 32°56'16"S; 27°27'56"E. The water is inundated by a thick mass of water hyacinth and aquatic weeds over a distance of more than 300 m. The water was also observed to be very turbid, possibly due to the impact of the discharge from the sewage treatment plant located upstream.

Figure 3.12: Map showing the Eluxolzweni site catchment

Bridle Drift Dam

The Bridle Drift Dam site (Figure 3.13) is located in Mdantsane at geographical coordinates 32°58'30"S; 27°42'22"E. With a population of more than 250 000 Mdantsane is still expanding. Bridle Drift Dam is not protected and there is visible evidence of anthropogenic activities here. Cattle dung litters the shoreline, and a significant stretch of the water is heavily polluted with substances suspected to be chemicals discharged into the water body.

Figure 3.13: Map showing the Bridle Drift Dam site catchment

Parkside (East London)

The Parkside sampling site (Figure 3.14) is located at geographical coordinates 33°01'23"S; 27°51'31"E in East London on the estuary where this river empties into the Indian Ocean. East London is one of the 11 metropolitan areas in South Africa with a population of half a million or more people living in the city and the surrounding townships. Parkside is a suburb located next to East London's notorious Second Creek dumping site.

Figure 3.14: Map showing the Parkside site catchment

CHAPTER 4: PHYSICOCHEMICAL SURVEY AND ASSESSMENT

4.1 INTRODUCTION

Physicochemical parameters have major influences on biochemical reactions that occur within river systems (Bezuidenhout et al., 2002). Natural forces such as rainfall and drought together with **a**nthropogenic activities are responsible for most of the major changes to the physicochemical qualities of in-stream water since they usually offset the delicate balance in the chemical composition of water. Such a departure from the natural state comes with ecological, economic and health implications. Major sources of pollutants in river systems include agricultural, industrial and municipal activities (Ouyang et al., 2006). We here present the results of the physicochemical qualities of the Tyume River and the Buffalo River.

4.2 SAMPLING AND ANALYTICAL PROCEDURES

Water sampling from the study sites was done on a monthly basis for 12 months (August 2010 to July 2011) to shed light on the effect of season on the parameters. Sample processing and analyses were conducted within 6 h of sample collection following the procedure recommended by the American Public Health Association (*Standard Methods*, 2005). In all, a total of 12 samples per site were collected giving a total of 72 samples per river. Collected samples were then transported in cooler boxes to the Applied and Environmental Microbiology Research Group (AEMREG) Laboratory at the University of Fort Hare, Alice for analyses.

A total of 13 recognised physicochemical parameters in water quality control and pollution studies were determined. Temperature (°C), pH, electrical conductivity (EC) (µS/cm), and total dissolved solids (TDS) (mg/ℓ) were determined using a digital multi-parameter system (Hanna; HI 9828). Turbidity (NTU [nephelometric turbidity units]) was determined using a digital turbidimeter (Hach; 2100P). Phosphate (mg/ℓ PO_4-P), nitrite (mg/ℓ NO_2-N), nitrate (mg/ℓ NO_3-N) and chloride (mg/ℓ) were determined using a spectrophotometer (Merck; Spectroquant NOVA 60). Chemical oxygen demand (COD) (mg/ℓ) was determined by the standard photometric method after samples had been digested in a thermo-reactor (for 2 h at 148^{o}C). Dissolved oxygen (DO) (mg/ℓ) and 5-day biochemical oxygen demand (BOD_5) were determined using a BOD meter (Hach; HQ 40d).

4.3 STATISTICAL ANALYSIS

All data were subjected to descriptive statistical analysis (95% confidence limit). The generalised linear model (GLM) of SAS was used to generate analysis of variance (ANOVA), means, standard errors and ranges. Tukey's Studentised Range (HSD) Test was used to test differences among all possible pairs of treatments. The correlation coefficients were computed using the PROC CORR procedure in SAS (SAS Version 8, SAS Institute, Cary, NC).

4.4 RESULTS AND DISCUSSION

4.4.1 Tyume River catchment

Physicochemical parameters were individually analysed on a monthly basis from September 2010 to July 2010. Generally, BOD levels observed in this study ranged as follows: Hala (0.78 mg/ℓ to 1.36 mg/ℓ), Khayalethu (1.03 mg/ℓ to 2.73 mg/ℓ), Sinakanaka (0.98 mg/ℓ to 1.46 mg/ℓ), Alice (1.33 mg/ℓ to 2.14 mg/ℓ), Drayini (1.26 mg/ℓ to 2.44 mg/ℓ) and Manqulweni (1.44 mg/ℓ to 2.76 mg/ℓ) and fell within the stipulated BOD guideline of 10 mg/ℓ for surface waters where full contact use is allowed and ≤ 30 mg/ℓ where public access is prohibited, restricted, or infrequent (EPA, 2004). Figure 4.1 shows the monthly BOD levels observed over a period of 11 months.

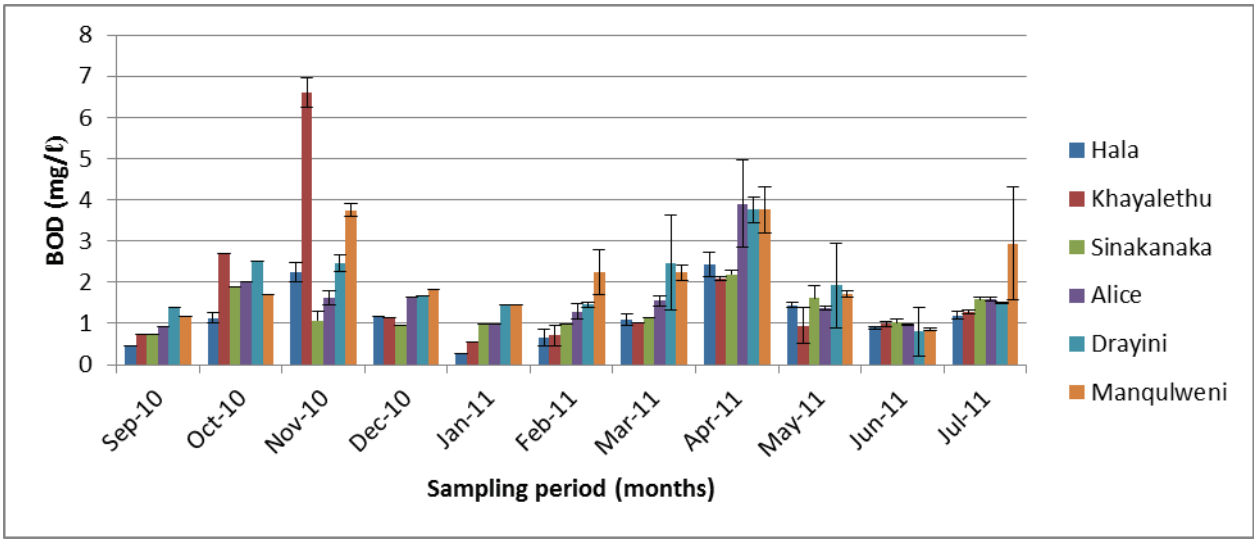

Figure 4.1: Monthly BOD levels for Tyume River

BOD levels differed from one month to the other at each of the sites and from one sampling site to the other in each month (P<0.05). Elevated BOD levels were observed in April 2011 across sampling sites as compared to other months. Variations in BOD levels of surface water may arise from changes in the organic matter content of such waters either as a result of point sources like sewage effluent discharges or non-point sources like stormwater runoff with suspended organic matter.

Also, DO concentrations generally ranged between 7.47 mg/ℓ and 10.42 mg/ℓ. In unpolluted surface waters, dissolved oxygen concentrations are usually close to saturation. Typical saturation concentrations at sea level and at TDS values below 3 000 mg/ℓ, are: 12.77 mg/ℓ at 5°C; 10.08 mg/ℓ at 15°C; 9.09 mg/ℓ at 20°C (DWAF, 1996e). A temperature range of between 6°C and 28°C was obtained in this study. Given that a target water quality range for the DO level in aquatic ecosystems in the range 80% to 120% of saturation will protect all life stages of most Southern African aquatic biota endemic to, or adapted to, aerobic warm water habitats (DWAF, 1996e), the DO concentrations obtained in this study were well within the criteria standard for warm- and cold-water biota. A pattern was observed in which DO concentrations did not significantly differ within the sampling period (P>0.05) of September 2010 to February 2011 at each sampling site. The same trend was observed for the period between April 2011 and July 2011. However, when compared by sampling point on a monthly basis, DO levels differed significantly (P<0.05) with the exception of April, May

and June 2011 where monthly inter-sampling point differences in DO levels were not significant. Seasonal variations in DO concentrations arise from changes in temperature and biological productivity (DWAF, 1996e). BOD and DO results imply that Tyume River is clean with respect to organic pollution (Bhutiani and Khanna, 2007; Kannel et al., 2007). Monthly DO levels for the period under study are displayed in Figure 4.2.

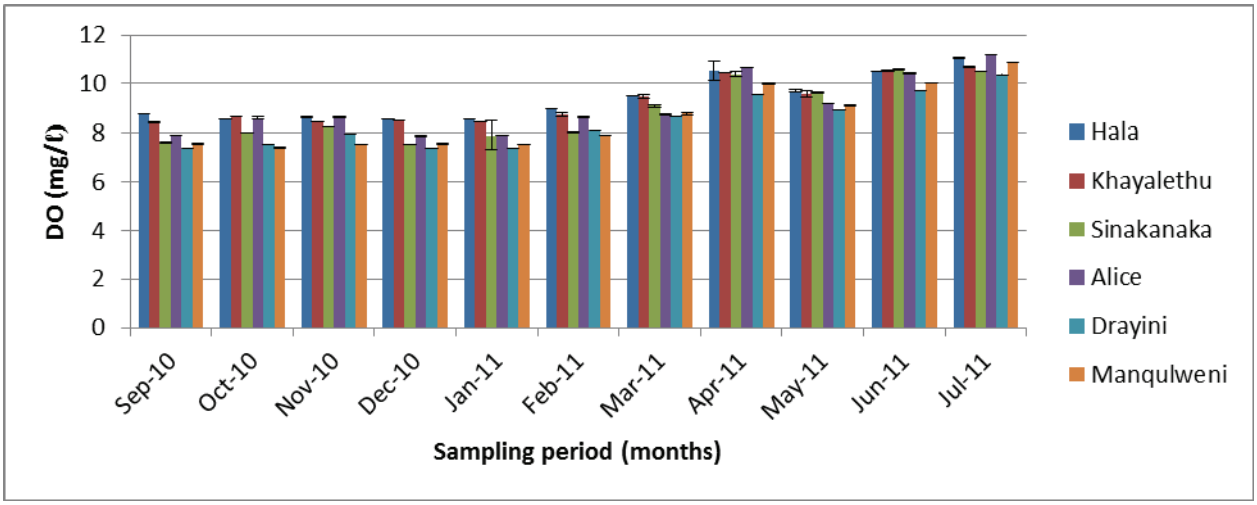

Figure 4.2: Monthly DO levels for Tyume River

The temperature range for Hala was 6°C to 20°C, while those for Khayalethu, Sinakanaka, Alice, Drayini and Manqulweni were 8°C to 23°C, 10°C to 28°C, 9°C to 25°C, 10°C to 27°C and 10°C to 25°C, respectively. Temperature regimes varied significantly (P<0.05) with month of sampling at each sampling site and from one sampling site to the other. For all sampling sites, a general trend was observed where water temperature kept increasing from September 2010 onwards, reaching a peak in February 2011 after which the temperature began to drop, reaching the lowest in July 2011, reflecting the seasonal patterns in South Africa. With the exception of March 2011, the lowest water temperatures for the duration of the sampling period were recorded at Hala, a site which receives the least insulation as it is located at the foot of high mountains. The temperature regimes for most sampling sites were within the acceptable limit of no risk (≤ 25°C) for domestic water uses in South Africa (DWAF and WRC 1995). While it is not possible to define a single cut-off point below which water temperatures are dangerous for recreational activities, unintentional exposure to cold water at temperatures of below 16°C can result in a debilitating shock response and hypothermia (excessive heat loss) (National Health and Medical Research Council, 2008). However, this will vary according to the specific circumstances and physical condition of the person involved and the duration of their exposure. A temperature regime pattern for the period under study is displayed in Figure 4.3.

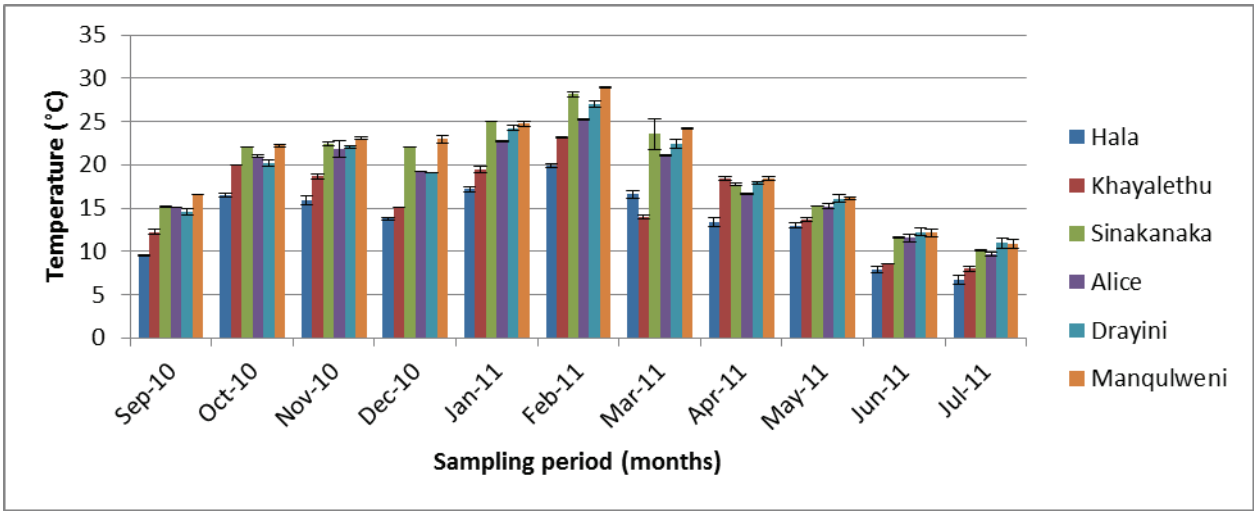

Figure 4.3: Monthly temperature profiles for Tyume River

Electrical conductivity (EC) which generally ranged between 47 µS/cm and 408 µS/cm showed significant variability among sampling points in each month (P<0.05). While EC levels at Hala and Khayalethu were not significantly different from each other (P<0.05) both within and between months, progressively higher EC values were observed moving from Khayalethu (upstream) to Manqulweni (downstream). While the downstream sampling sites Alice and Drayini are directly impacted by sewage effluent from Alice Town and the University of Fort Hare, Manqulweni is indirectly impacted by virtue of being located further downstream of both. It is possible that sewage disposal into the Tyume River could have influenced the EC levels in the downstream sites as has been suggested elsewhere (Suthar et al., 2010). However, EC levels were within the recommended target water quality range (TWQR) of no risk for domestic water uses which is set at 0 µS/cm to 700 µS/cm (DWAF, 1996b). However, this guideline is for treated drinking water. Figure 4.4 shows the EC pattern observed after 11 months of sampling in Tyume River.

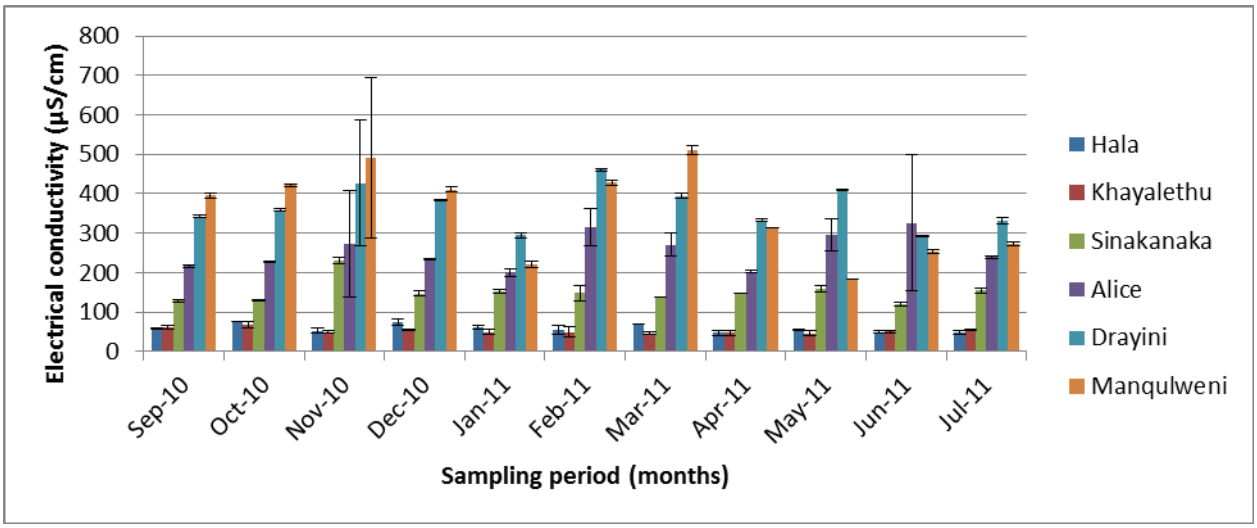

Figure 4.4: Monthly EC values for Tyume River

The South African TWQR for pH in water for domestic use is pH 6 to pH 9 (DWAF, 1996b), which is the same as that of the European Union tolerance limit for pH in water for the support of fisheries and aquatic life

(Chapman, 1996). The pH of Tyume River during the study period partly satisfies the South African water quality guideline for both recreational and domestic water use. In the period beginning September 2010 to January 2011, the pH of Tyume River water was consistently below pH 9, but from February 2011 through to June 2011 the pH significantly increased to between pH 10 and pH 11 at most sampling sites. The pH of an aquatic system is affected by several factors including the geology and geochemistry of the rocks and soils of a catchment, temperature, effluent discharges, algal growth, acid mine drainage, acidic precipitation, runoff, microbial activity and decay processes (DWAF, 1996a). The absence of mining ventures in the Tyume River catchment suggests that the observed fluctuations in the pH of Tyume River water could be a result of a combination of the other factors. Since the recorded water pH between February and June 2011 at some sampling sites was >pH 9, this could lead to eye, skin, ear and mucous membrane irritations among swimmers and bathers in the river. Adverse aesthetic and taste effects could also have been expected in cases where the water was accidentally consumed (DWAF, 1996a). Figure 4.5 shows the pH profile of Tyume River water between September 2010 and July 2011.

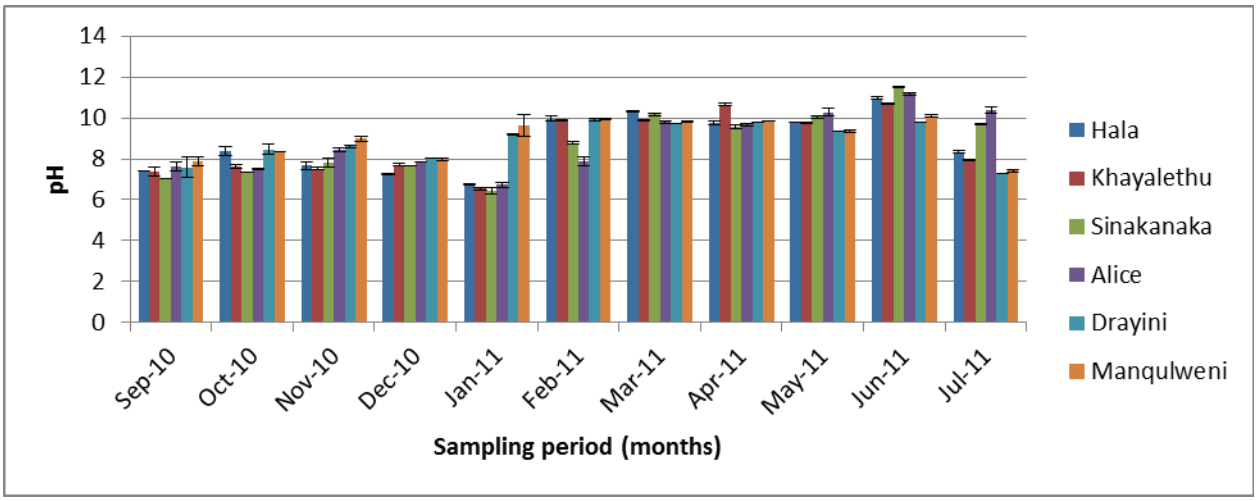

Figure 4.5: Monthly pH levels for Tyume River

The turbidity of the river differed significantly during the study period with respect to sampling sites and months. With respect to sampling sites, turbidity was always lower at Khayalethu and, with the exception of April 2011, and always higher at Manqulweni which is the furthest point downstream. This could be expected since the flow volume becomes cumulatively larger with distance downstream, and so becomes the erosive power of the river also and hence the silt. Suspended silt and clay, organic matter, and plankton can contribute to turbidity; hence turbidity in a stream will fluctuate before, during and after stormflow (Igbinosa and Okoh, 2009). Highly turbid water has an altered odour, taste and its visual properties are negatively impacted and will significantly increase water-treatment costs due to the amount of flocculants needed to clarify the water (Osode and Okoh, 2009). The turbidity of the river water ranged between 6 NTU and 281 NTU and fell short of the target water quality range (0 NTU to 1 NTU) of no risk for domestic water uses in South Africa (DWAF, 1996b). While untreated river water in South Africa is not considered as 'drinking water' (National Water Act, 1998 (Republic of South Africa, 1998); Water Services Act, 1997 (Republic of South Africa, 1997)), some communities in South Africa are still without access to potable water and therefore rely on direct surface water for domestic use (National Climate Change Response, 2010; Hippo Water Roller

Project, 2011). In OR Tambo district, for example, more than 60% of households use water from dams, springs or rivers (Jeenes and Steele, 2010). Turbidity can have a significant effect on the microbiological quality of water. Microbial growth in water is most extensive on the surface of particulates while river silt also readily adsorbs viruses and bacteria. For domestic water use, turbidity levels of 5 NTU to 10 NTU are visible and may be objectionable to users while turbidity levels of >10 NTU will also increase chances of transmission of disease by microorganisms associated with particulate matter, particularly for agents with a low infective dose such as viruses and protozoan parasites (DWAF, 1996b). Reliance on such sources of water as wells and rivers led to a well-publicised cholera case in KwaZulu-Natal in the year 2000 where more than 200 residents died after contracting cholera from the nearby uMhlathuze River (Cottle and Deedat, 2002) suggesting the need for communities, which solely depend on untreated river water for domestic use, to boil such water before use in order to minimise the chances of infection. For full-contact recreational water use, DWAF (1996a) proposes that the turbidity of the water should not increase by more than 5 NTU above natural background turbidity when that turbidity is low (less than 50 NTU). Elevated turbidities are often associated with the possibility of microbiological contamination. Figure 4.6 shows the observed turbidities of Tyume River between September 2010 and July 2011 though turbidity was not measured from November 2010 through to January 2011.

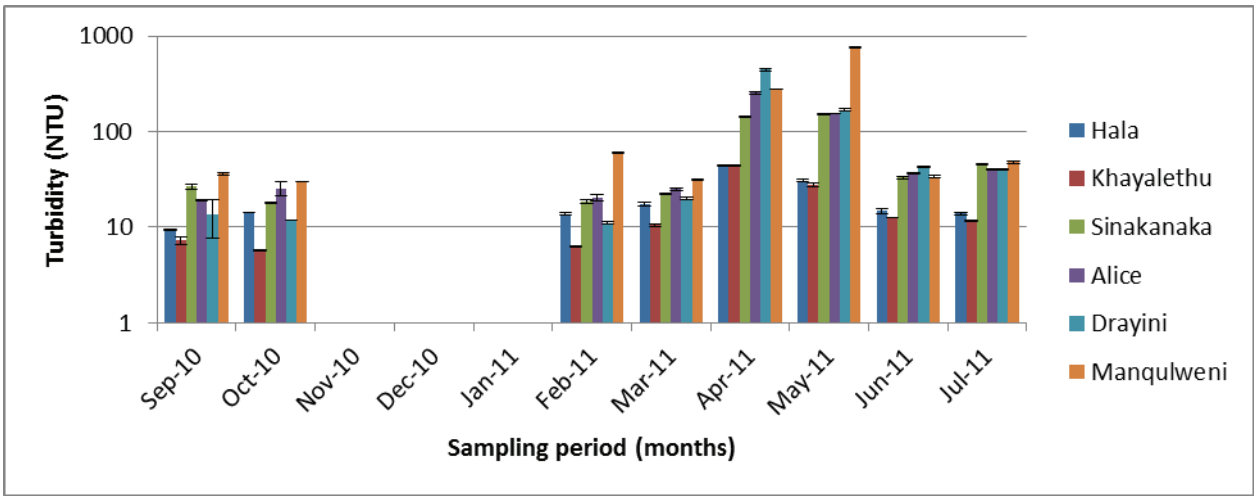

Figure 4.6: Monthly turbidity values for Tyume River

Like water with high turbidity levels, water with high total dissolved solids (TDS) is unpalatable and potentially unhealthy. Dissolved substances in water also interfere with the normal workings of sanitizers by forming a chemical 'shield' around bacteria and algae (Hoko, 2005). The TDS target water quality range is set at 0 mg/ℓ to 450 mg/ℓ for domestic use (DWAF, 1996b). The TDS levels observed in this study fell within this range. There is a remarkable similarity between the pattern displayed in Figure 4.4 (monthly EC values) and the pattern displayed in Figure 4.7 (monthly TDS values), which confirms that the TDS concentration is directly proportional to the electrical conductivity (EC) of water (DWAF, 1996b).

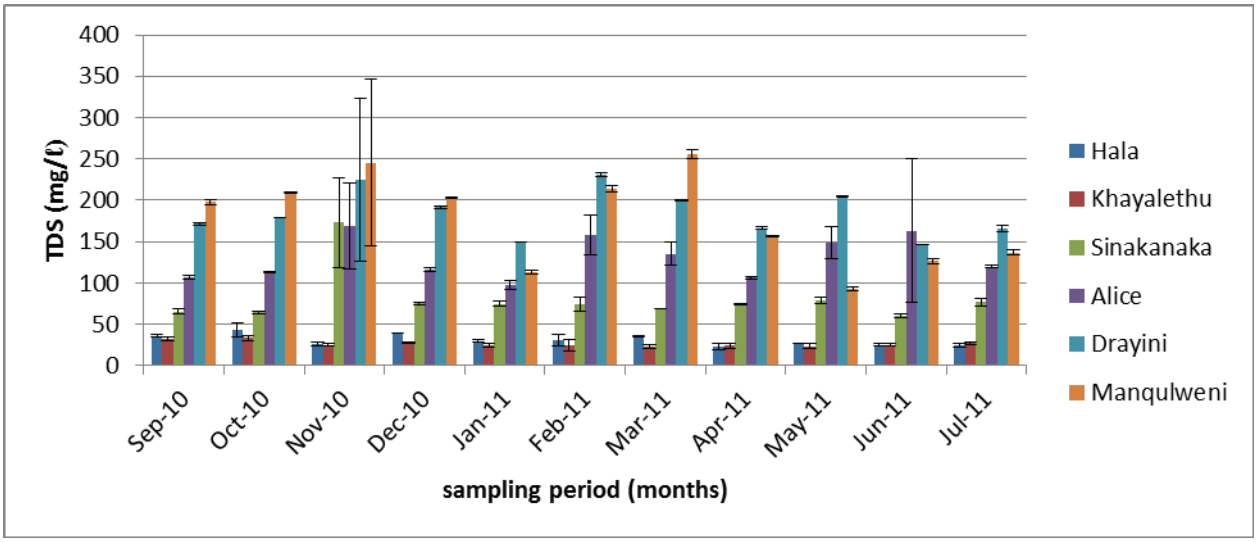

Figure 4.7: Monthly TDS values for Tyume River

Nitrate, nitrite and orthophosphate concentrations are remarkably similar in that significantly higher values (P<0.05) were obtained in September and October 2010 compared to other months. While sampling in September and October was done in dry weather, sampling in November 2010 was done 2 d after rain had fallen. The observed trends shown in Figure 4.8, Figure 4.9 and Figure 4.10 suggest that these nutrients originated from a point source, possibly due to sewage effluent disposal into the river. The absence of a dilution effect in September and October may have caused the nutrient concentrations to be higher than were observed in November when sampling was done 2 d after it had rained. Similar trends have been reported elsewhere (Castillo et al., 2000; Ferrier et al., 2001). While agricultural activities can result in high levels of these nutrients due to runoff, there is little agricultural activity in the Tyume catchment. However, it is worth noting that in addition to point sources of river pollution, nutrient loadings also arise from non-point source pollution from contaminated runoff during flash storms, particularly in urban areas (Brainwood et al., 2004). The final nutrient concentrations found in river water will therefore be a balance between the dilution effect of stormwater runoff and the concentration of the same nutrients in the incoming runoff.

All three nutrients are naturally present in the environment and natural nutrient cycling processes prevent accumulation of very high concentrations of the nutrients. However, human activities have increased environmental nitrate and nitrite concentrations, with agriculture being the major source (Castillo et al., 2000; Ferrier et al., 2001). This includes increased use of nitrogen-containing fertilisers as well as concentrated livestock and poultry farming; the latter two produce millions of tons of nitrate-containing manure each year (EPA, 2007). Nitrate and nitrite compounds are very soluble in water and quite mobile in the environment. They have a high potential for entering surface water during rainfall events, as nitrates in applied fertilisers can dissolve in runoff that flows into streams (Brainwood et al., 2004). Nitrates themselves are relatively nontoxic and normal individuals have low levels (0.5% to 2%) of methaemoglobin in their blood (EPA, 2007). When in excess, nitrates may also result in excessive nutrient enrichment in water systems (eutrophication) leading to loss of diversity in the aquatic biota and overall ecosystem degradation through algal blooms, excessive plant growth, oxygen depletion, and reduced sunlight penetration (Odjadjare and Okoh, 2010). The South African Water Quality Guidelines for nitrate consider the effect of this compound on the health of infants and pregnant women and thus set the safety limit for water meant for human consumption at 6 mg NO^-_3 as N/ℓ (DWAF 1996b). The nitrate concentrations ranged between 0.18 mg/ℓ and 4.21 mg/ℓ and fell

within the South African TWQR for domestic water. In September and October 2010 as well as in January 2011, nitrate concentrations were significantly higher at all sampling sites than in any other month. During the period from September 2010 through to January 2011 sampling was done under relatively dry weather conditions except in November when sampling was done 2 d after it had rained. From February 2011 through to July 2011, rainfall activity was much more frequent than in the preceding period. The observed effect is that nutrient concentrations significantly dropped at the upstream sampling sites (Hala, Khayalethu and Sinakanaka). At the downstream sites of the Tyume River, however, nitrate concentrations remained significantly higher than upstream, owing to the discharge of sewage effluent from Alice and Fort Hare Wastewater Treatment Plants into the river. Figure 4.8 shows the monthly concentrations of nitrate in the Tyume River.

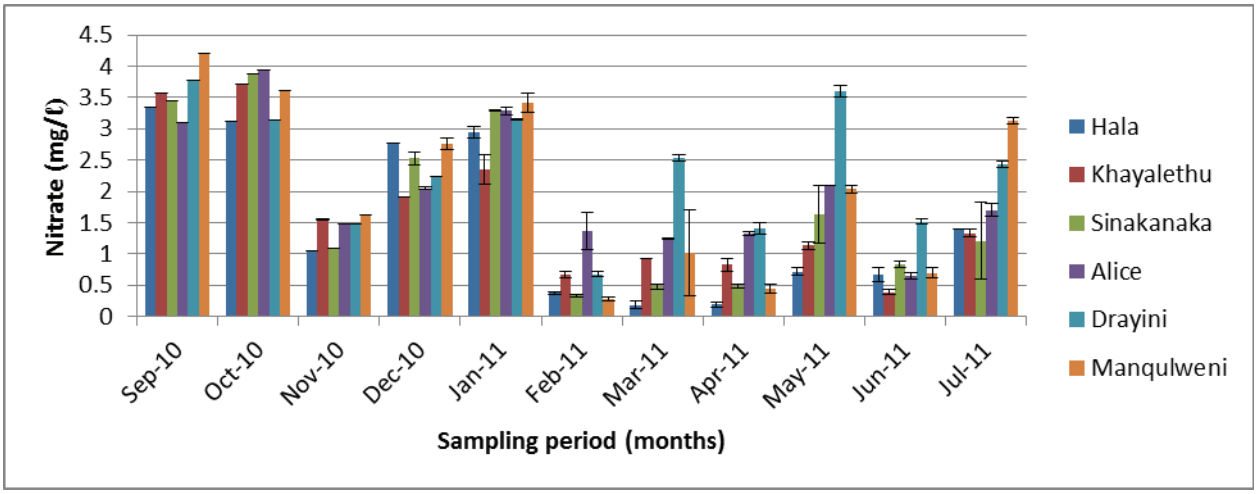

Figure 4.8: Monthly nitrate concentrations in Tyume River

Nitrite levels ranged between 0.02 mg/ℓ and 2.35 mg/ℓ across months and sampling points. A target water quality range of 0.5 mg/ℓ to 2.5 mg/ℓ is indicative of mesotrophic conditions in aquatic ecosystems (DWAF, 1996e). In September, October and December 2010 as well as in January and May 2011, nitrite levels were in the mesotrophic range at most sampling sites. In the remainder of the months, nitrite concentrations were within the oligotrophic range (<0.5 NO^-_2 as mg N/ℓ) (DWAF, 1996c) and also within the drinking water limits of 0.5 mg N/ℓ and 1 mg N/ℓ for the EU and USA, respectively (Figure 4.9). Nitrite easily changes to nitrate as the end-product of the oxidation of organic nitrogen and ammonia (DWAF, 1996b). The detected nitrite concentrations may therefore not have posed a health risk in the case of people imbibing the raw water since the detected nitrate levels in the same period were within the safety guideline of 6 mg NO^-_3 as N/ℓ (DWAF, 1996b) set for water meant for human consumption.

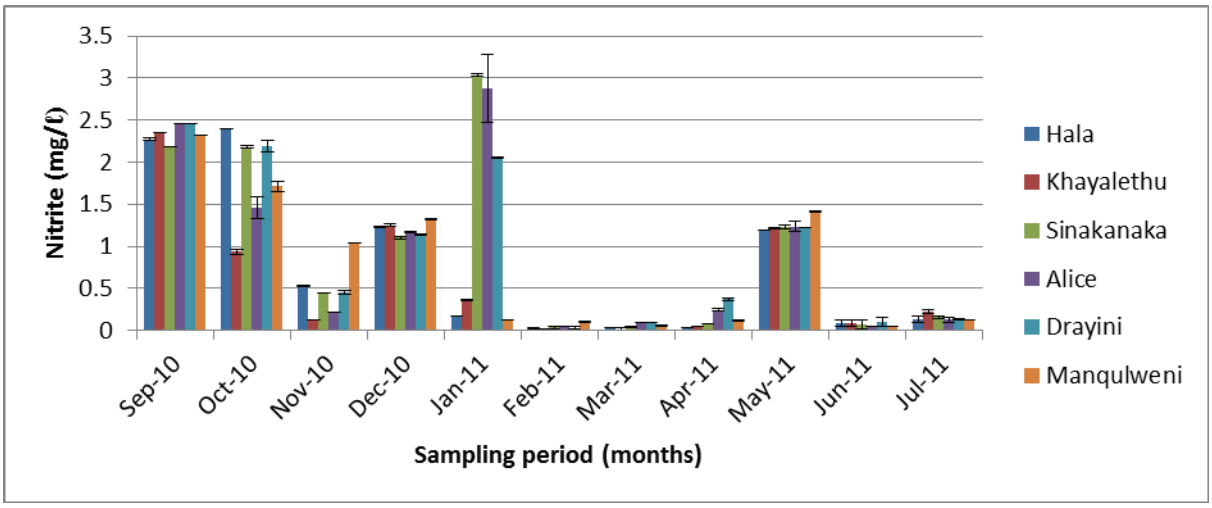

Figure 4.9: Monthly nitrite concentration for Tyume River

Orthophosphate (as P) concentrations in this study varied from 0.06 mg PO_4^{3-}/ℓ to 2.72 mg PO_4^{3-}/ℓ across months and sampling points. From September 2010 to January 2011, phosphate concentrations were above the standard limit (0.1 mg/ℓ) of the US Public Health Standards (Solaraj et al., 2010) in water systems that will not encourage the growth of algae and other plants. Since municipal wastewater contains substantial amount of phosphorus contributed by human urine and detergents (Ekholm and Krogenus, 1998), disposal of municipal sewage into the river may account for the observed trend in September and October 2010. The dilution effect on the sewage disposed into the river could have been less in this period, hence the higher concentrations of nutrients in this period than in all the other months. In the presence of sufficient available phosphorus as was the case in the spring months (September and October) of this study, nitrogen-fixing organisms will be able to fix atmospheric nitrogen, thereby compensating for any deficit caused by low inorganic nitrogen concentrations culminating in aquatic eutrophication (DWAF, 1996b). Figure 4.10 shows the phosphate concentrations observed in Tyume River during the study period.

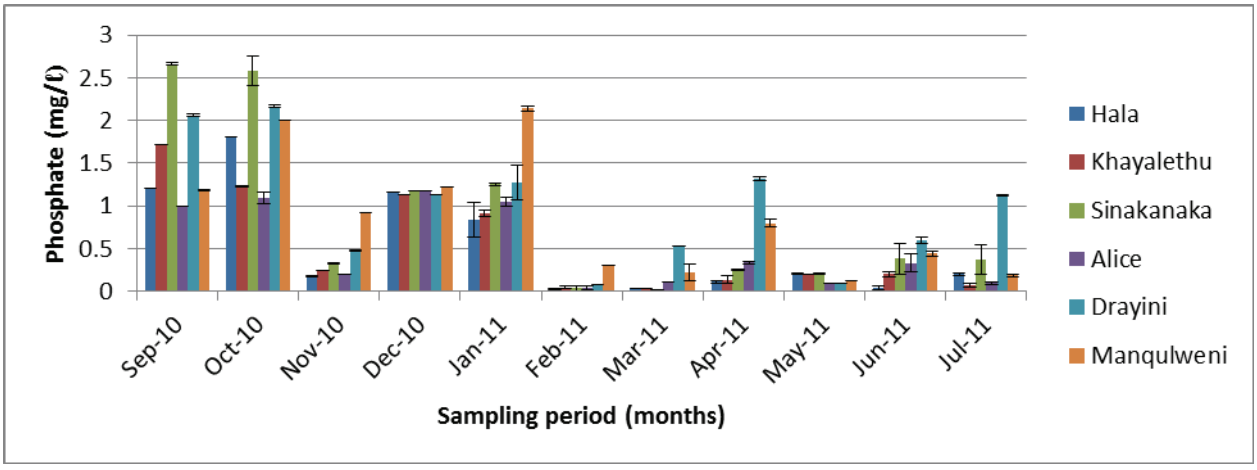

Figure 4.10: Monthly phosphate concentrations for Tyume River

Conclusions

Physicochemical parameters are major determinants of water quality that directly or indirectly affect its use. All physicochemical parameters were within water quality guidelines for the duration of the study period. This has major health, ecological and economic implications for a water-scarce country like South Africa where every flowing river is a precious natural resource that needs to be safeguarded against pollution. Since the Tyume River is the source water for a drinking-water-treatment plant (DWTP), its relative purity in respect of physicochemical pollutants means that water-treatment costs will be kept at a minimum.

4.4.2 Buffalo River catchment

Figures 4.11 to 4.22 show the monthly variation in the physicochemical characteristics of the Buffalo River. Mean water temperature values ranged between 13.7^{o}C and 27.9^{o}C (Fig. 4.11). Water temperatures increased as sampling progressed from the spring months (September, October and November) and peaked in the summer months of December through February. A steady decrease in temperatures was then recorded throughout the autumn months (March, April and May) into the winter months (June, July and August). The significantly lower (P<0.05) temperatures recorded in winter, across the sites and at Maden Dam (the first sampling point closest to the river source in the Amathole Mountain range) suggest that water temperature was largely determined by the seasons and altitude.

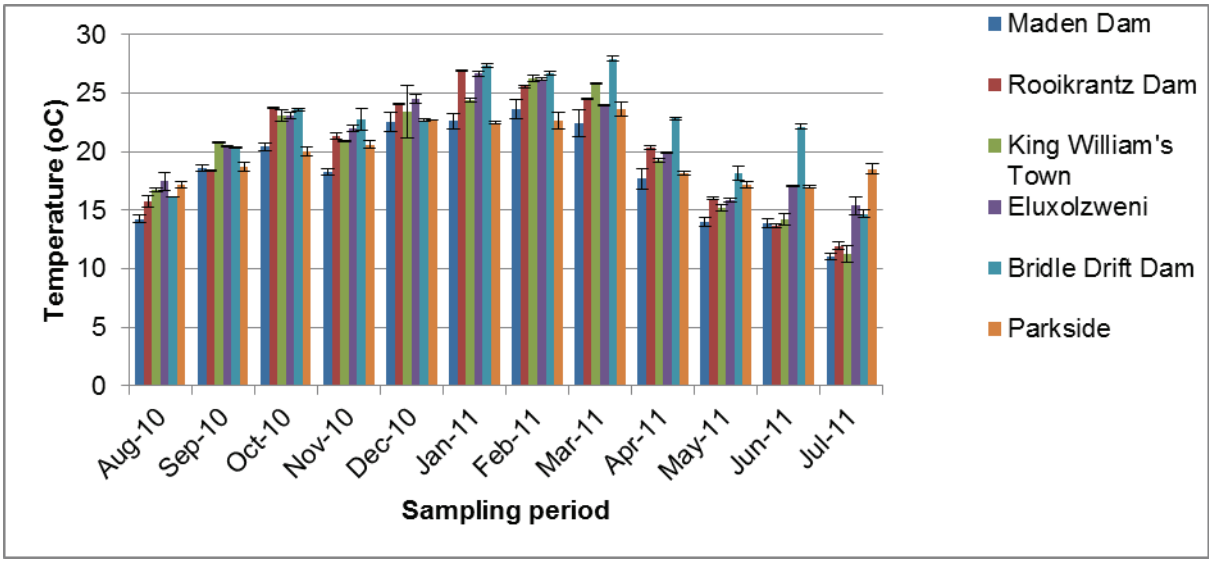

Figure 4.11: Monthly variation in water temperature for Buffalo River

The pH of an aquatic system is an important indicator of water quality and the extent of pollution in the watershed area. Figure 4.12 shows the monthly variation in pH values recorded at the 6 sampling sites on the Buffalo River.

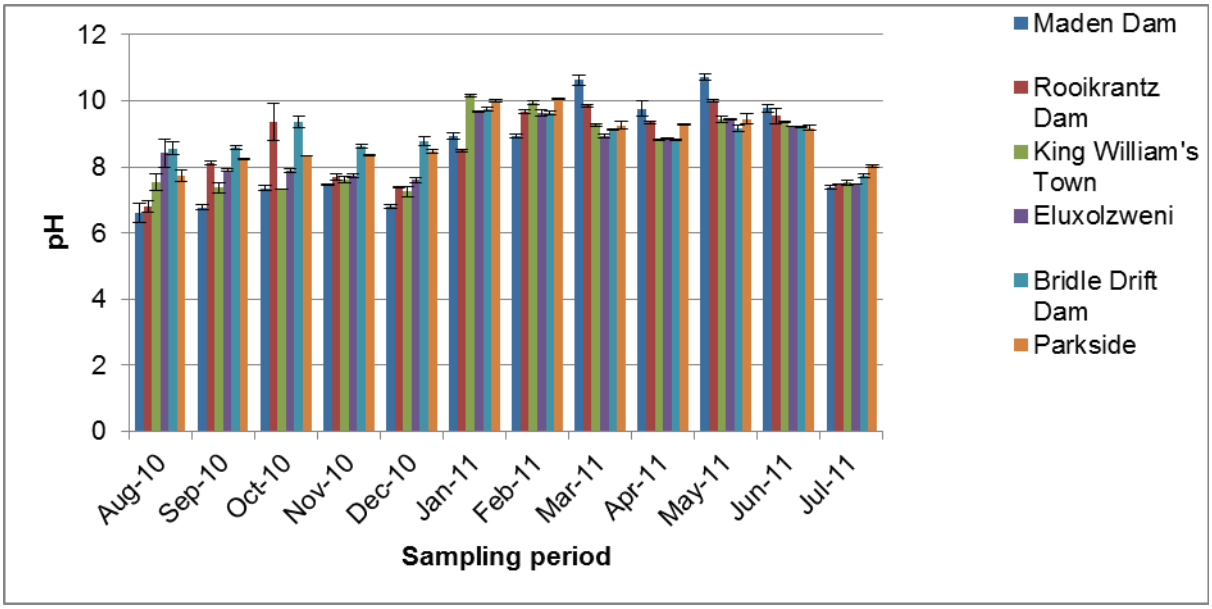

Figure 4.12: Monthly variation in pH values for Buffalo River

While unpolluted waters normally show a pH of about pH 6.5 and pH 8.5 (DWAF, 1996b; WHO 2008), most of the pH values observed in this study lie between pH 8.5 and pH 10.8 which is not far off from the upper level guideline of pH 9.0 for domestic use. Mean monthly pH levels ranged between pH 6.62 and pH 10.73. Generally, significantly higher pH values were recorded at Eluxolzweni than the rest of the sites, though in broad terms, all pH values observed fell within South African water quality guidelines for aquaculture.

Figure 4.13 shows the mean monthly variation in turbidity values at Buffalo River. The observed turbidity levels (range: 1.71 NTU to 132.7 NTU) were mostly above 5 NTU, the upper international limit for drinking water. Across the months, turbidity generally increased as the river flowed from its source downstream through settlements. Consequently, significantly higher (P<0.05) turbidities were encountered at King William's Town, Eluxolzweni and Bridle Drift Dam sites. The lower turbidities recorded at Parkside (the site located on the Buffalo Estuary) can be explained to be due to the dilution effect of sea water.

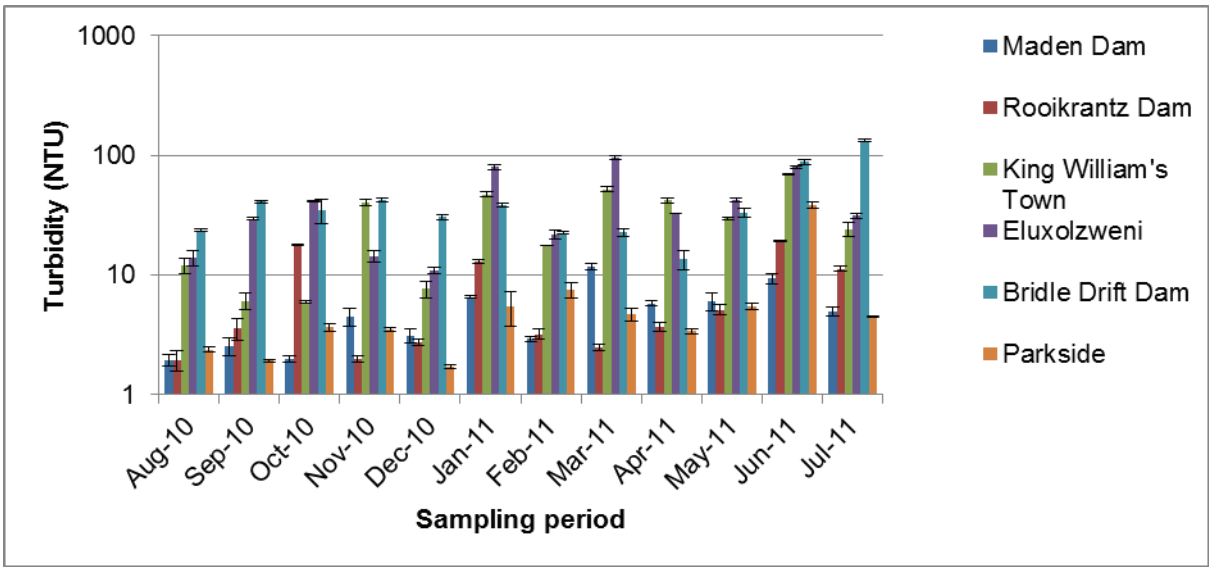

Figure 4.13: Monthly variation in turbidity values for Buffalo River

Turbidity could have been affected by storm events as was reported by Kistemann et al. (2002) who studied water reservoir tributaries in Germany during extreme rainfall and runoff and observed a remarkable increase in turbidity and microbial loads following rainfall and runoff events. This could also help explain the lack of seasonal variation at most sites, across the seasons. Variations recorded within the seasons could be attributable to possible settling that occurred, depending on the time lapse between storm event and sampling, as soil runoff is the major source of turbidity in surface waters.

Figure 4.14 shows the mean monthly variation in salinity values at sampling sites on the Buffalo River. Salinity is an important measurement in estuaries where freshwater from rivers and streams mixes with salty ocean water. *In situ* salinity of Buffalo River showed a very wide range of values, from a mean value of 0.02 recorded at Maden Dam to 33.78 at Parkside. Salinity varied with sampling sites, increasing as the water flows from its source in the Amathole Mountain range down to where it empties into the sea at East London. Freshwater salinity is usually less than 0.5, and mean salinity levels recorded at five of the six studied sites (including Maden Dam, Rooikrantz Dam, King William's Town, Eluxolzweni and Bridle Drift Dam) in Buffalo River were generally within this limit. Values ranged between 0.02 at Maden and Rooikrantz Dams and 0.37 at King William's Town. Salinity at Parkside was significantly higher (P<0.0001) than those of the other sites. For the 12 months and 4 seasons, the mean salinity values at this site (27.6 to 33.8) were much higher, and fall within the range for salt water. This reflects the influence of sea water at the Buffalo River estuary. The significantly lower (P<0.05) salinity recorded at this site in June (mean value, 4.3) could be attributable to possible discharge of sewage as other parameters including bacteriological indicators appear to suggest.

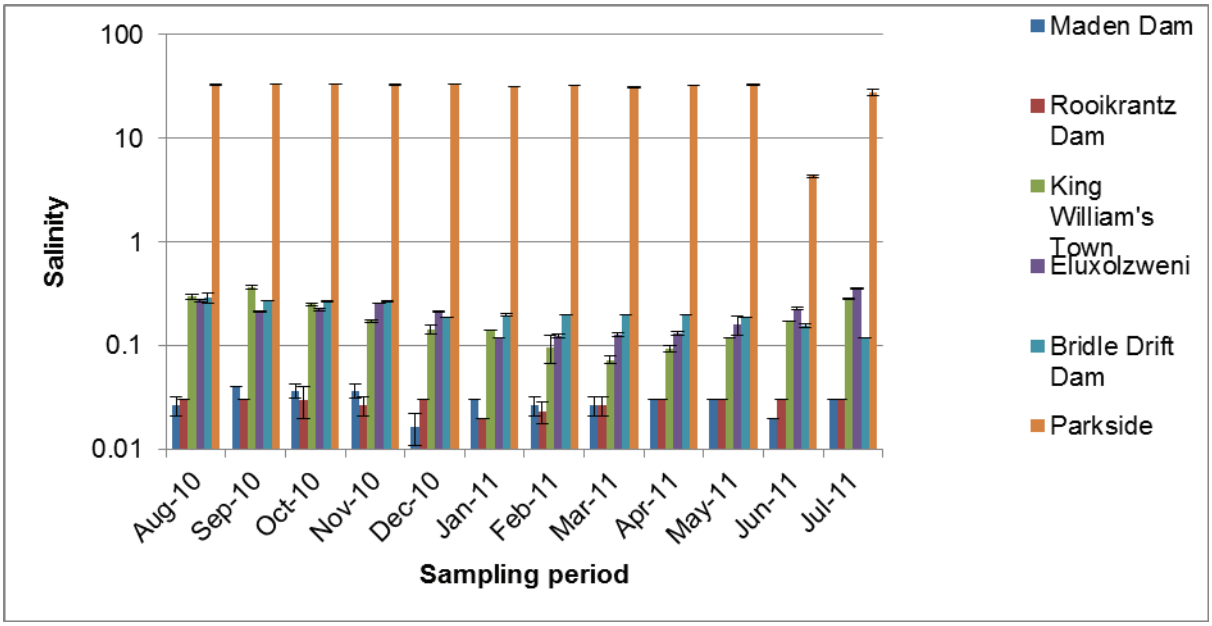

Figure 4.14: Monthly variation in salinity values for Buffalo River

Figure 4.15 shows the mean monthly variation in electrical conductivity in the Buffalo River. The range of values recorded in respect of electrical conductivity (EC) was relatively wide (38.6 µS/cm to 6 947 µS/cm).

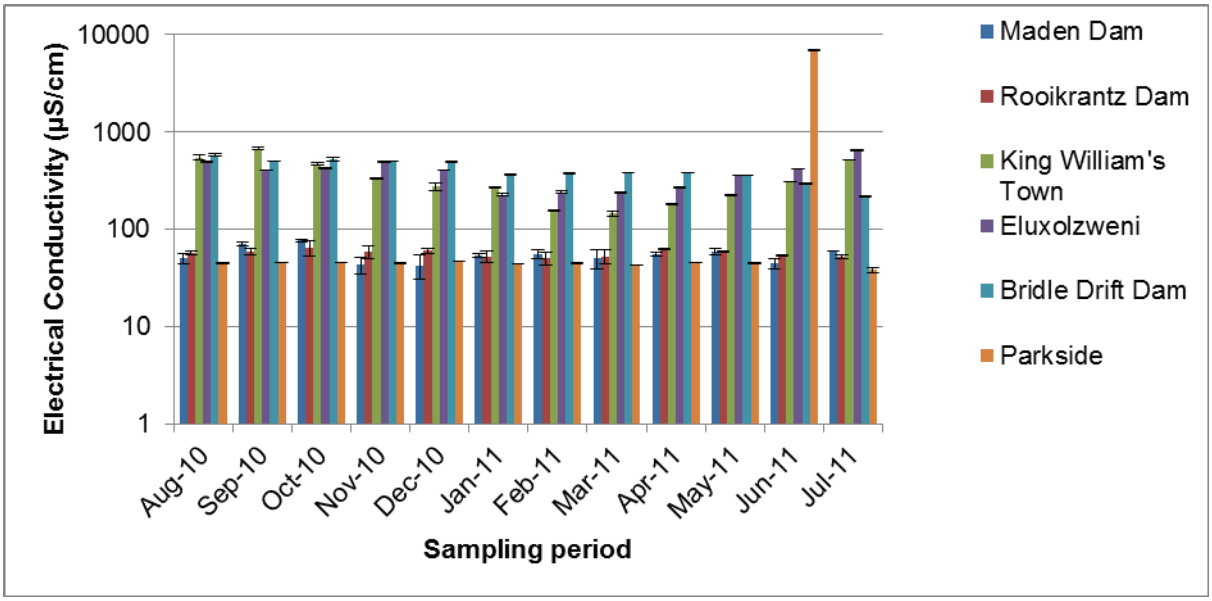

Figure 4.15: Monthly variation in electrical conductivity for Buffalo River

Electrical conductivity, however, increased as the river flowed through settlements. Although the lowest mean EC values were consistently obtained at Parkside within the study period, the highest mean EC value (6 947 µS/cm) was recorded at the same site in the winter month of June. This significantly higher (P<0.05) value in electrical conductivity with respect to other sites, months and seasons may be due to dumping in June of sewage (also suggested by the bacteriological indicators). It should be pointed out that while EC values far exceed the South African target water quality EC limit of no risk for domestic water uses (700 µS/cm; DWAF 1996a) at Parkside in winter, it generally fell within this range of values at all the other sites with values ranging between 42 µS/cm and 679 µS/cm.

Figure 4.16 shows the mean monthly variation in total dissolved solids in the Buffalo River. The level of TDS reflects the total concentration of all dissolved ions in the water, some of which may be contaminants depending largely on anthropogenic activities in the catchment. The range of TDS was wide (20.3 mg/ℓ to 23 350 mg/ℓ). High levels of dissolved and suspended solids in water systems increase the biological and chemical oxygen demand (Jonnalagadda and Mhere, 2001). TDS concentrations increased as the Buffalo River flowed from its source through settlements and down to the estuary. This could be due to runoffs, municipal wastewater discharges and low water flow at the lower reaches compared to the upper reaches as studies have demonstrated that TDS correlates with water flow (Albek, 2003; Calijuri et al., 2011).

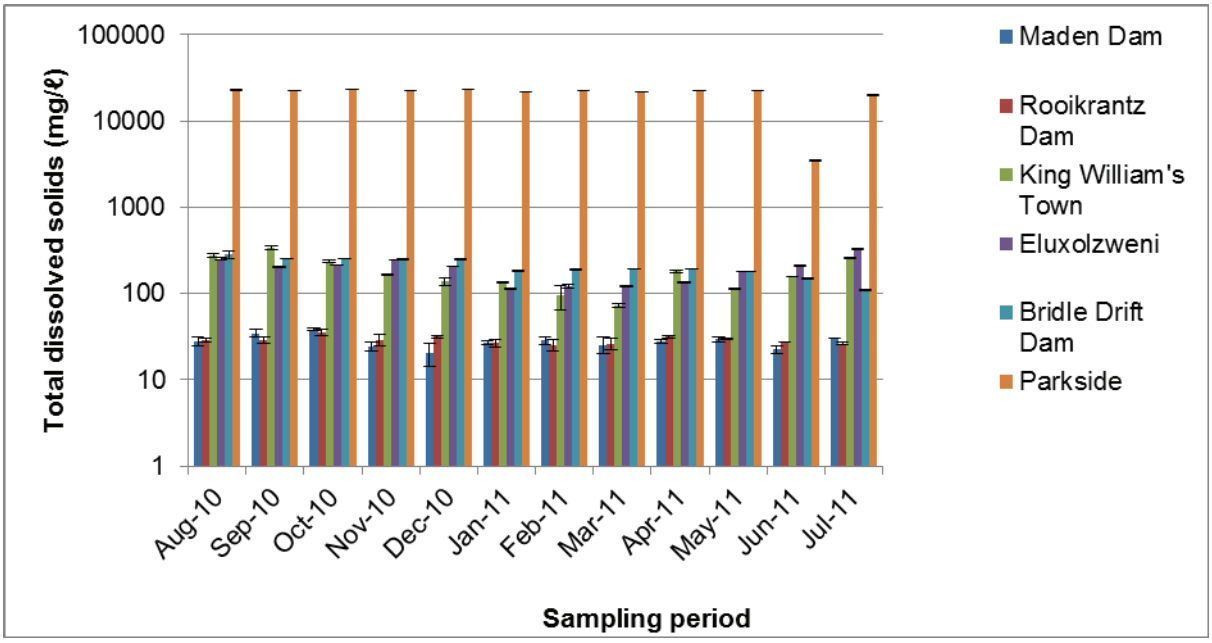

Figure 4.16: Monthly variation in total dissolved solids for Buffalo River

The highest TDS levels were obtained at Parkside (range: 3 473 mg/ℓ to 23 350 mg/ℓ), where the mean TDS was significantly lower (P<0.05) in winter compared to other months. Although elevated TDS levels are known to be toxic to freshwater animals, most of the TDS concentrations (5 out of 6 sites) fell within acceptable guidelines (0 mg/ℓ to 450 mg/ℓ) for domestic use (DWAF, 1996d) except at Parkside.

Figure 4.17 shows the monthly variation in dissolved solids concentrations in the Buffalo River. The DO concentrations in this study varied between 6.88 mg/ℓ and 11.14 mg/ℓ across the sampling sites and showed both spatial and seasonal variations.

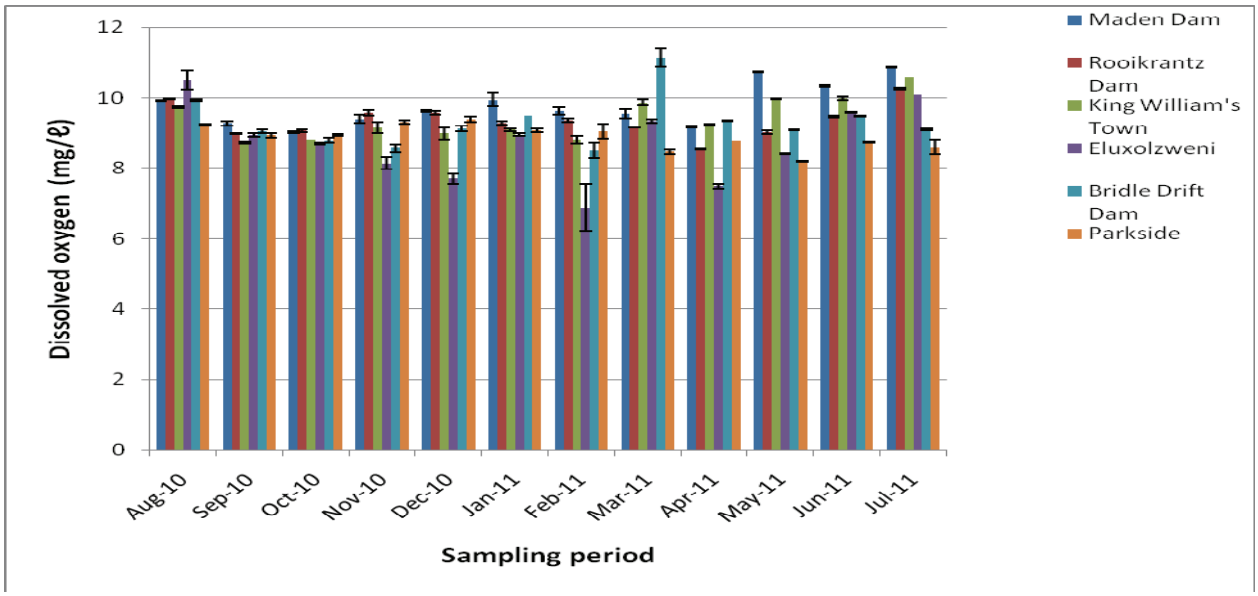

Figure 4.17: Monthly variation in dissolved oxygen concentrations for Buffalo River

Dissolved oxygen (DO) was significantly higher at Maden Dam compared to Eluxolzweni and Parkside (Figure 4.17). The significantly higher DO concentration recorded at Maden Dam could be attributable to

higher flow and colder water temperature. The DO concentration at Eluxolzweni is attributable to the high organic loading from a wastewater treatment plant upstream from this point as well as to the presence of water hyacinths which reduced flow significantly. Dissolved oxygen is vital in maintaining the oxygen balance in aquatic ecosystems; and concentrations below 5 mg/ℓ will have harmful effects on aquatic life (DFID, 1999). The DO concentrations encountered in this study (6.88 mg/ℓ to 11.14 mg/ℓ) contrast the 2.7 mg/ℓ to 3.8 mg/ℓ reported for Keiskamma River and Sandile Dam (Fatoki et al., 2003), Dissolved oxygen concentrations in unpolluted water normally range between 8 mg/ℓ and 10 mg/ℓ (DFID, 1999). In winter, the water temperature was significantly lower (Figure 4.11). The highest DO concentrations were encountered in winter. No significant correlation was discernible between water temperature and DO, although it is known that the temperature of water determines the level of dissolved oxygen in an inverse relationship (Manasrah et al., 2006).

Figure 4.18 shows the monthly variation in biochemical oxygen demand (BOD) in the Buffalo River. The BOD levels were generally higher at the lower reaches of the Buffalo River from King William's Town sampling point down to Parkside (1.13 mg/ℓ to 5.54 mg/ℓ) compared to those in the upper reaches of the river (0.84 mg/ℓ to 1.27 mg/ℓ) (Table 4.3).

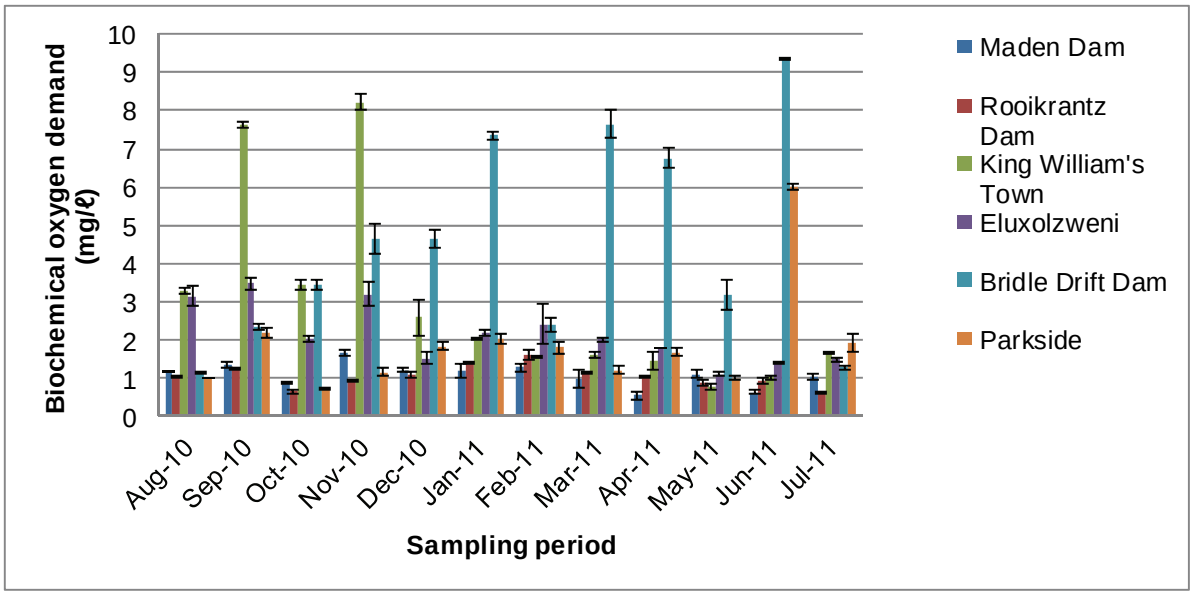

Figure 4.18: Monthly variation in biochemical oxygen demand for Buffalo River

Also, the BOD was significantly higher (P<0.05) at Bridle Drift Dam in the summer, winter and autumn months than at the other sampling sites, indicating that the dam compared to the sites was of significantly inferior quality during the three seasons. However, the differences observed amongst the seasons were not significant. At King William's Town, the BOD values recorded in August through to November were significantly different from those recorded in the other months and at the other sites. The lower BOD values (<5.00 mg/ℓ) observed at Maden and Rooikrantz Dams reflect a lower burden of organic pollution. However, the lower BOD values (<5.00 mg/ℓ) recorded at Parkside, however, could be attributable not to reduced pollution or self-purification process but to increased harsh conditions (like high salinity) at the estuary that are limiting the survival of many bacterial species. BOD indicates the extent of organic pollution in the

aquatic systems, which adversely affects the water quality, and a river is said to be unpolluted if it has a BOD of 2 mg/ℓ or less (Hobson and Poole, 1988). The BOD of Buffalo River during this study period ranged between 0.5 mg/ℓ and 9.39 mg/ℓ. Based on the BOD values, the Bridle Drift Dam is the most polluted site along the Buffalo River course. Due to the high volume of water, the extent of pollution therein may not appear severe. The pollution potential gains significance when it is considered that the dam is the major source water for some drinking-water-treatment plants.

Figure 4.19 shows the monthly variation in chemical oxygen demand in the Buffalo River. COD is a measure of the oxygen equivalent of the usually organic and sometimes inorganic matter content of a sample that is susceptible to oxidation by a strong chemical oxidant. The COD of the water samples generally ranged between 3.5 mg/ℓ and 45.9 mg/ℓ and increased down the river course in the following order: Maden Dam (23.19 mg/ℓ), Rooikrantz dam (13.62 mg/ℓ), King William's Town (23.19 mg/ℓ), Eluxolzweni (24.98 mg/ℓ), Bridle Drift Dam (30.8 mg/ℓ) and Parkside (34.83 mg/ℓ).

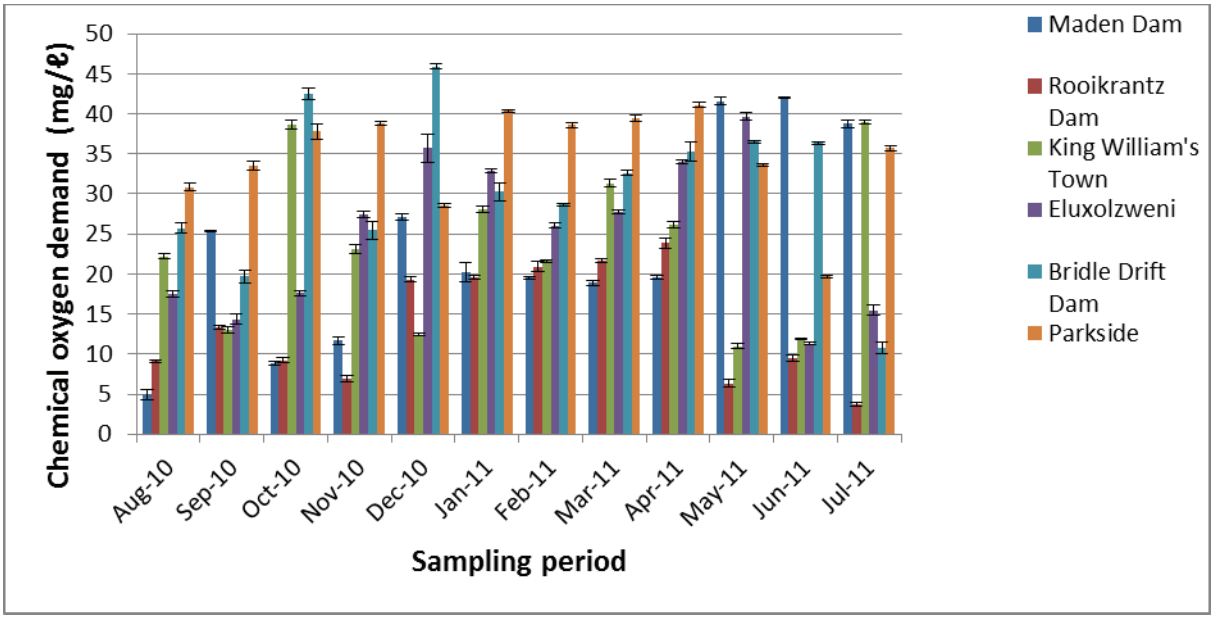

Figure 4.19: Monthly variation in chemical oxygen demand for Buffalo River

This trend of steady increase in COD could be attributed to an increase in both organic and inorganic substance from the environment, as well as organic loading from the municipal sewage treatment plants. Elevated levels of COD in water systems lead to drastic oxygen depletion which adversely affects aquatic biota (Fatoki et al., 2003). Apart from the Rooikrantz Dam, Eluxolzweni and Parkside sites, where mean COD levels were significantly lower (P<0.05) in winter, no distinct pattern was observed across the seasons.

Figure 4.20 shows the monthly variation in nitrite concentrations in the Buffalo River. Nitrite concentrations ranged between 0.02 mg/ℓ and 0.21 mg/ℓ. Nitrite (NO_2^-) is the inorganic intermediate, and nitrate (NO_3^-) the end-product, of the oxidation of organic nitrogen and ammonia (DWAF, 1996b). Nitrite concentrations <0.5 NO_2^- as N/ℓ are symbolic of oligotrophic conditions (DWAF, 1996c).

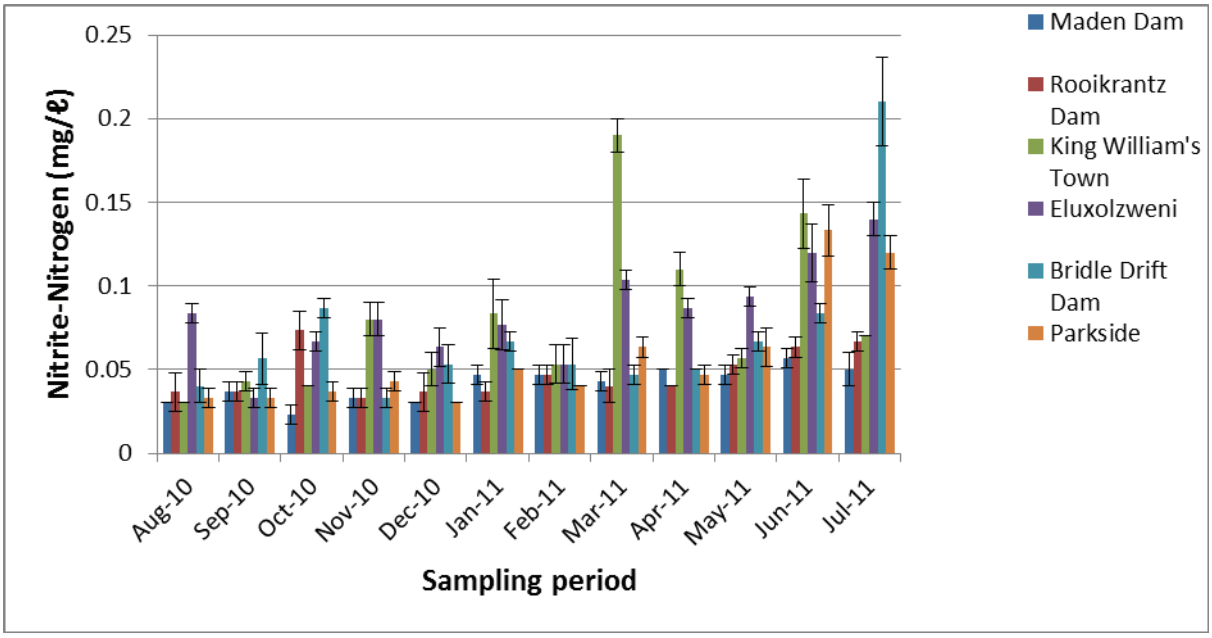

Figure 4.20: Monthly variation in nitrite concentrations for Buffalo River

Figure 4.21 shows the monthly variation in nitrate concentrations in the Buffalo River. Nitrate levels varied significantly with sites (P<0.05) as shown by the higher concentrations observed at Eluxolzweni (1.4 mg/ℓ to 4.5 mg/ℓ) compared to 1.3 mg/ℓ to 2.4 mg/ℓ, 1.0 mg/ℓ to 3.2 mg/ℓ, 1.3 mg/ℓ to 4.0 mg/ℓ, 1.1 mg/ℓ to 3.2 mg/ℓ and 1.1 mg/ℓ to 3.5 mg/ℓ observed at Maden Dam, Rooikrantz Dam, King William's Town, Bridle Drift Dam and Parkside, respectively.

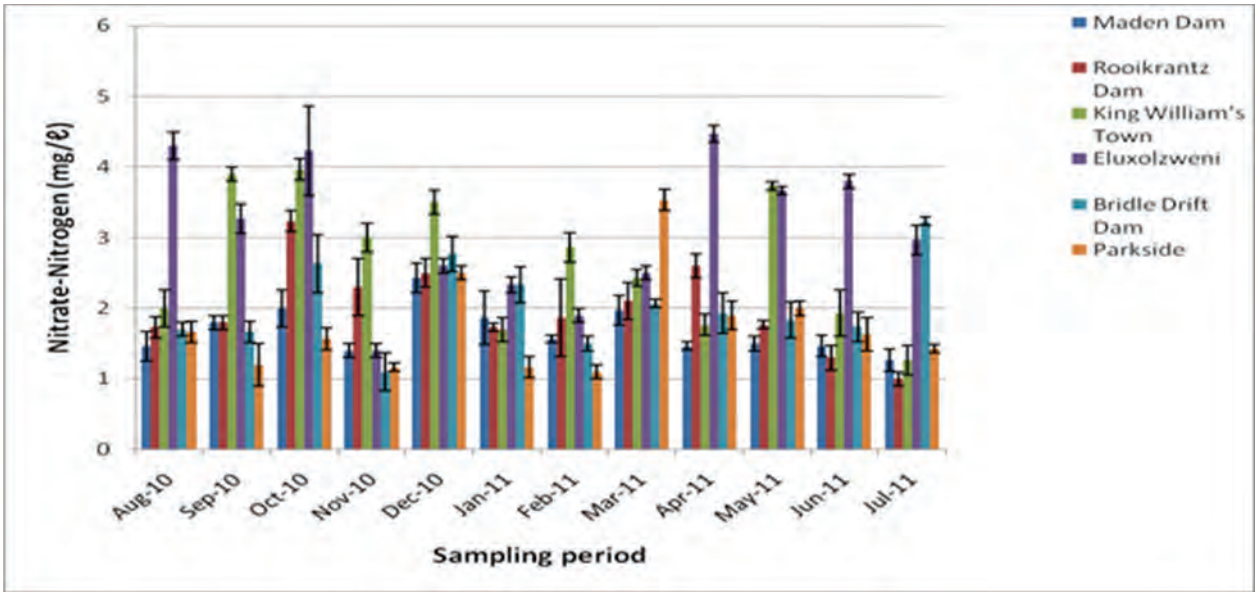

Figure 4.21: Monthly variation in nitrate concentrations for Buffalo River

The Eluxolzweni sampling site is impacted with organic matter from municipal wastewater discharging effluents into the river. The level of algal growth observed at Eluxolzweni should not be overlooked. Blooms of various planktonic species are directly or indirectly hazardous to human and animal health (Hitzfeld et al., 2000; Hunter, 2003; Harding et al., 2009). However, the observed nitrate levels (range: 1 mg/ℓ to 4.47 mg/ℓ) did not exceed the standard limits, and therefore, it appears that the waters represent no nitrate-mediated

public health hazard. According to the Water Institute of Southern Africa (WISA), nitrate concentrations of 6 mg/ℓ to 10 mg/ℓ would cause insignificant risk.

Figure 4.22 shows the monthly variation in phosphate concentrations in the Buffalo River. The concentrations of phosphate recorded in this study ranged between 0.01 mg/ℓ and 1.72 mg/ℓ. The highest concentration of phosphate (1.72 mg/ℓ) was observed in the summer month of December at Eluxolzweni and the least (0.01 mg/ℓ), at Maden Dam in the winter month of August. Concentrations of total phosphate in the water samples exceeded the standard limit (0.1 mg/ℓ) of the US Public Health Standards (Solaraj et al., 2010) at 5 of the 6 sites. It has been documented (Ekholm and Krogenus, 1998) that municipal wastewater contains substantial amounts of phosphorus contributed by human urine and detergents. This may account for the significantly higher phosphate concentrations observed at Eluxolzweni, which is heavily affected by municipal sewage and the wastewater-treatment plant discharges. High concentrations of phosphates and nitrates increase the growth of vegetation in water systems and elevate oxygen demand (McEldowney et al., 1993). Stretches of Buffalo River downstream of King William's Town are characterised by extensive growth of water weeds and algal blooms which may be indicative of eutrophication. However, besides its contribution to eutrophication and toxic algal blooms, phosphate does not have notable adverse health effects (WHO, 2008).

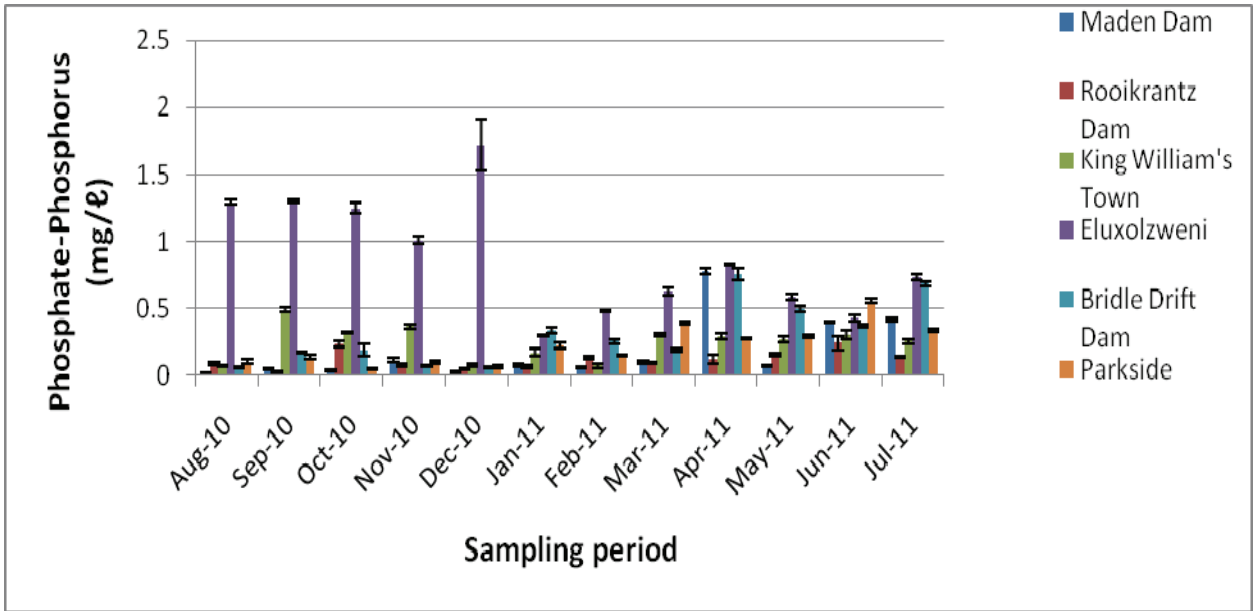

Figure 4.22: Monthly variation in phosphate concentrations for Buffalo River

Conclusions

Results indicate that the Buffalo River is negatively impacted by physicochemical pollutants, either directly or indirectly from settlements and/or anthropogenic activities.

CHAPTER 5: BACTERIAL SURVEY AND ASSESSMENT

5.1 INTRODUCTION

Water pollution remains a global problem. Contaminated surface waters used for domestic, agricultural and recreational purposes constitute public hazards (Chigor et al., 2012) and have been implicated in outbreaks of enteric infections (WHO, 2009; Chigor et al., 2010). South Africa is a semi-arid, water-stressed country (Muller et al., 2009) sourcing water largely from rivers and dams (Odjadjare and Okoh, 2010). These surface waters are susceptible to faecal pollution and contamination with a wide spectrum of human enteric pathogens. Available statistics (based on Census 2001 information) show that of the roughly 7.3 million residents living in the Eastern Cape, only 13.6% have access to piped water either in their dwelling place or within 200 m of their dwelling places (Municipal Demarcation Board, 2009). Consequently, many households rely solely on untreated river water (RHP, 2004).

Both the Tyume River and Buffalo River catchments support rapidly growing population bases on the east coast of South Africa that are dependent to a large extent on these rivers, hence information on the bacteriological qualities of the water becomes imperative. Members of the faecal coliform group and enterococci have been traditionally used to assess the microbiological safety of water since the advent of water research (Ahmed et al., 2010; Ahmed et al., 2006; Anderson et al., 2005). We here present the results of the bacteriological quality of the rivers.

5.2 METHODOLOGY

5.2.1 Bacteria

Analyses of total coliforms (TC), faecal coliforms (FC) and enterococci were carried out by membrane filtration method in accordance with *Standard Methods* (2005).

Total coliforms

For TC, samples were processed by making serial dilutions and filtering 100 mℓ of water through membrane filters (47-mm diameter, 0.45 µm pore size). Thereafter, the Millipore filter papers were placed on m-Endo agar and incubated at 37°C for 24 h. Typical red colonies with a metallic sheen were enumerated and reported as CFU/100 mℓ surface water.

Faecal coliforms

For the enumeration of FC, water samples were filtered as described above and the Millipore filter paper was placed on m-FC agar and incubated at 44.5°C for 24 h. Colonies that exhibiting any shades of blue were counted and reported as CFU/100 mℓ surface water.

Enterococci

Enterococcus selective agar (Merck), a selective medium for faecal enterococci containing bile and esculin, was used with the Millipore filtration technique as a one-step identification of faecal enterococci. After incubation at 37°C for 48 h, all brown to black colonies with a typical dark halo were counted as faecal enterococci. The *E. faecalis* ATCC 29212 was used as a positive control.

5.2.2 Statistical analysis

All data were subjected to descriptive statistical analysis (95% confidence limit). The generalized linear model (GLM) of SAS was used to generate analysis of variance (ANOVA), means, standard errors and ranges. Tukey's Studentized Range (HSD) Test was used to test differences among all possible pairs of treatments. Correlation was performed using PROC CORR procedure of SAS (SAS Version 8, SAS Institute, Cary, NC).

5.3 RESULTS AND DISCUSSION

5.3.1 Tyume River catchment

Figure 5.1 shows the average counts for total coliforms (TC). The average counts ranged as follows: 2.1×10^2 to 9.5×10^3 CFU/100 mℓ, 3.5×10^2 to 1.2×10^4 CFU/100 mℓ, 1.7×10^3 to 1.3×10^4 CFU/100 mℓ, 2.0×10^4 to 5.3×10^4 CFU/100 mℓ, 1.4×10^4 to 3.4×10^4 CFU/100 mℓ and 2.6×10^3 to 1.8×10^4 CFU/100 mℓ for Hala, Khayalethu, Sinakanaka, Alice, Drayini and Manqulweni, respectively. Total coliform counts differed significantly with sampling month on the same sites ($P<0.05$), as well as from site to site within months ($P<0.05$). However, TC counts were always highest at the Alice sampling site from September 2010 through to May 2011. Over the same period, the second highest TC counts per month were obtained at Drayini sampling site. The same trend characterised both the FC and enterococci counts. Both Alice and Drayini sites are located downstream from effluent discharge points at Alice Town and the University of Fort Hare Wastewater Treatment Plants, respectively. Also, the river stretch in which both the Alice and Drayini sampling points are located passes through settlements with higher population densities than at any other point under this study. The obtained results may therefore be a consequence of both these factors. Manqulweni, by virtue of being downstream from both Alice and Drayini sites, had higher coliform and

enterococci counts compared to each of the three upstream sampling sites (Hala, Khayalethu and Sinakanaka).

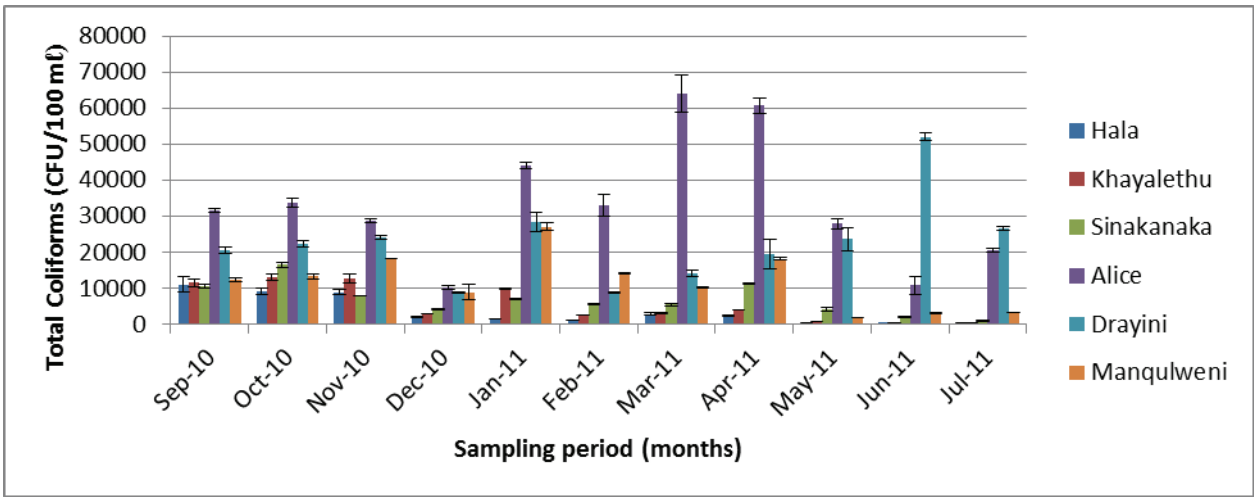

Figure 5.1: Monthly average TC counts for selected sites on Tyume River

Average counts for FC ranged as follows; 1×10^2 to 7.2×10^2 CFU/100 mℓ, 1.3×10^2 to 7.4×10^2 CFU/100 mℓ, 1.9×10^2 to 8.7×10^2 CFU/100 mℓ, 3.2×10^3 to 1.2×10^4 CFU/100 mℓ, 1.3×10^3 to 1.6×10^4 CFU/100 mℓ and 2.3×10^2 to 2.0×10^3 CFU/100 mℓ for Hala, Khayalethu, Sinakanaka, Alice, Drayini and Manqulweni, respectively. Results are displayed in Figure 5.2 below.

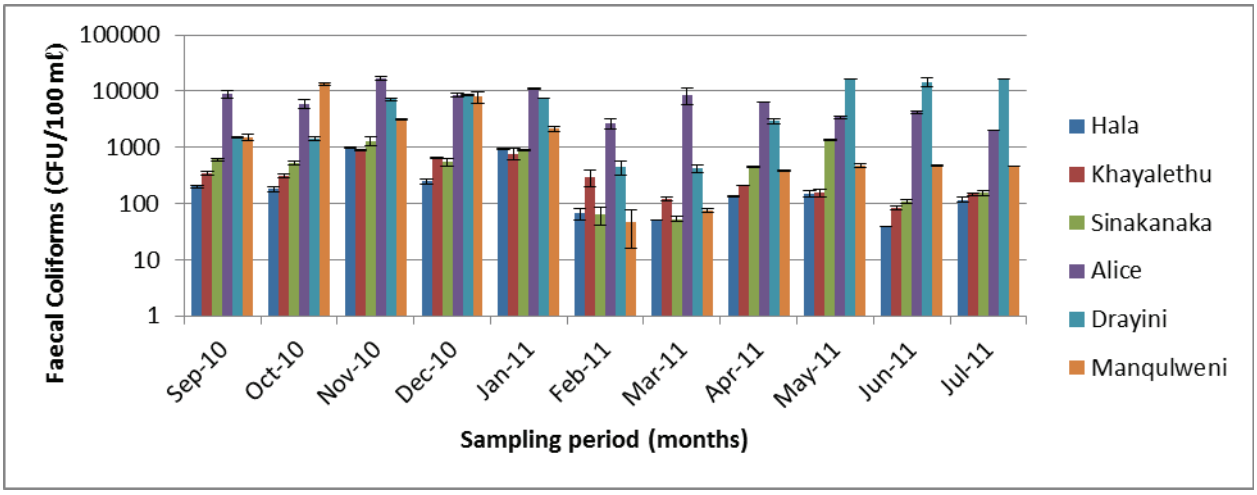

Figure 5.2: Monthly average FC counts for selected sites on Tyume River

For enterococci average counts ranged as follows; 3.3×10 to 5.2×10^2 CFU/100 mℓ, 4.3×10 to 1.3×10^2 CFU/100 mℓ, 8.2×10 to 2.0×10^2 CFU/100 mℓ, 2.1×10^2 to 5.1×10^3 CFU/100 mℓ, 1.4×10^2 to 3.0×10^3 CFU/100 mℓ and 9.2×10 to 1.2×10^3 CFU/100 mℓ for Hala, Khayalethu, Sinakanaka, Alice, Drayini and Manqulweni respectively. Figure 5.3 shows the average enterococci counts obtained at the selected sites from September 2010 through to July 2011.

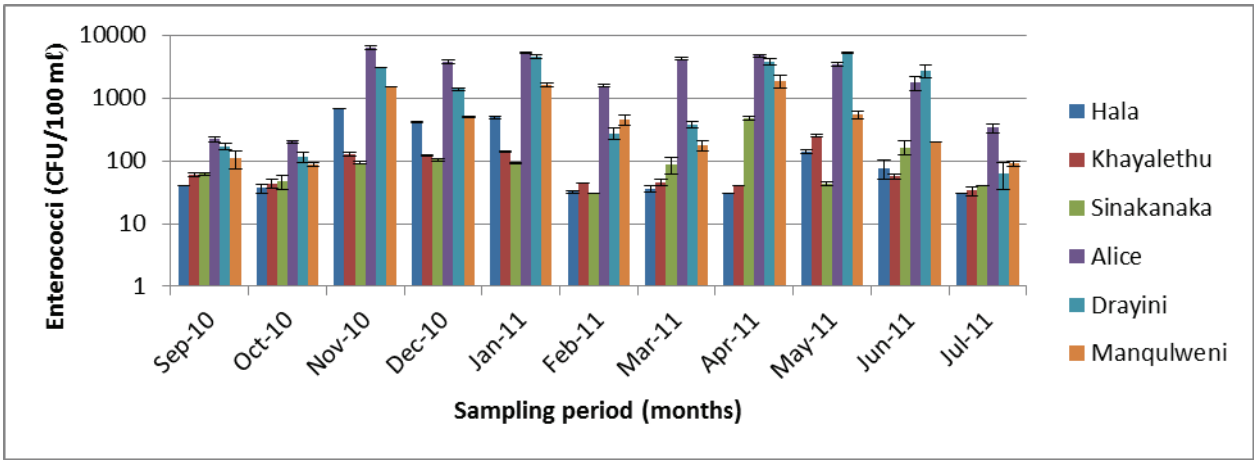

Figure 5.3: Monthly average enterococci counts for selected sites on Tyume River

The presence of FC and enterococci indicate faecal pollution as both indicators are associated with human and animal faeces. Faecal pollution, in turn, reveals the potential presence of pathogenic enteric microorganisms that are known to be the major cause of waterborne diseases, where diarrhoea is the primary manifestation of such infections worldwide (Griffin et al., 2003). The bacteriological quality of the water, as suggested by the FC and enterococci average counts, which exceeded the guideline of 600 CFU/100 mℓ and 100 CFU/100 mℓ for faecal coliforms and enterococci, respectively, for recreational water (DWAF 1996b), may be considered to be poor. The elderly or immunocompromised people may be at a higher risk of health damage from microbial deterioration of water quality because they are more susceptible to pathogenic organisms (National Health and Medical Research Council, 2008).The microbial quality of recreational water may be strongly influenced by factors such as rainfall in the river catchment. This can potentially lead to relatively short periods of elevated faecal pollution arising from animal wastes washed from forests, pastures and urban land as well as the re-suspension of sediment-trapped pathogens; a particular problem in freshwater river catchments (National Health and Medical Research Council, 2008).

Though indicator bacteria are usually harmless, more plentiful, and easier to detect than pathogens, they are used to determine the relative risk of the presence of enteric pathogenic microorganisms like viruses, *Shigella*, *Salmonella* and *Vibrio* in a water sample (Toze, 1998; Ahmed et al., 2006; Wilhelm and Maluk, 1998). Presence of faecal indicator bacteria also indicates contamination with sewage effluent or with runoff from soil and other land surfaces (Toze, 2005; Ahmed et al., 2010). Bathing water with more than 126 *E. coli* or 33 enterococci per 100 mℓ over a 30-day period presents a health risk (Alm et al., 2003). An emerging issue on the bacterial pathogen scene is various haemorrhagic *Escherichia coli* found in faecal matter of domestic or farm animals, such as type O157:H7, which are highly infectious in low numbers (Teunis et al., 2004). At the level of bacterial contamination detected in this study, there is a risk of contracting gastrointestinal illness as a result of full-contact recreation or direct consumption of untreated water. Based on visual observations made during sampling visits, public defecation is a common practice, especially around Alice and Drayini where population density is high. According to Ashbolt et al. (2001), the higher the number of people contributing to sewage or faecal contamination, the more likely the presence of a range of pathogens.

The Tyume River is also open to access by cattle and other livestock adding to the deterioration of the bacterial quality of the water since cattle (and cattle dung) were usually visible at some spots along the river during sampling visits. Rivers can provide a constant supply of oxygen, organic, and inorganic compounds as well as nutrients essential for bacterial growth and survival (Bernier et al., 2009). This can result in persistence especially of faecal bacteria.

Also, enterococci concentrations were always lower than FC concentrations, probably because enterococci survive less easily in river water, in part because of their sensitivity to photo-oxidation (Bernier et al., 2009). However, enterococci and *E. coli* have been deemed to be better indicators of faecal pollution than faecal bacteria in general since these bacteria are exclusively found in the intestine of warm-blooded animals. Their presence in surface waters indicates that recent contamination has occurred by humans or other warm-blooded animals (Ahmed et al., 2006).

Moving forward, since faecal contamination of surface waters has been affirmed, there may be a need for future research to focus on the assessment of these surface waters for the presence of bacterial pathogens. Microbial source tracking (MST) may also help to ascertain the origin of this faecal pollution, which data may be useful in pollution-mitigation measures. Educational campaigns aimed at reducing risks of contracting waterborne illnesses may also need to be conducted in communities who rely directly on surface waters for domestic uses. Recreational water managers may take steps to identify periods when water quality is poor, and issue advisory notices warning the public of increased risk. Another way of protecting public health may be to permanently discourage its recreational use, for example by fencing or signposting.

Conclusions

The presence of FC and enterococci indicates that Tyume River water is faecally polluted. This means that the water may not be fit for human consumption and full-contact recreational activities.

5.3.2 Buffalo River catchment

Figures 5.4 to 5.6 show the profiles of faecal indicator bacterial counts of the Buffalo River water samples. Across the sites, mean TC counts ranged between 1.9×10^2 CFU/100 mℓ and 3.8×10^7 CFU/100 mℓ, while FC and enterococci counts ranged between 3.0×10 CFU/100 mℓ and 3×10^5 CFU/100 mℓ and 1.2×10^1 - 5.3×10^5 CFU/100 mℓ, respectively. Faecal indicator bacteria counts recorded in this study are high. In the Buffalo River catchment, blockages in the sewerage systems, inadequate treatment capacity and poor management result in the discharge of partially treated and untreated sewage into the river and dams (RHP, 2004). Also implicated sources are urban wastewater and stormwater runoffs. It is worth noting that rainfall and storm events occurred across the seasons during the study period. Previous reports have shown that extreme rainfall and runoff result in significant increases in microbial loads (Kistemann et al., 2002; Chigor et al., 2012).

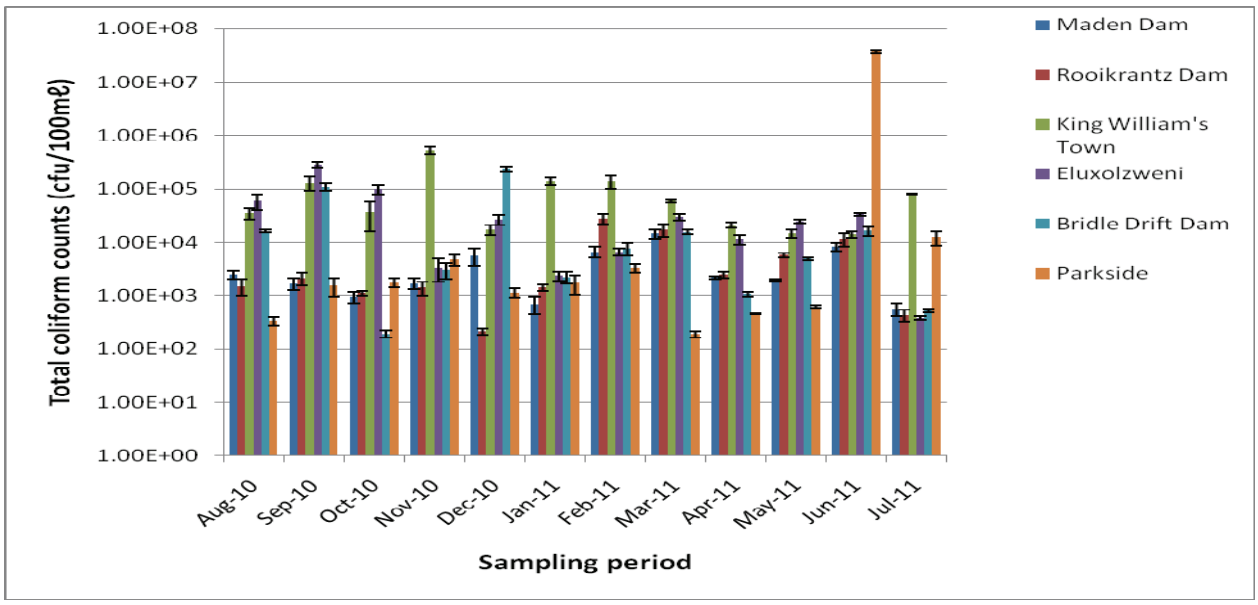

Figure 5.4: Monthly variation in concentrations of total coliforms in Buffalo River

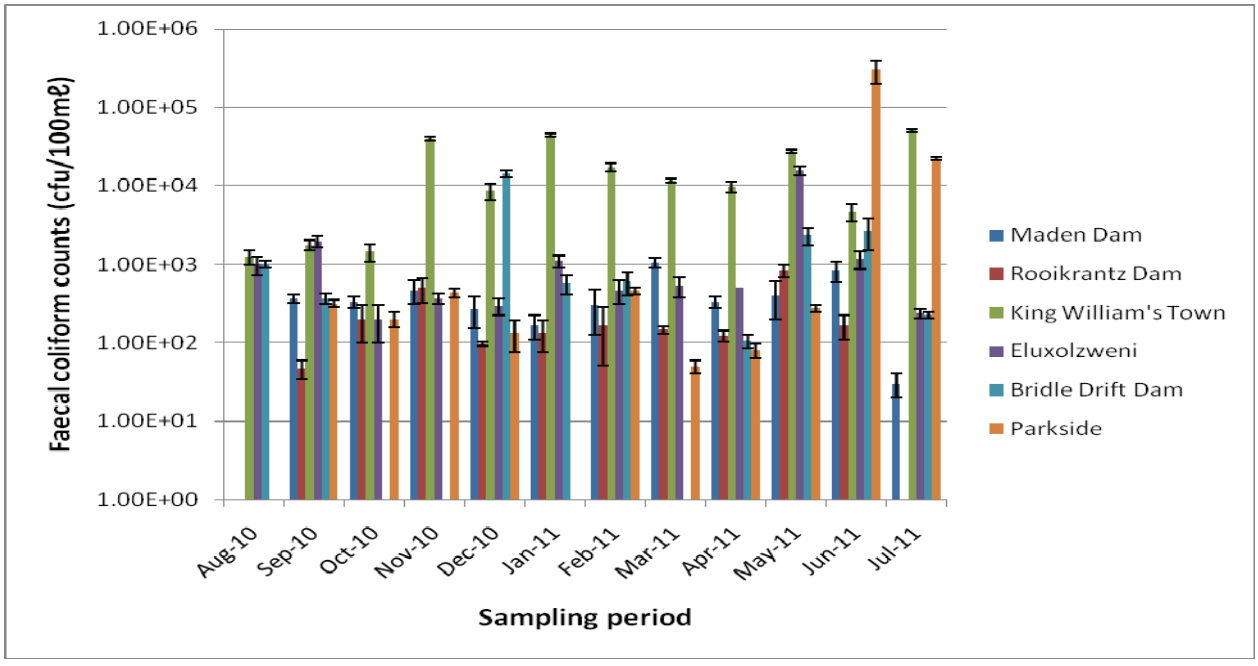

Figure 5.5: Monthly variation in concentrations of faecal coliforms in Buffalo River

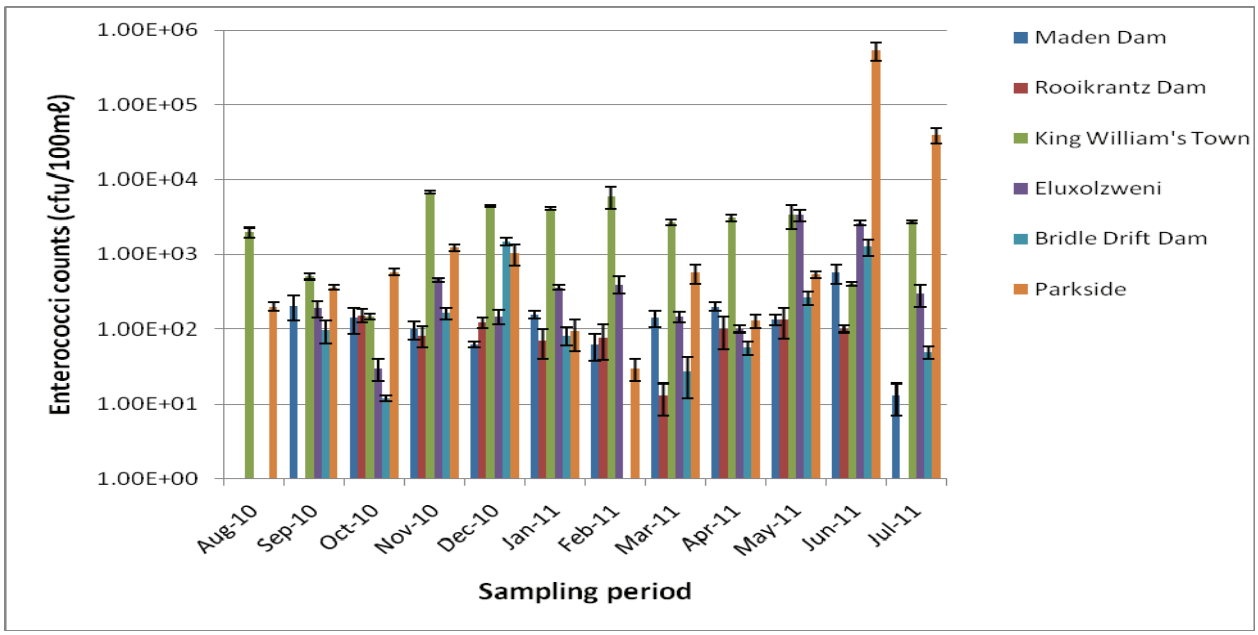

Figure 5.6: Monthly variation in concentrations of enterococci in Buffalo River

A uniform trend was observed for the three bacteriological parameters tested. Generally, higher counts of TC, FC and enterococci were recorded at the sampling sites located at the lower reaches of the river compared to Maden Dam and Rooikrantz Dam at the upper reaches. This could be attributable to anthropogenic activities and increased populations. Expectedly, the general trend at all the sites was that TC counts were significantly (P<0.05) higher than FC counts. Across the sites, FC counts in this study were generally higher than the enterococci concentrations, except at Parkside where the mean enterococci counts were higher than FC counts. Enterococci survive the harsh environments and extremes of salinity recorded in this study (range 32.47 mg/ℓ to 33.62 mg/ℓ) that are associated with river estuaries (He and Jiang, 2005). The finding that enterococci concentrations were always lower than FC concentrations is in agreement with previous studies and has been attributed, in part, to the sensitivity of enterococci to photo-oxidation (Bernier et al., 2009) that allows enterococci to survive less easily in river water, compared to faecal coliforms. In a report on the persistence and differential survival of faecal indicator bacteria in subtropical waters and sediments, Anderson et al. (2005), who measured persistence by decay rates (change in culturable counts over time), showed that faecal coliform decay rates were significantly lower than those of enterococci in freshwater.

The average counts of indicator bacteria were compared per month across sampling sites and also per sampling site across months. The FC counts were significantly (P<0.05) higher at King William's Town in 8 of the 12 months including October, November, January, February, March, April, May and July than at all the other sites. In August 2010 and September 2010, although FC counts at King William's Town and Eluxolzweni were significantly (P<0.05) higher than the counts recorded at the rest of the sites, the difference between the means at both sites was not significant. For enterococci, significantly higher counts were recorded at King William's Town than at all the other sites throughout the study period, with the exception of June and July, during which counts at Parkside were significantly higher. The significantly higher counts of the indicator bacteria recorded at King William's Town compared to the other sites suggests that this is the most contaminated site. The general trend suggests that Buffalo River is continuously being

polluted with faecal matter throughout the study period. The unexpectedly high concentrations of indicator bacteria recorded at Parkside (TC, 3.8×10^7 CFU/100 mℓ; FC, 3.0×10^5 CFU/100 mℓ; and enterococci, 5.3×10^5 CFU/100 mℓ) in June, which were also signicantly ($P<0.05$) higher than counts encountered that month at all the other sites, suggest that sewage was discharged into the Buffalo River.

Faecal coliform count should be zero (per 100 mℓ) of sample in all drinking-water supplies, piped or un-piped, treated or untreated, and less than 1 000 CFU/100 mℓ for water used in fresh produce irrigation (WHO, 2008; Chigor et al., 2010a). Bathing water with more than 33 enterococci per 100 mℓ presents a health risk (DWAF, 1996c; Alm et al., 2003). Therefore, Buffalo River water is not suitable for human consumption, without treatment, and for full-contact recreation and irrigation of crops and vegetables that are eaten raw or partially cooked.

Conclusions

The distinct increase in bacterial indicators as the Buffalo River flows from its source downstream through settlements reveals the deterioration in the water quality and reflects the degradatory impact of settlements and anthropogenic activities on the quality of the river water.

CHAPTER 6: VIRAL SURVEY AND ASSESSMENT

6.1 INTRODUCTION

Surveillance of source waters for viral pathogens is necessary to protect public health and although the culture-propagation procedure is still the best method to enumerate viruses and demonstrate their infectivity, they are unsuitable for the detection of hepatitis A virus and other enteric viruses, for which appropriate cell cultures are not available or their growth is limited (Schvoerer et al., 2000). Molecular techniques have been successfully applied on environmental samples, allowing a rapid and specific detection of human enteric viruses (De Paula et al., 2007; Costafreda et al., 2006; Bosch et al., 2008). This chapter reports on the virus component of this study.

6.2 METHODOLOGY

6.2.1 Concentration of viruses in water

Viruses in water samples were concentrated following the adsorption-elution method as described by Haramoto et al. (2005), with some modifications. This method showed recovery yields of 56% ± 32% (*n* = 37) for surface-water samples inoculated with polioviruses, and it is based on electrostatic interactions. Under neutral pH conditions viruses are negatively charged and are positively charged under acidic conditions. Multivalent cations (Mg^{2+}, Al^{3+}) can change the surface charge of viruses thereby allowing adsorption to negatively charged membranes. An aliquot of 5 mℓ of 250 mM $AlCl_3$ was passed through an HA filter (0.45 µm pore size and 47 mm diameter, Millipore) attached to a glass-filter holder, to form a cation (Al^{3+})-coated filter. Subsequently, 1 ℓ of the water sample was passed through the filter. A volume of 200 mℓ of 0.5 mM H_2SO_4 was then passed through the membrane and viral particles were eluted with 10 mℓ of 1 mM NaOH. Eluates were carefully placed in a tube containing 0.1 mℓ of 50 mM H_2SO_4 and 0.1 mℓ of 100x Tris-EDTA (TE) buffer for neutralisation before further concentration. The concentrate was subjected to further concentration using Centriprep YM-50 ultrafiltration device (Millipore) to obtain a final volume of approximately 700 µℓ. Further filtration and concentration of more water samples were done to have a final volume of concentrate of about 2 mℓ. The concentrates were pooled together per sample and stored at -80°C until ready for use. Storage of viruses at temperatures of below -60°C has been shown to result in insignificant loss of both titre and infectivity for periods longer than a decade (Gould, 1999; Merrill et al., 2012).

6.2.2 Extraction of viral nucleic acids

Two sample aliquots (200 µℓ each) of concentrated virus samples (per target virus) were prepared one set was spiked with the specific virus controls for quality assurance while the other set was not, and both sets

were used for the extraction of viral nucleic acids and purification with commercially available kits following the manufacturer's protocol. RNA (for the RNA viruses: EnVs, NoVs, RoVs and HAV) was extracted using 200 µℓ of the final concentrated sample using commercial RNA purification kits, Quick-RNA^{TM} MiniPrep (Zymo Research, USA) to obtain a final volume of 60 µℓ. This method (Boom et al., 1990) is based on the lysing and nuclease-inactivating properties of the chaotropic agent guanidinium thiocyanate (GSCN) together with the nucleic acid-binding properties of silica particles in the presence of this agent. The viruses were first lysed in a column containing silica gel-based membrane and GSCN that inactivates RNase to ensure isolation of intact viral RNA. The mixture was then centrifuged to aid the selective adsorption of the viral RNA to the silica gel membrane. A two-step wash to free the bound RNA of contaminants was followed by elution in RNase-free water containing sodium-azide to prevent microbial growth and subsequent recontamination with RNase. To remove contaminating DNA, the eluate was treated with RNase-free DNase followed by heating at 70^{o}C to in activate the DNase. For adenoviruses, DNA was extracted using Quick-gDNA^{TM} MiniPrep (Zymo Research, USA). Purified viral RNA/DNA was eluted in 60 µℓ of RNase-free water.

6.2.3 Quantification of viral genomes by real-time PCR Assays

The concentrations of human enteric viruses in the river water samples were estimated by using quantitative PCR (qPCR) with TaqMan probes. For specific RNA viruses (RoVs, HAV, EnVs), TaqMan reverse transcription QPCR (RT-QPCR) were performed using a StepOnePlus PCR System (OPTIPLEX 755, Applied Biosystems). The primer pair and TaqMan probes used for detection of each virus are summarised in Table 6.1.

Table 6.1: Primers and probes for one-step real-time RT-PCR and qPCR

Enteric Virus	Primers and labelled TaqMan Probe	Reference
Hepatitis A Virus	HAV68 (F): 5'-TCA CCG CCG TTT GCC TAG-3' HAV240 (R): 5'-GGA GAG CCC TGG AAG AAA G-3' HAV150 (P): 5'-FAM-CCT GAA CCT GCA GGA ATT AA- MGBNFQ-3'	Costafreda et al., 2006; Pinto et al., 2009
Enterovirus	EV1 (F): 5'-CCCTGAATGCGGCTAAT-3' EV1 (R): 5'-TGTCACCATA AGCAGCCA-3' EV-BHQ (P): 5'–FAM–ACGGACACCCAAAGTAGTCGGTTC–MGBFQ-1–3'	Gregory et al., 2006; Noble et al., 2006
Rotavirus	JVK (F): 5'-CAGTGGTTGATGCTCAAGATGGA-3' JVK (R): 5'-TCATTGTAATCATATTGAATACCCA-3' JVK (P): 5'-FAM-ACAACTGCAGCTTCAAAAGAAGWGT-MGBFQ-3'	Logan et al., 2006; Jothikumar et al., 2009
Adenovirus	JTVX(F): 5'-GGACGCCTCGGAGTACCTGAG-3' JTVX(R): 5'-ACIGTGGGGTTTCTGAACTTGTT-3' JTVX(P): 5'-FAM-CTGGTGCAGTTCGCCCGTGCCA-MGBFQ-3'	Jothikumar et al., 2005; Xagoraraki et al., 2007; Fong et al., 2009

Abbreviations: F, forward/sense; R, reverse/antisense; P, probe; FAM, 6-carboxyfluorescein (reporter dye); MGBNFQ, minor groove binder/non-fluorescent quencher

Quantification of AdV by qPCR was done following a one-step reaction in a 96-well plate. An aliquot of 5 µℓ of sample DNA was mixed with 20 µℓ of a reaction buffer (containing 12.5 µℓ of 2× TaqMan universal PCR MasterMix [Applied Biosystems], 400 nM sense primer, 400 nM antisense primer, and 250 nM TaqMan probe and PCR grade water (Haramoto et al., 2008) to give a 25-µℓ total reaction mixture. Subsequently, the mixture was added into a well of a 96-well micro-plate and loaded into the thermocycler. Fluorescence data were collected at the end of each cycle.

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

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

The thermal cycling protocols used for the respective viruses are given below:
HAV: 10 min at 95°C for *Taq* activation, and 45 cycles of denaturation at 95°C for 15 s, annealing at 60°C for 1 min, and extension at 70°C for 1 min.

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

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

Adenovirus: 15 min at 95°C for *Taq* activation, followed by 45 cycles of denaturation at 95°C for 10 s, annealing at 55°C for 30 s, and extension at 72°C for 20 s.

Standard curves for all viruses were obtained as described by Haramoto et al. (2008). Briefly, nucleic acids (DNA/RNA) were extracted from positive ATCC strains of each target virus using commercially available extraction kits. RNA extracts would then be converted into cDNA. The DNA/cDNA was then quantified using a Qubit® fluorometer (probes.invitrogen.com/qubit) and diluted by serial tenfold dilution. The sample extracts and standards samples were subjected to real-time PCR simultaneously, followed by analysis using SDS software (Applied Biosystems™) to obtain quantitative data on the titre of viral DNA in a well. Two wells each

were used for the standard, negative control (no template control) and sample, and the average used for subsequent calculations. The total number of viruses in the viral suspensions and eluted samples were estimated by multiplying the titre of viruses per millilitre by the volumes of the samples.

6.2.4 Detection of viral species and serotypes

Adenovirus species and serotypes

Serotype-specific multiplex PCR assays as described by Metzgar et al. (2005) were used to detect the epidemiologically important serotypes, Ad3, Ad7 and Ad21 (belonging to species B), Ad1, Ad2, Ad5, and Ad6 (belonging to species C), and Ad4 (belonging to species E). The primers used are shown in Table 6.2 below. The F species serotypes Ad40 and Ad41 were detected using serotype specific primers K402 and K403. AdV serotypes 40 and 41 were separated by digesting the PCR product with restriction enzyme ACC1 which cannot digest the AdV41 PCR product but restricts the AdV40 PCR product to band size of approximately 94 bp while the AdV41 product remains 152 bp. For quality assurances, the specific virus strains were used as controls.

Table 6.2: Primers for detection of adenovirus serotypes

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

Source: Tiemessen and Nel (1996); Metzgar et al. (2005)

Norovirus genogroups

The norovirus genogroups GI and GII were detected by semi-nested PCR as described by Victoria et al. (2010 b). Obtaining NoV positive control was a major challenge as we could not find it in American Type Culture Collection (ATCC) (nor any other agency) but NoV genogroup GI and GII specific primer sets which target the viral RNA-dependent RNA polymerase gene (Boxman et al., 2006) were used, and reliability of the results was pinned on the expected amplicon band sizes being obtained and also on the fact that these primer sets have been used in previous studies for detecting NoV GI and GII in environmental samples (Victoria et al., 2010a; b). The primer sets are shown in Table 6.3.

Table 6.3: Primers for detection of norovirus genogroups

Genogroup	Primer Sequence	Band size	Reference
Norovirus	JV13I 5'-TCA TCA TCA CCA TAG AAI GAG- 3'	327 bp	Boxman et al*.,* 2006
	JV12Y 5'-ATA CCA CTA TGA TGC AGA YTA- 3'		Victoria et al., 2010a
GI	JV13I 5'-TCA TCA TCA CCA TAG AAI GAG- 3'	187 bp	
	G1 5'-TCN GAA ATG GAT GTT GG- 3'		
GII	JV12Y 5'-ATA CCA CTA TGA TGC AGA YTA- 3'	236 bp	
	Noro11-R 5'-AGC CAG TGG GCG ATG GAA TTC- 3'		

PCR cycling conditions were as follows: 1st round PCR; 3 min at 94°C to activate the *Taq* polymerase followed by 40 cycles of 1 min at 94°C, 1.5 min at 37°C, 1 min at 72°C, and a final extension of 72°C for 7 min. The 2nd round PCR was run under the same conditions as the first round, except that initial *Taq* activation temperature time was increased from 3 min at 94°C to 5 min at 94°C. Amplified products were analysed on ethidium bromide-stained 2% agarose gels.

Enterovirus serotypes

Restriction fragment length polymorphism (RFLP) analysis of PCR products was used to determine the serotype of enteroviruses. Samples positive for enteroviruses were subjected to a RT-PCR-RFLP assay as described by Siafakas et al. (2002, 2003). An aliquot of 5 µℓ of RNA template, 50 pmol of each primer, sense UC53 (5'-TTGTCACCATAACCAGCCA-3') and antisense UG52 (5'-CAAGCACTTCTGTTTCCCCGG-3') and a reaction mixture (containing 1.5 mM $MgSO_4$; 0.2 mM dNTPs, and 5U of AMV reverse transcriptase) was subjected to 45 min of reverse transcription at 48°C. The PCR conditions involved a denaturation step for 1 min at 94°C, annealing for 1 min at 56°C and extension for 1.3 min at 72°C. An aliquot of 10 µℓ of each amplicon was analysed by electrophoresis in 2.5% agarose gel containing 1 µg/mℓ ethidium bromide in Tris-borate-EDTA (TBE) buffer. An aliquot of 20 µℓ of the amplicon was reacted with 20 units of the restriction enzyme *Hpa*II in a reaction buffer prepared with distilled, RNase-free sterile water. The samples were incubated at 37°C for 2 h and the products subjected to electrophoresis in 3% high resolution agarose gel containing 1 µg/mℓ ethidium bromide. The precise length of the restriction fragments produced was determined with the aid of analysis software such as GelPro Analyzer Version 3.0 based on the number and length of restriction fragments produced by the enzyme *Hpa*II. The digestion with this enzyme generates

fragments that divide the enteroviruses into 5 distinct clusters that also correlate with serotype (Siafakas et al., 2003) as shown in Table 6.4.

Table 6.4: Restriction profile of UC53/UC52 RT-PCR amplicons of enterovirus strains cut by *Hpa*II

Serotypes	Genetic cluster	Restriction profile fragments (BP)
CAV2-10,12,14,16; CBV1-4,6; ECV1,2,4,5,6,7,9,13,14,16,17, 18,19,20,21,24,25,27,29,33	Cluster I	213+149+55+18
ENV73; CAV1,13,15,17,18,19,20,21,24	Cluster II	161+148+108+18
PV1;ENV70;ECV3,11,12,15,26	Cluster III	268+149+18
CBV5; ENV71; CAV11,22	Cluster IV	148+121+108+40+8
PV2; PV3	Cluster V	148+148+121+18

Source: Siafakas et al*.,* 2003

Rotavirus group determination

The detection of the epidemiologically important human rotaviruses, species A, B and C was done by a PCR amplification of the antigen-specific inner capsid protein VP6. The primers, Beg9 and End9, RGB-1F and RGB-1R, as well as G8S and G8A were used for the identification of the groups A, B and C respectively following the descriptions of Lai et al. (2005). The primers are as listed in Table 6.5.

Table 6.5: Primers for detection of rotavirus species

Species	Primer	Primer sequence (5'-3')	Band size (bp)	Reference
A	Beg9 End9	GGCTTTAAAAGAGAGAATTTCCGTCTGG GGTCACATCATACAATTCTAATCTAAG	1062	Gouvea et al., 1991
B	GRB-1F GRB-1R	CTATTCAGTGTGTCGTGAGAGG CGTGGCTTTGGAAAATTCTTG	498	Gouvea et al., 1991
C	G8S G8A	GGCATTTAAAAAAGAAGAAGCTGT AGCCACATGATCTTGTTTACGC	1063	Gouvea et al., 1991

PCR cycling conditions for group A and B rotavirus were as follows: 3 min at 95^{o}C followed by 30 cycles of 1 min at 95^{o}C, 2 min at 42^{o}C and 1 min at 72^{o}C and a final extension step of 7 min at 72^{o}C. The PCR assay for group C rotavirus was run at 3 min at 95^{o}C followed by 30 cycles of 1 min at 95^{o}C, 2 min at 48^{o}C and 2 min at 72^{o}C and then a final extension step of 7 min at 72^{o}C. The PCR product was visualised on an ethidium bromide-stained 1% agarose gel.

6.2.5 Controls

Each test included two controls; a positive control consisting of a spiked sample containing a viral concentration near the detection limit of the method and a negative control consisting of PCR-grade water and MasterMix formulation. The entire control virus strains (Table 6.6) used were obtained from ATCC and preserved at -80^{o}C.

Table 6.6: Control viral strains

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

6.2.6 Statistical analysis

Analyses were made using the Statistical Package for the Social Sciences (IBM SPSS Statistics release 19; IBM, USA). One-way ANOVA and Tukey's Studentized Range (HSD) Test were used to test differences among all possible pairs of treatments while Pearson's correlation coefficient and Spearman's rank correlation test were used for correlation studies.

6.3 RESULTS AND DISCUSSION

6.3.1 Tyume River catchment

Human adenovirus (HAdV)

The concentrations of HAdV detected in this study ranged between 1.0 genome copies/ℓ and 8.49×10^{4} genome copies/ℓ. Adenovirus was more prevalent at Alice, Drayini and Manqulweni sampling sites, which are downstream locations, than at the upstream locations (Hala, Khayalethu and Sinakanaka). This may be expected considering the level of anthropogenic activities in areas along the lower stretch of Tyume River,

with the major one being the effluent of treated and semi-treated sewage from Victoria Hospital, Alice Town and the University of Fort Hare being discharged into the river. Figures 6.1, 6.2 and 6.3 show the amplification plot, standard curve and $\log_{10}$genome copies/ℓ obtained after real-time PCR assay for AdV.

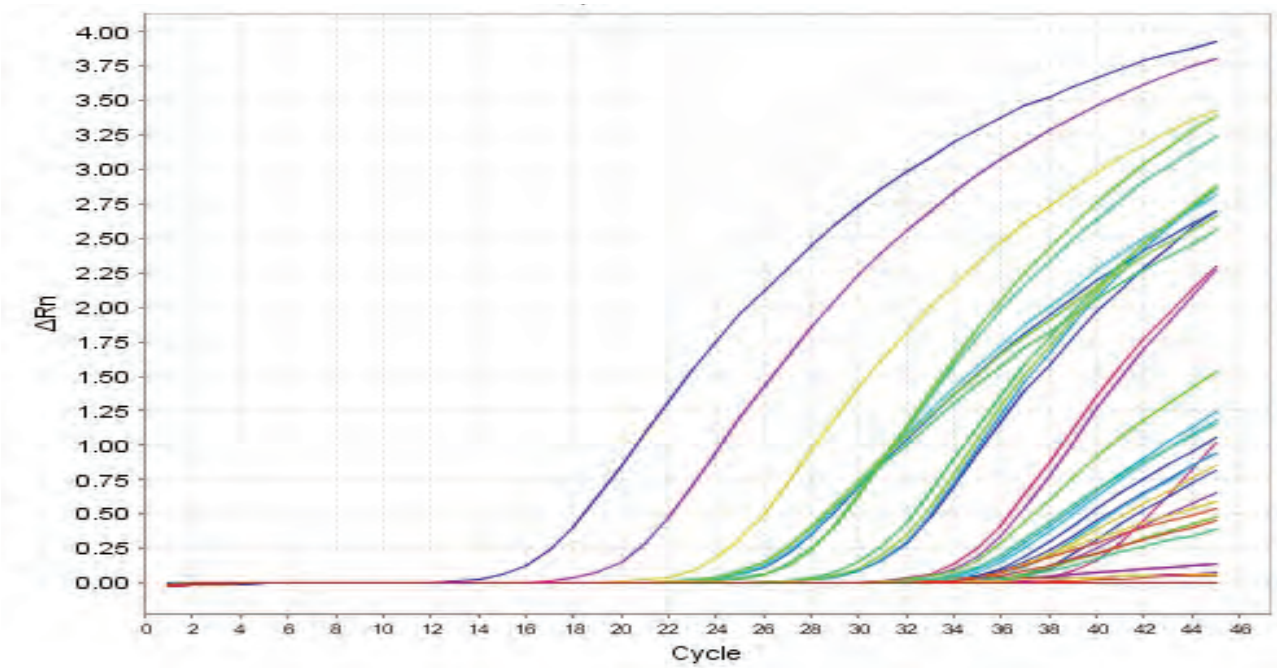

Figure 6.1: Amplification plot for AdV quantitation in Tyume River

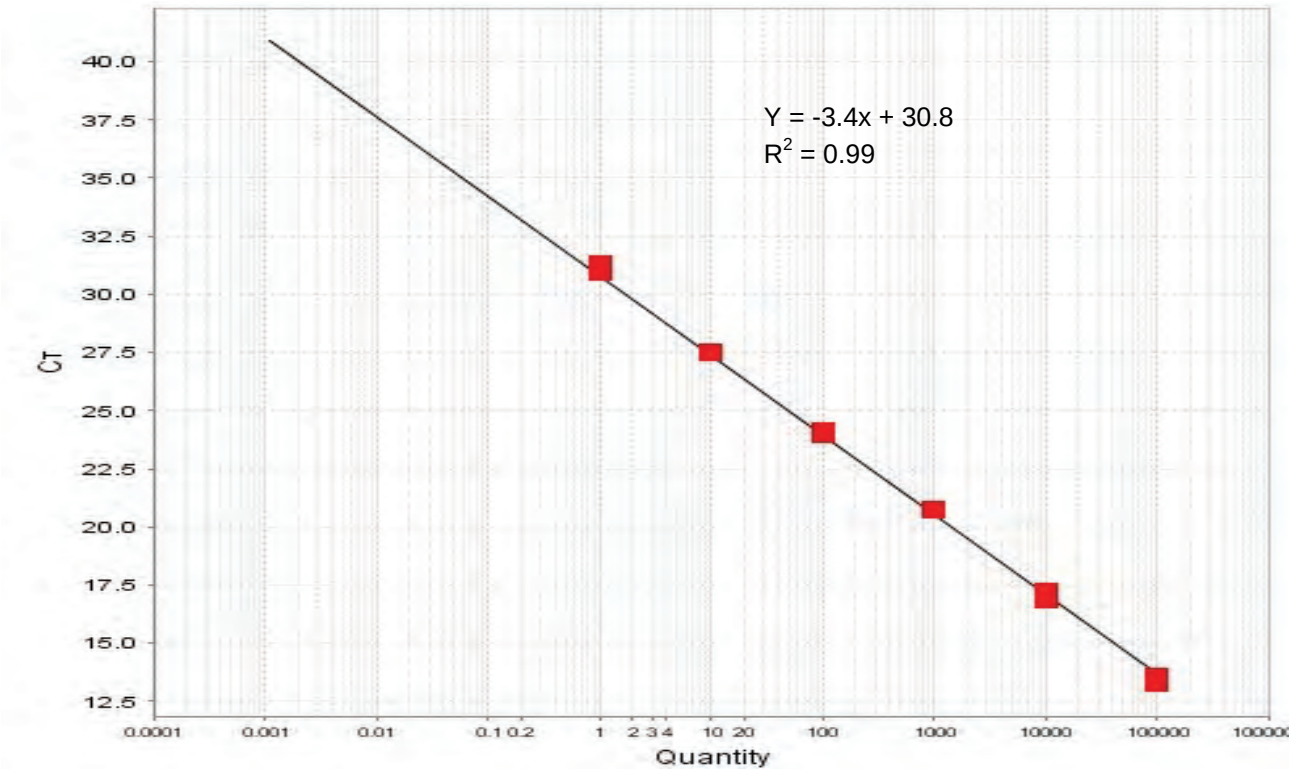

Figure 6.2: Standard curve for AdV quantitation in Tyume River

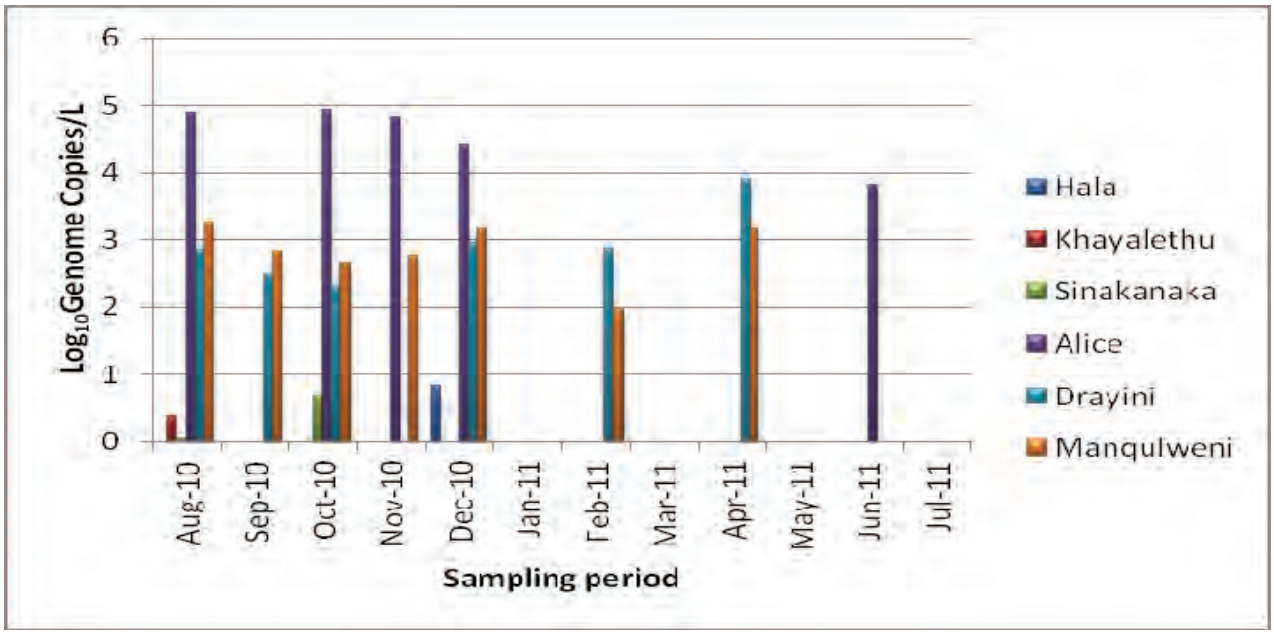

Figure 6.3: Quantitative detection of AdV at the sampling sites on Tyume River using qPCR

Of the 72 samples collected over a 1-year period, 22 samples were positive for adenovirus giving a detection rate of 31%. Of these, 82% (18/22) were collected from downstream sampling points (Alice, Drayini and Manqulweni). Statistical analysis showed that adenovirus detection was significantly higher in the downstream sampling points (P<0.05) compared to the upstream points (Hala, Khayalethu and Sinakanaka). The downstream stretch of Tyume River flows through areas of high population density characterised by the presence of the small town of Alice and the University of Fort Hare whose combined population is approximately 18 000. The higher prevalence of adenovirus in the downstream stretch of the river could therefore be explained in terms of increased human pressure on the environment, notably from the discharge of effluent of domestic and municipal sewage into the river.

The highest concentrations of adenovirus ranging between 6.54×10^3 genome copies/ℓ and 8.49×10^4 genome copies/ℓ were recorded in samples collected from the Alice sampling point between August 2010 and June 2011. This sampling point lies immediately downstream from sewage outfall points from Victoria Hospital and the northern surburbs of Alice which include Ntselamantsi, Lower and Upper Gqumashe. Among the dowstream sampling points, AdV detection rate increased with distance downstream, being 28%, 33% and 39% for Alice, Drayini and Manqulweni, respectively. This trend has been noted in previous studies and attributed to the fact that HAdVs are likely able to survive in effluents of wastewater-treatment plants discharged upstream in the river, survive sunlight inactivation, and be transported to the downstream areas (Xagoraraki et al., 2007).

One-way ANOVA analyses of results showed that adenovirus detection did not differ by season in most sampling points except at Drayini and Manqulweni where its detection significantly differed between winter and spring (P<0.05). This suggests that adenovirus was an all-season contaminant of the river, in agreement with previous findings (Fong et al., 2009), and also probably encouraged by the temperature of the river

which was generally within the range of optimal virus survival throughout the year (<23^{o}C) (Lipp et al., 2001). Also, there was no direct relationship between adenovirus detection in this study and rainfall events as was the case with coliform studies suggesting that the pollution of the river from human sources may be sporadic throughout the year, which is independent of rain events. Similar results have been reported elsewhere (Choi and Jiang, 2005).

Adenovirus characterisation

Because HAdV are double-stranded DNA viruses, they have remarkable stability with regards to several physical conditions such as pH, temperature and moisture. In addition, their resistance to commercially available disinfectants or wastewater treatments contributes significantly to their persistence in the environment (Jiang et al., 2001; Maier et al., 2000; EPA, 1998; Harm, 1980). Real-time PCR positive samples for HAdV were further subjected to multiplex PCR for detection of clinically important adenovirus species B, C and F and their serotypes. Results are displayed in Figures 6.4, 6.5 and 6.6.

Of the 22 samples which were positive for adenovirus by real-time PCR, 59% were positive for Species C adenovirus and of this, 54% were positive for both adenovirus serotypes 6 and 7, 38% were positive for adenovirus serotype 2, while 46% were positive for adenovirus serotype 1 (Figure 6.4). None of the samples were positive for adenovirus species B or A while 18% of the samples were positive for adenovirus Species F, all of which belonged to serotype 41 (Figure 6.5). The HAdV serotypes 40 and 41 have long been recognised as etiological agents of viral gastroenteritis in children (Jothikumar et al., 2005; Cruz et al., 1990; Unhoo et al., 1986). Species B and C adenoviruses have been linked to outbreaks of pharyngoconjunctivitis (Papapetropoulou and Vantarakis, 1998) and may play an important role in the transmission of respiratory diseases in recreational waters through aerosol transmission (Castignolles et al., 1998).

AdC serotypes are also associated with a wide variety of illnesses in immunocompromised patients and, on rare occasions, in healthy adults (Metzgar et al., 2005). The presence of these HAdV serotypes in the river suggests that a significant portion of the human population in this catchment could have suffered from AdV-induced illness especially between August and December 2010 which is the period with 85% of all AdV detections. Since enteric viruses, of which HAdV is one, are present in the faeces of infected patients in high concentrations (Haramoto et al., 2008; Fong and Lipp, 2005), the decline in the detection rate of HAdV in the year 2011 may also be an indication of the declining incidence of HAdV infections among the human population living in the Tyume River catchment. Adenovirus detection in this study seemed to be strongly associated with point-source human faecal pollution, an observation that agrees with previous findings (Aslan et al., 2011).

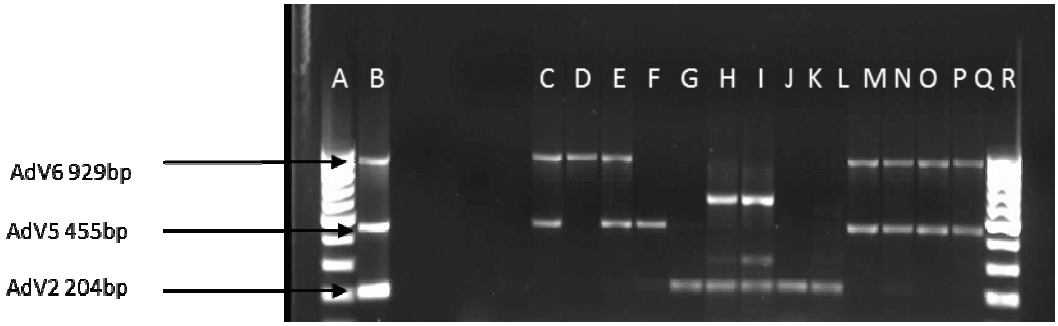

Figure 6.4: EtBr stained agarose gel picture showing HAdV Species C serotypes 2, 5 and 6.
Lane A and R = DNA Ladder; Lane B = positive control; Lane C = negative control; Lane E = Alice (Dec 2010); Lane F = Alice (Nov 2010); Lane G = Alice (Oct 2010); Lane H = Alice (Aug 2010); Lane I = Manqulweni (Dec 2010); Lane J = Manqulweni (Oct 2010); Lane K = Manqulweni (Sept 2010); Lane L = Manqulweni (Aug 2010); Lane M = Manqulweni (March 2011); Lane N = Drayini (Dec 2010); Lane O = Drayini (Sept 2010); Lane P = Drayini (Aug 2010); Lane Q = Drayini (Feb 2011).

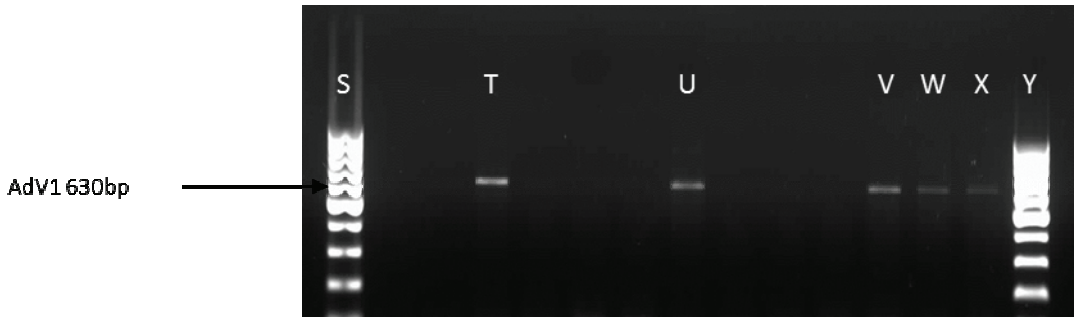

Figure 6.5: EtBr stained agarose gel picture showing HAdV Species C serotype 1.
Lanes S and Y = DNA ladder; Lane T = positive control; Lane U = Manqulweni (Oct 2010); Lane V = Drayini (Dec 2010); Lane W = Drayini (Sept 2010); Lane X = Drayini (Feb 2011).

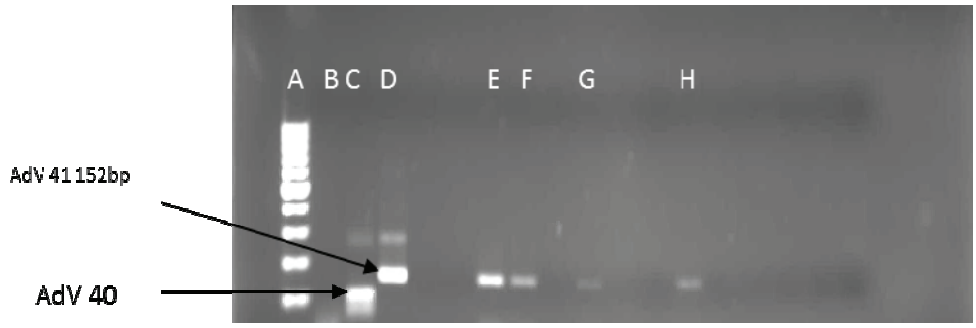

Figure 6.6: EtBr stained agarose gel picture showing HAdV Species F serotypes 40 and 41.
Lane A = DNA Ladder; Lane B = negative control; Lane C = positive control (HAdV 40); Lane D = positive control (HAdV 41); Lane E = Alice (Oct 2010); Lane F = Alice (Aug 2010); Lane G = Drayini (Dec 2010); Lane H = Manqulweni (Sept 2010).

Hepatitis A virus (HAV)

Hepatitis A virus (HAV) was detected in about 13% of the samples in concentrations ranging between 1.67×10^3 genome copies/ℓ and 1.64×10^4 genome copies/ℓ. Of these positive samples, about 56% were collected between October 2010 and January 211 while the other 44% were collected between June and July 2011. Results for real-time PCR detection of HAV are displayed in Figures 6.7, 6.8 and 6.9.

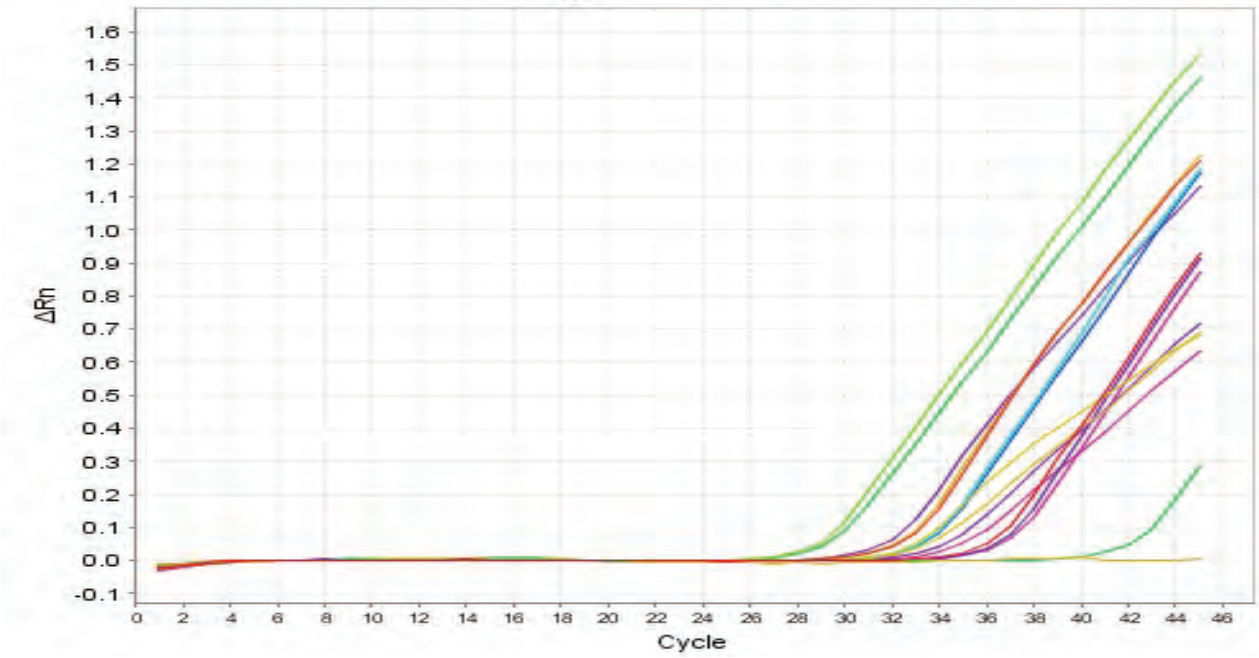

Figure 6.7: Amplification plot for HAV quantitation in Tyume River

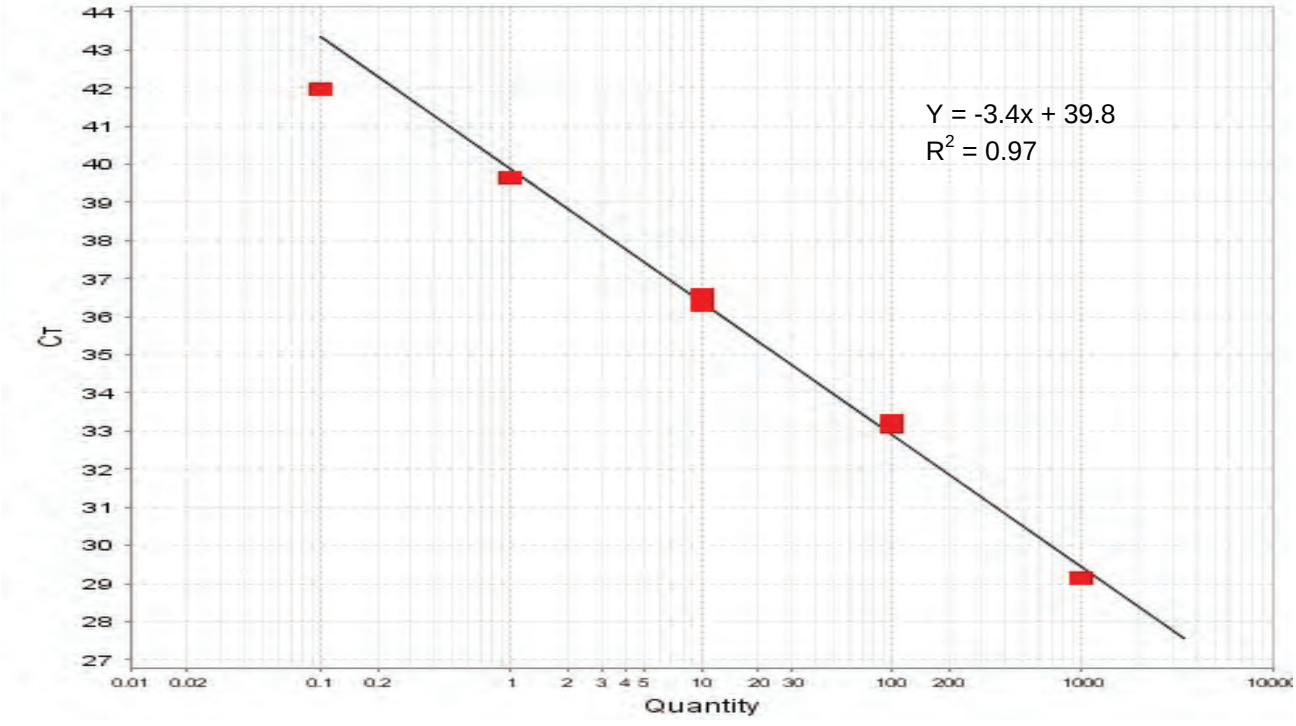

Figure 6.8: Standard curve for HAV quantitation in Tyume River

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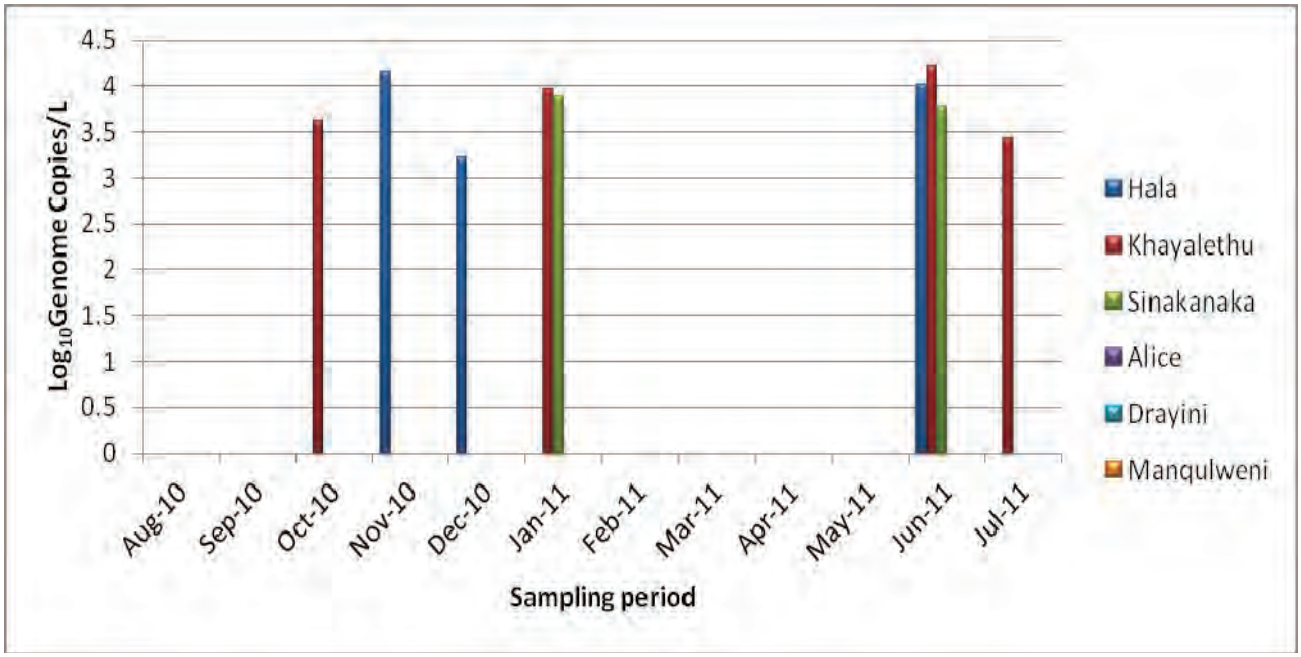

Figure 6.9: Quantitative detection of HAV at the sampling sites on Tyume River using real-time RT-PCR

Statistical analysis showed that HAV detection in this study was not affected by season. HAV infection is the leading cause of acute viral hepatitis throughout the world (Costafreda et al., 2006). One interesting observation from this study is that the HAV was detected only in samples collected from the upstream sampling sites where population pressure is less compared to the downstream stretch of Tyume River. The communities along this stretch are rural with homes situated further apart unlike in urban settlements (which characterises communities along the downstream stretch of the river). They may therefore not have an established sewer reticulation system which could help to reduce or remove viruses (especially RNA viruses since they are more susceptible to disinfection processes) from faecal matter before discharging into the environment. Untreated or insufficiently treated wastewater plays an important role in the transmission of HAV (Mara, 2000; Espigares et al., 1999; Graff et al., 1993).

It is well documented that the distribution patterns of HAV in different geographical areas of the world have been found to be closely related to socioeconomic development, with endemicity being high in less developed regions (Costafreda et al., 2006; Fernandez-Molina et al., 2004). Viral contamination of water sources has been frequently reported as a primary source of gastro-enteritis or hepatitis outbreaks (Brassard et al., 2005). The structural characteristics of HAV make it a very stable virus, largely resistant to physical-chemical agents (De Paula et al., 2007; Ferna´ndez-Molina et al., 2004). Consequently, HAV can survive in water for long periods of time (Soule et al., 1999).

Because only a few viral particles are needed to cause disease, detection of low concentrations of the virus in water becomes significant (De Paula et al., 2007). Survival of naked RNA is limited in the environment

(Tsai et al., 1995) and this, coupled with the fact that only viral capsids and not naked RNA bind to the membrane used for the selective recovery of viruses (Katayama et al., 2002), implies that the RNA found in the water is most likely accompanied by virus particles and would most probably cause infection. Also, because viruses have been reported to survive and remain infective for up to 130 d in seawater, and for up to 120 d in freshwater and sewage (Fong and Lipp, 2005), this may further buttress the opinion that RNA viruses detected by PCR could be infectious and the data may as well be used for risk assessment. The real-time PCR technique is an efficient tool in detecting HAV because it combines PCR amplification with the use of a probe to confirm the identity of the PCR product (De Paula et al., 2007).

Rotavirus (RoV)

RoV RNA was detected in about 4% (3/72) of the samples collected over a 1-year period in concentrations ranging between 9 genome copies/ℓ and 5.64×10^3 genome copies/ℓ. Results are displayed in Figures 6.10, 6.11 and 6.12.Of the positive samples about 67% were collected during the winter months (June and July 2011) while 33% were collected in September 2010 (spring), in support of previous findings which noted that whereas RoV infections are common all-year round in tropical climates (Cook et al., 1990), RoV levels in the environment are generally higher during winter and spring (Hejkal et al., 1984), corresponding to seasonal variations of rotaviral diarrhoea in the population (Mehnert and Stewien, 1993).

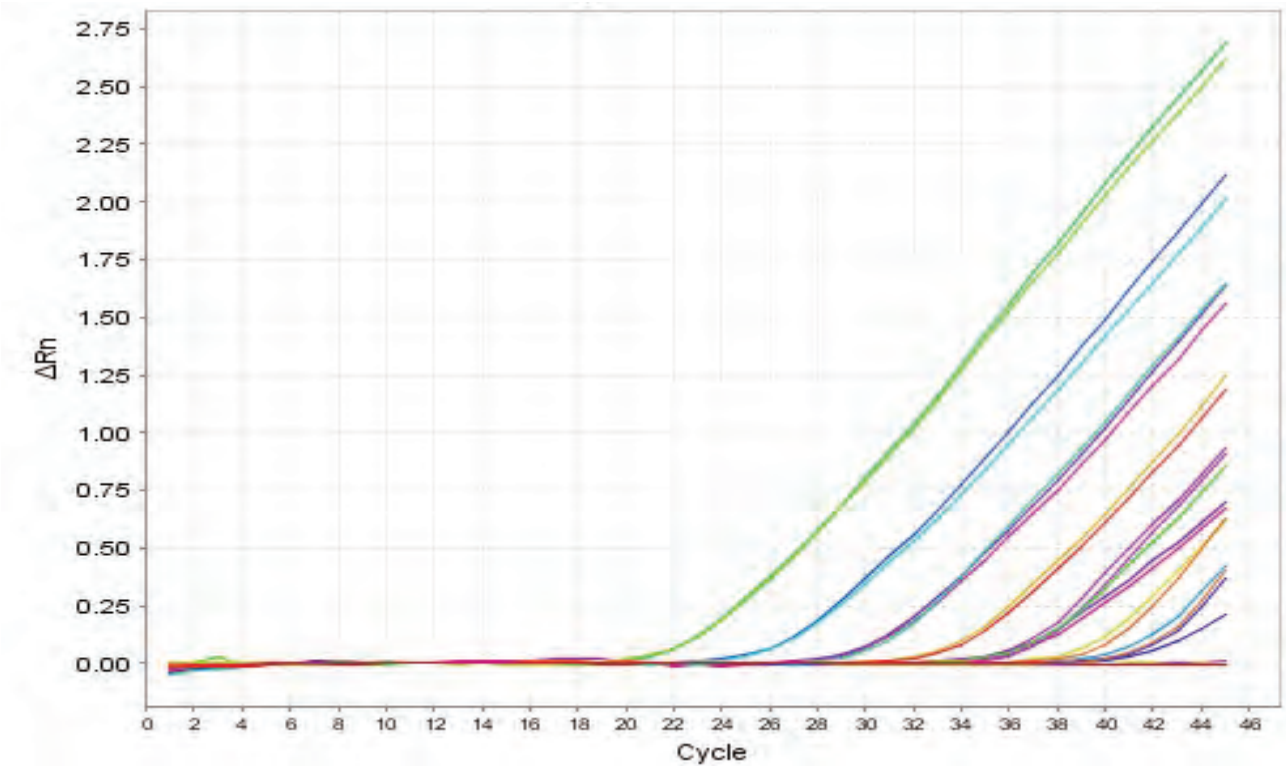

Figure 6.10: Amplification plot for RoV quantitation in Tyume River

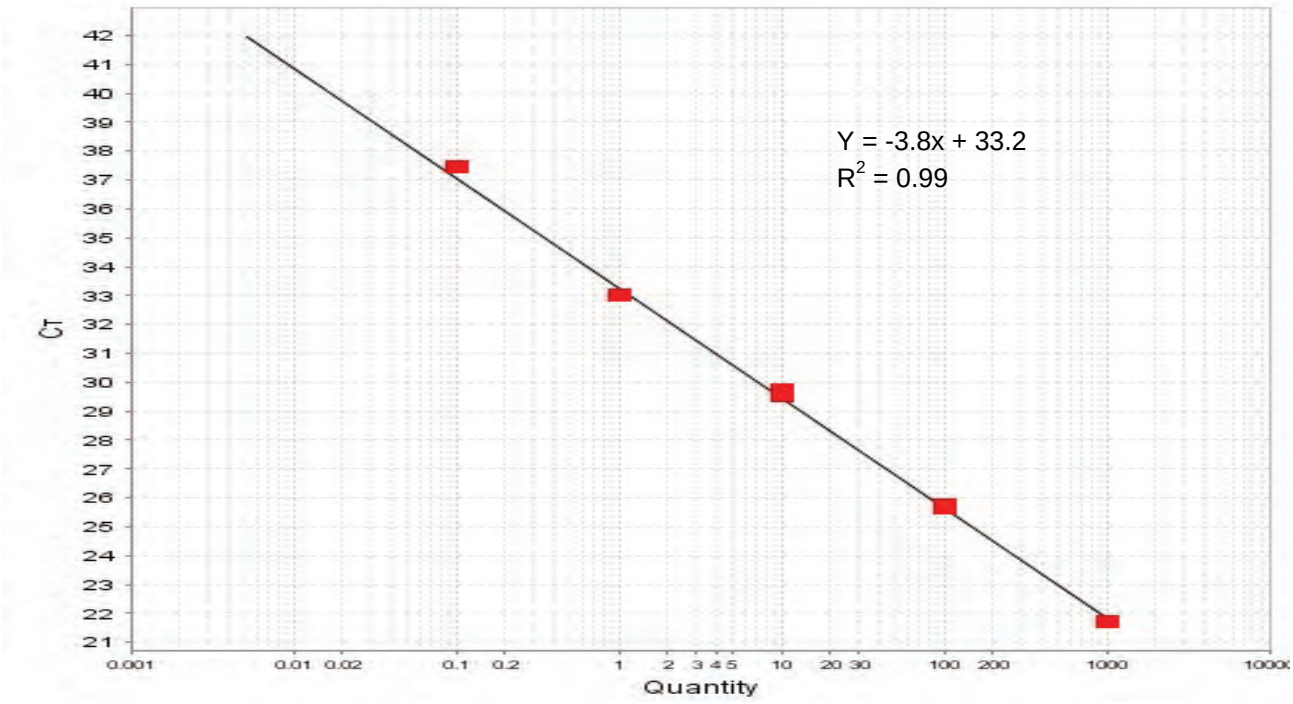

Figure 6.11: Standard curve for RoV quantitation in Tyume River

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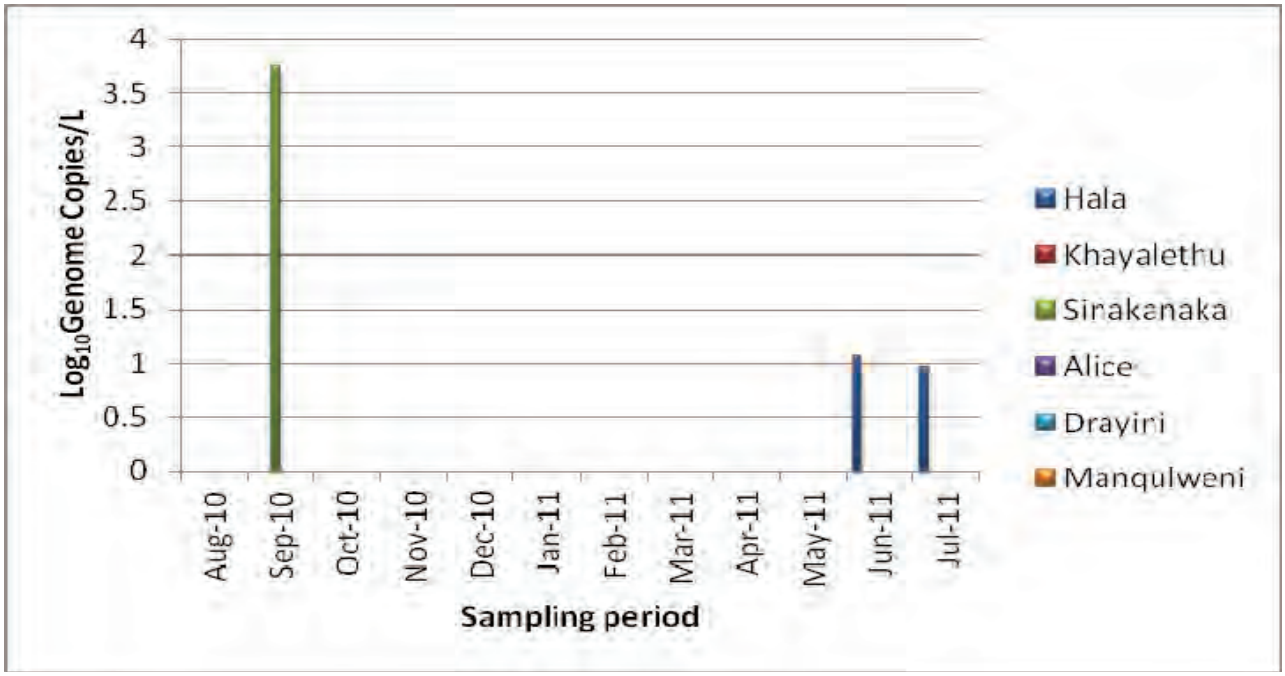

Figure 6.12: Quantitative detection of RoV at the sampling sites on Tyume River using real-time RT-PCR

Environmental transmission of RoV occurs mainly through shellfish grown in polluted surface waters and in contaminated drinking water (Koroglu et al., 2011; Van Zyl et al., 2006).The virus has been found to be stable in environmental conditions. Fischer et al. (2002) have reported that in ambient tropical temperatures (30^{o}C), RoV particles can survive for more than 2 months and can maintain infectivity for more than 32 months at ≤10^{o}C. Group A RoVs have been detected in untreated and treated drinking-water samples in

Southern Africa (Van Zyl et al., 2006). When real-time PCR positive samples were then screened for RoV Groups A-C, none of the samples were positive for any of those groups suggesting that the detected RoV may not have belonged to any of the RoV Groups A, B or C, but to some other groups which were not screened for in this study.

To the best of our knowledge, the detection (by real-time PCR) of RoV in surface waters is the first such study to be undertaken in the Eastern Cape Province, and underscores the need for risk analysis profiling in the Province, more so when considering that the outcome of RoV infection is more serious in developing countries where an estimated 600 000 deaths occur annually and surviving children may fail to thrive (Kang et al., 2004).

Norovirus (NoV)

Since our efforts to purchase a norovirus control strain were unsuccessful, real-time PCR detection could not be done, and as such we carried out our detection of norovirus using semi-nested PCR. The prevalence of NoV was very low (4%) as only three samples tested positive. Of these, two were positive for NoV GI, while one was positive for NoV GII (Table 6.7).

Table 6.7: Norovirus detection frequency along Tyume River

Sampling points	Sampling Period											
	Aug-2010	Sep-2010	Oct-2010	Nov-2010	Dec-2010	Jan-2011	Feb-2011	Mar-2011	Apr-2011	May-2011	Jun-2011	Jul-2011
Hala	x	x	x	x	x	x	x	x	x	x	x	x
Khayaleth	x	x	x	x	x	x	x	x	x	x	x	x
Sinakanak	x	x	x	x	x	x	x	x	x	x	x	x
Alice	GI	x	GII	x	x	x	x	x	x	x	x	x
Drayini	x	GI	x	x	x	x	x	x	x	x	x	x
Manqulwei	x	x	x	x	x	x	x	x	x	x	x	x

X = not detected; GI = NoV GI; GII = NoV GII

Unlike other RNA viruses detected in this study, all NoV detections were in samples collected from the downstream sampling sites of Tyume River which is more impacted by wastewater effluents than the upper stretch of the river. NoVs have been previously detected in wastewater and surface water (Haramoto et al., 2005; Lodder and Husman, 2005; Ueki et al., 2005). It has also been noted that NoVs in the urban environment may be transported by stormwater runoff, combined and sanitary sewer overflows, and discharge of wastewater treatment plant effluent (Arnone and Walling, 2007). NoV detection in samples from the downstream Tyume River might have been influenced by their relatively close proximities to sewage outfall points from Victoria Hospital, Alice Town and the University of Fort Hare. Similar results have been reported by Aw et al. (2009), who reported detection of human NoVs in downstream waters of urban rivers and the receiving estuarine bay, suggesting urban runoff as a source of viral contamination. Considering that

viral RNA was concentrated from only 1 ℓ of sample, it is possible that more positive samples would have been detected had larger volumes been used, since previous findings have shown that there are low ambient concentrations of viruses in environmental waters (Aw et al., 2009), necessitating the concentration of NoVs from larger volumes of water.

NoVs, like most other enteric viruses, are able to survive treatment processes if there is inadequate chlorination. It is reported that NoVs can be destroyed by 'adequate chlorination' (Shin and Sobsey, 2008). However, their physicochemical stability helps them to pass through sewage treatment without inactivation and reach many kinds of environmental waters (Victoria et al., 2010 a; b). Considering the low infectious dose of NoVs and further taking into account the probability of PCR inhibition (Chandler et al., 1998), the obtained results could be indicative of a potential health risk to users of raw water from the Tyume River.

Correlation analysis of Tyume River data

Table 6.8 shows the correlation half-matrix of all physicochemical and microbiological parameters assessed in this study. Significant negative correlations existed between water temperature and each of the nutrients (nitrite, nitrate and phosphate). This agrees with the findings of Badran (2001) and Manasrah et al. (2006) which explained this to be the result of increased nutrient consumption by primary producers in favourable temperature conditions.

Dissolved oxygen (DO) was also negatively correlated with temperature ($P<0.05$) while TDS and EC showed positive correlation to temperature ($P<0.05$). This is expected since high water temperatures result in less DO in the water (DWAF, 1996c; Papafilippaki et al., 2008; Rounds, 2002; Vega et al., 1998). TDS was inversely correlated with DO ($P < 0.01$). All nutrients were positively correlated with each other at the 99% confidence level suggesting that they are from the same source. The positive correlation between DO and nutrients ($P<0.05$) observed in this study is in agreement with the findings elsewhere. Morgan et al. (2006) and Arheimer and Liden (2000) attributed this relationship to nutrient input which promotes primary productivity and contributes to the water's DO concentrations.

The concentrations of total coliforms, faecal coliforms and enterococci were positively correlated ($P< 0.01$) to each other indicating that they probably come from the same source. The highly significant positive correlation between DO and pH ($P<0.01$) is an interesting observation which seems to indicate that the bacteria responsible for decomposition of organic material, and hence utilisation of DO in water, do not thrive at a pH range of over pH 7.0. This will mean that as the pH increases, more and more bacteria die off and the oxygen concentration is maintained at a high level (Araoye, 2009; Swaminathan, 2005).

Enteric viruses were inversely correlated with temperature ($P<0.01$). Previous studies on the persistence of enteric viruses in groundwater and surface waters found that temperature was the single most important predictor of virus persistence in well water (Yates et al., 1985). It has also been observed that though enteric viruses can survive for prolonged periods in the aquatic environment (Rzezutka and Cook, 2004), their survival depends on different factors, such as UV, temperature, and pH (de Roda Husman et al., 2009). Inactivation rates for poliovirus 1 have been observed to range from 1 log reduction in titre (LRT) per day to 2

LRT in 0.25 day in river-water temperatures of between 16.5°C and 27°C, respectively (O'Brien and Newman, 1977; Cubbage et al., 1979).

Table 6.8: Correlation half-matrix of physicochemical and microbiological indicators for Tyume River

Parameters	pH	WT	TDS	TBD	EC	NO_3^-N	NO_2^-N	$PO_4^{3-}P$	TCC	FCC	EntC	BOD	DO	EntVC
pH	1													
WT	0.014	1												
TDS	0.077	**0.412****	1											
TBD	0.021	-0.052	-0.052	1										
EC	0.037	**0.150***$^{}$	**0.593****	0.101	1									
NO_3^-N	-0.046	-0.223**	-0.078	**0.135***	**0.288****	1								
NO_2^-N	-0.004	-0.320**	-0.125	**0.240****	**0.154***	**0.747****	1							
$PO_4^{3-}P$	-0.013	-0.387**	-0.047	**0.266****	**0.223****	**0.717****	**0.701****	1						
TCC	-0.104	0.052	**0.185****	0.070	**0.358****	**0.212****	**0.162***	**0.184****	1					
FCC	-0.112	.0146*	0.103	-0.046	**0.226****	**0.139***	0.103	-0.110	**0.418****	1				
EntC	**-0.148***	0.004	**0.157***	-0.063	**0.293****	0.086	-0.002	-0.095	**0.641****	**0.525****	1			
BOD	-0.110	-0.016	**-0.481****	0.006	**-0.278****	0.032	0.086	-0.101	0.061	**0.203****	0.032	1		
DO	**0.550****	-0.562**	**-0.333****	-0.003	**-0.246****	0.088	**0.166***	**0.271****	**-0.146***	**-0.138***	**-0.154***	0.088	1	
EntVC	-0.201	**-0.231***	0.058	-0.185	-0.098	0.163	**0.292***	0.116	**0.235***	0.066	0.030	-0.008	-0.144	1

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

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

Abbreviations: WT, water temperature; EC, electrical conductivity; TDS, total dissolved solids; TBD, turbidity; DO, dissolved oxygen; BOD, biochemical oxygen demand; TCC, total coliform count; FCC faecal coliform count; EntC, enterococci count; EntVC, enteric virus concentration

Like in previous findings (Harwood et al., 2005) enteric viruses, in this study, showed a very weak positive correlation with total coliforms (P<0.01; r = 0.24), This finding concurs with the universally agreed belief that there is no direct correlation between faecal indicator bacteria and pathogenic viruses in water (Grabow, 1996) thereby necessitating the need for direct assessment of surface water and underground water sources for the presence of enteric viruses as a more reliable tool to determine faecal pollution of water resources.

Conclusions

This is the first study that has been conducted on the prevalence and distribution of enteric viral pathogens in surface waters in the Eastern Cape Province. All viruses assayed for, except enterovirus, were detected suggesting that incidences of viral gastroenteritis could be common occurrences among the people living in the Tyume River catchment. The detection of enteric viruses in the river is of public health concern for a population which relies on the river for various uses, thus exposing them to risk of infection through contact and accidental ingestion of the river water.

6.3.2 Buffalo River catchment

Human adenovirus (HAdV)

Figures 6.13 and 6.14 show the amplification plot and standard curve for the quantitation of HAdV in Buffalo River water samples. Figure 6.15 shows the results for quantitative detection of adenoviruses at the six sampling sites on Buffalo River. Human AdV was detected in about 35% (25/72) of the samples analysed in concentrations ranging from 1 genome copy/ℓ to 470 genome copies/ℓ throughout the study period. This observation is consistent with other studies. Choi and Jiang (2005), in a real-time PCR study that showed high prevalence of AdV genomes in California urban rivers, reported that no significant seasonal variability was observed for adenoviruses. In their study on seasonal occurrence of human enteric viruses in river water samples collected from rural areas of South-East Poland, Kozyra et al. (2011) reported that human adenoviruses were detected in 28.3% of samples, and that they were present in all seasons of the year. However, in another recent study, Silva et al. (2011) detected AdV in 44.4% (24 of 54) of the samples collected from rivers and lakes used for recreation and as a source for the public water supply in the City of Goiania, Brazil, and reported that the occurrence of AdV showed a seasonal influence.

Most of the AdV-positive samples were those from Parkside where about 92% (11/12) of the samples were positive in concentrations ranging between 3.4×10^{1} genome copies/ℓ and 4.71×10^{2} genome copies/ℓ. Human AdVs are known to be more stable than RNA viruses in the environment, especially in sea water (Calgua et al., 2008). The King William's Town samples yielded the second most abundant (50%) AdV-positive samples with concentrations ranging between 1.5×10^{1} genome copies/ℓ and 4.56×10^{2} genome copies/ℓ. Thirty-three percent (4/12) and 25% (3/12) of the samples from Eluxolzweni and Bridle Drift Dam, respectively, were

positive for AdV. Only 8.3% (1/12) of the samples from Rooikrantz Dam were positive for AdV while none of the samples from Maden Dam were positive.

The low detection rate of AdV at Rooikrantz Dam and no detection at Maden Dam is not surprising. The upper reaches of the Buffalo River catchment are sparsely populated compared to the lower reaches that comprise largely of urban areas including King William's Town, Zwelitsha, Mdantsane and East London (RHP, 2004). Also, it is estimated that more than 90% of the human population is seropositive for one or more serotypes of adenoviruses (Fong et al., 2010). Human AdVs are excreted in high concentrations from infected patients (up to 1 011 viral particles per gram of faeces) and are constantly found in raw sewage of large populations and in environmental waters impacted by faecal contamination (Wu et al., 2011) and stormwater, urban runoff and wastewater effluents (Choi and Jiang; Fong et al., 2010). In the lower reaches of Buffalo River, blockages in the sewerage systems, inadequate treatment capacity and poor management result in the discharge of partially treated and untreated sewage into the river and dams (RHP, 2004).

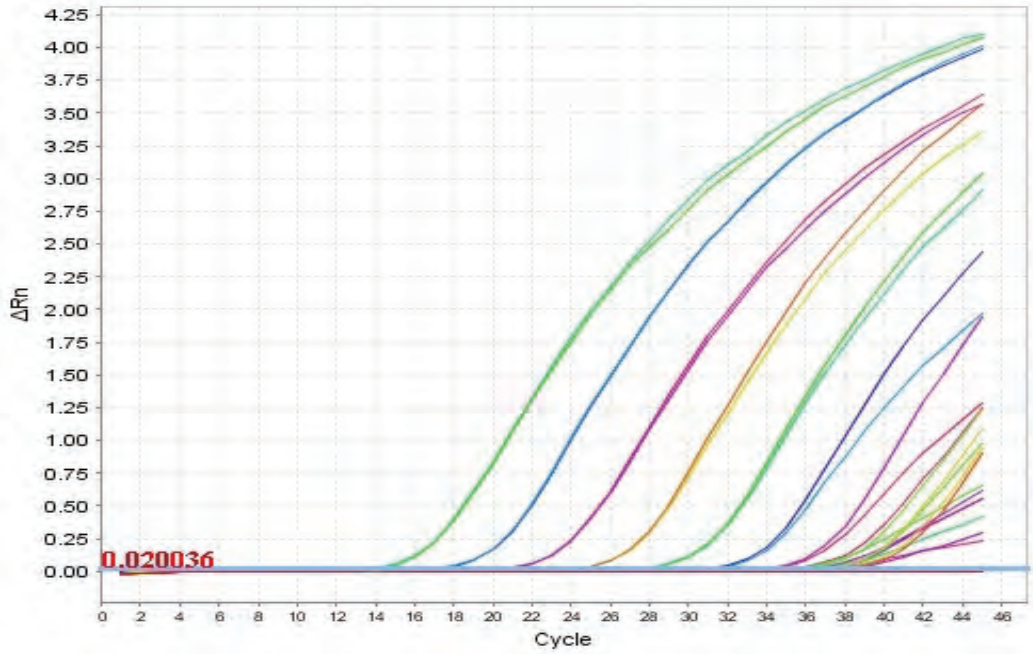

Figure 6.13: Amplification plot for AdV quantitation in Buffalo River

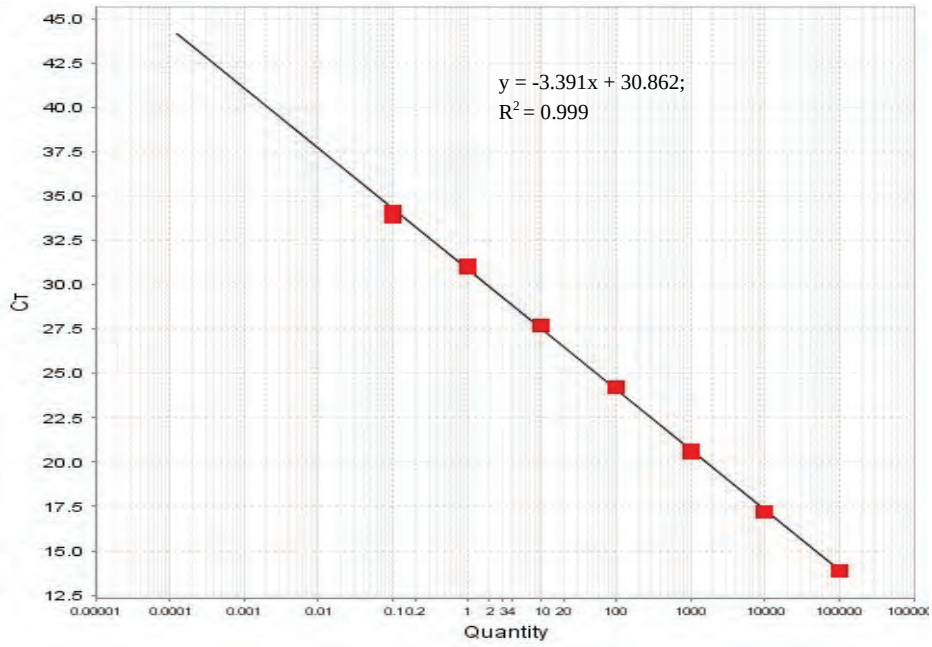

Figure 6.14: Standard curve for AdV quantitation in Buffalo River

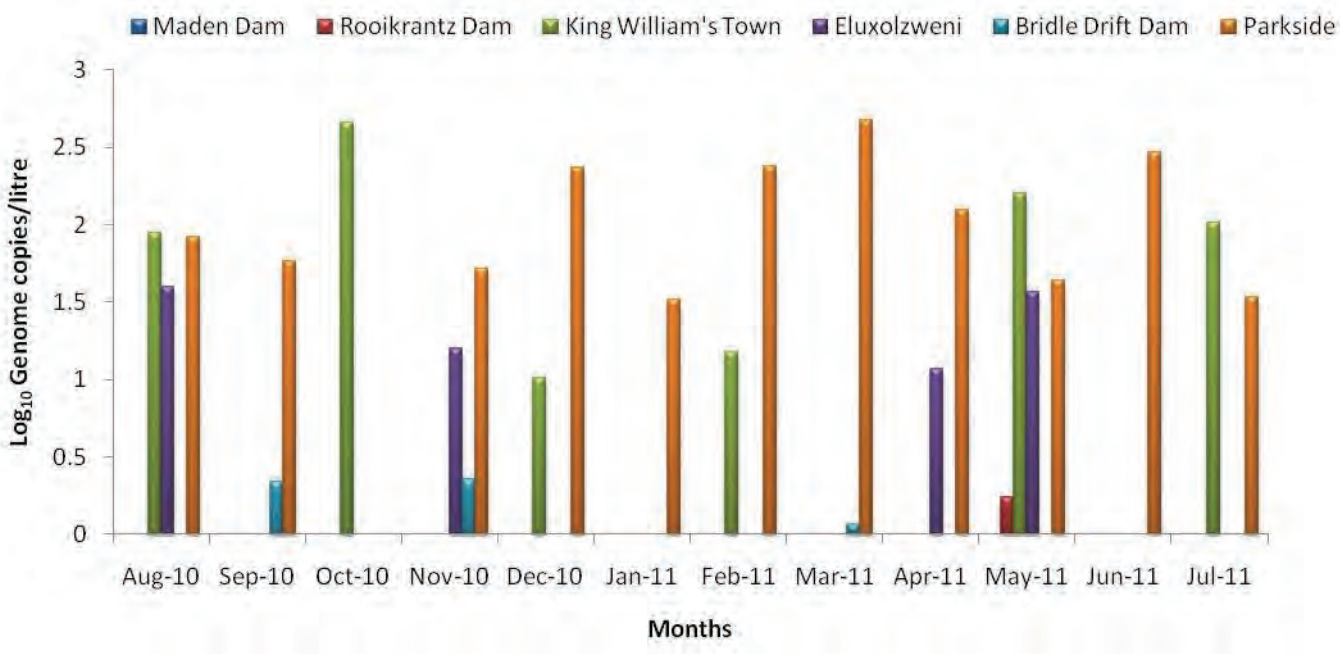

Figure 6.15: Quantitative detection of adenovirus at the sampling sites on Buffalo River using qPCR

Table 6.9 shows the outcomes of the PCR detection of the epidemiologically important species and serotypes of adenovirus amongst the 25 RT-PCR positive adenovirus samples. AdF was detected in 5 samples, while AdB was detected in one, thus suggesting that about 8.3% of the entire 72 samples analysed, and only 24% of the 25 AdV positive samples are of epidemiological importance. In a previous South African study on the incidence of adenoviruses in raw and treated water, Van Heerden et al. (2003) used a combination of cell culture and nested PCR and reported that human adenoviruses were present in 13 (12.8%) of the raw and 9 (4.4%) of the treated water samples tested.

The presence of AdV in Buffalo River water samples constitutes a public health problem. This is heightened by the fact that 83.3% (5/6) of the detected AdV belong to the 40/41 serotypes (Table 6.9) responsible for most cases of childhood diarrhoeal disease (Fong and Lipp, 2005; Jiang 2006). In this study, Ad40/41 constitutes 83.3% of all the human AdVs detected. This is similar to an earlier study by Van Heerden et al. (2005a) in which adenovirus serotypes 2, 40 and 41 were each detected in different treated drinking- and raw-water samples in South Africa, and most (70%) of the human AdVs detected in river-water samples were serotypes 40 and 41.

Table 6.9: Characterisation of AdVs detected in Buffalo River

Sample code	Sampling site	Sampling period	AdV Species (A-F)	AdV Serotype
RKD5	Rooikrantz Dam	May	—	—
KWT2	King William's Town	February	—	—
KWT5	King William's Town	May	—	—
KWT7	King William's Town	July	—	—
KWT8	King William's Town	August	AdF	40/41
KWT10	King William's Town	October	—	—
KWT12	King William's Town	December	—	—
ELX4	Eluxolzweni	April	—	—
ELX5	Eluxolzweni	May	AdF	40/41
ELX8	Eluxolzweni	August	—	
ELX11	Eluxolzweni	November	—	
BDD3	Bridle Drift Dam	March	AdF	40/41
BDD9	Bridle Drift Dam	September	—	—
BDD11	Bridle Drift Dam	November	—	—
PKS1	Parkside, East London	January	—	—
PKS2	Parkside, East London	February	—	—
PKS3	Parkside, East London	March	—	—
PKS4	Parkside, East London	April	—	—
PKS5	Parkside, East London	May	—	—
PKS6	Parkside, East London	June	AdB	21
PKS7	Parkside, East London	July	AdF	40/41
PKS8	Parkside, East London	August	—	—
PKS9	Parkside, East London	September	AdF	40/41
PKS11	Parkside, East London	November	—	—
PKS12	Parkside, East London	December	—	—

The human adenovirus, Ad21 serotype was also detected in one sample. This serotype belongs to homology cluster B1 (which includes Ad3 and Ad7) that is recognised as among the most pathogenic of the 51 known

human Ad serotypes (both in severity and extent of clinical illness). Although more commonly associated with mild respiratory illnesses and conjunctivitis, Ad3, Ad7, and Ad21 can cause severe and fatal lower respiratory tract infections, primarily in infants and young children and occasionally in adults (Xu and Erdman, 2001).

Hepatitis A virus (HAV)

Figures 6.16 and 6.17 show the amplification plot and standard curve, respectively, for the quantitation of HAV in Buffalo River samples. Hepatitis A virus (HAV) is a small, single-stranded RNA virus belonging to the family Picornaviridae and is the only member of the genus *Hepatovirus*. HAV is the most common cause of infectious hepatitis throughout the world with more than 1.5 million clinical cases reported annually (Roque-Afonso et al., 2010). The actual incidence of hepatitis A is probably underestimated owing to the high number of asymptomatic infections. Outbreaks of hepatitis A associated with contaminated water supply have been reported in various countries (Tallon et al., 2008; Pinto et al., 2009), although South Africa is reported to be a country of intermediate to high hepatitis A endemicity (Venter et al., 2007).

Figure 6.18 shows the results for quantitative detection of HAV at the 6 sampling sites on the Buffalo River. HAV was detected in about 43% (31/72) of the samples with concentrations ranging between 7×10^5 genome copies/ℓ and 1.4×10^5 genome copies/ℓ. HAV was detected in all but one (January 2011) sampling month. The Bridle Drift dam samples yielded the highest detection rate (91.67%) with HAV concentrations of 1.1×10^2 genome copies/ℓ to1.4×10^5 genome copies/ℓ. HAV was detected at the remaining 5 sites at various times ranging between 1 month and 6 months. Also, HAV was detected in 50% (6/12) of samples from Parkside at concentrations ranging between 9×10^1 genome copies/ℓ and 5.2×10^3 genome copies/ℓ. Samples collected from Rooikrantz Dam were only positive in 5 months of sampling with concentrations raging between 1.7×10^3 genome copies/ℓ and 2.9×10^4 genome copies/ℓ. Samples collected from Maden Dam were positive for HAV in four months of the sampling period with concentrations ranging between 3.8×10^2 genome copies/ℓ and 3.9×10^4 genome copies/ℓ. Samples from King William's Town were positive for HAV in 33.3% (4/12) of the times, 75% (3/4) of which were in August through October 2010 while 25% (1/4) was in July 2011. The presence of HAV at all the sites may suggest that the virus is consistently present in Buffalo River.

HAV was detected more often in winter and spring months. In general terms, the detection of hepatitis A virus in Buffalo River is similar to the findings in other studies in other provinces in South Africa (Taylor et al., 2001; Venter et al., 2007) and elsewhere (De Paula et al., 2007). While the detection rate for HAV in this study was about 43% (31/72), in the study by Taylor et al. (2001), HAV was detected in 18 (35.3%) of river-water and 19 (37.3%) of dam-water samples, with a seasonal peak being evident in both the river and dam water in early spring (August and September). It was also during these two months in this study that HAV was detected most often. Although Taylor and colleagues (2001) detected HAV less often in the months of May, June and July, it was actually during the months of January and February that the least HAV detection was recorded in this study. Considering that we used real-time quantitative reverse transcriptase-polymerase chain reaction (RT-PCR) which is more sensitive compared with the combination of cell-culture amplification and qualitative RT-PCR used by Taylor, the variations were not unexpected.

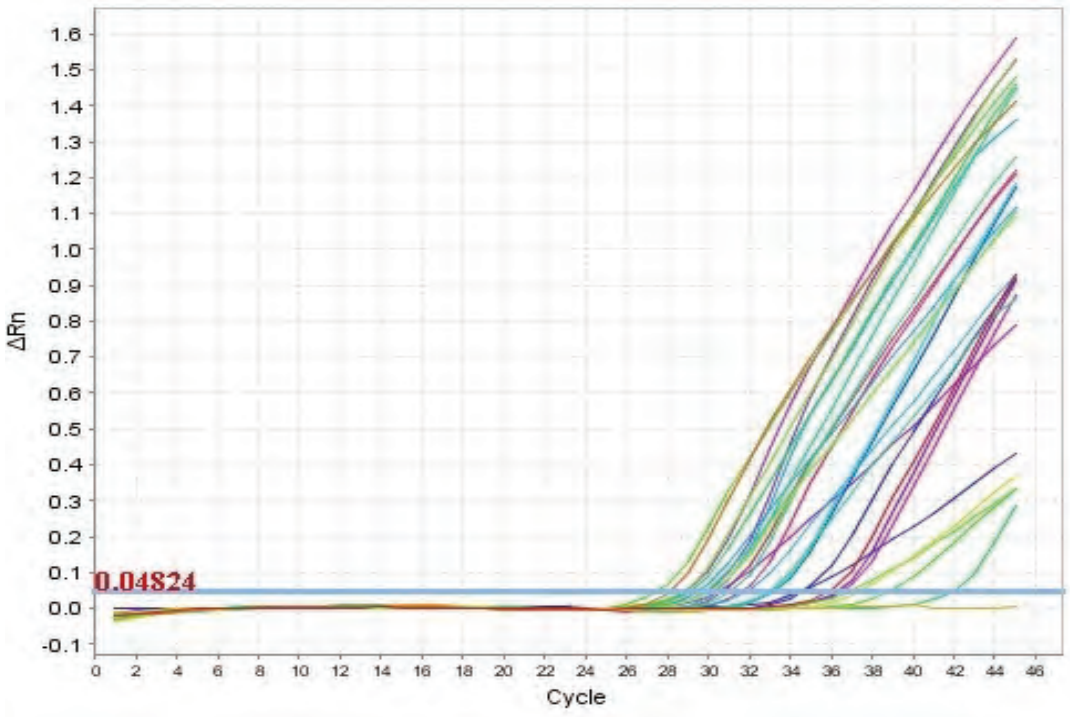

Figure 6.16: Amplification plot for HAV quantitation in Buffalo River

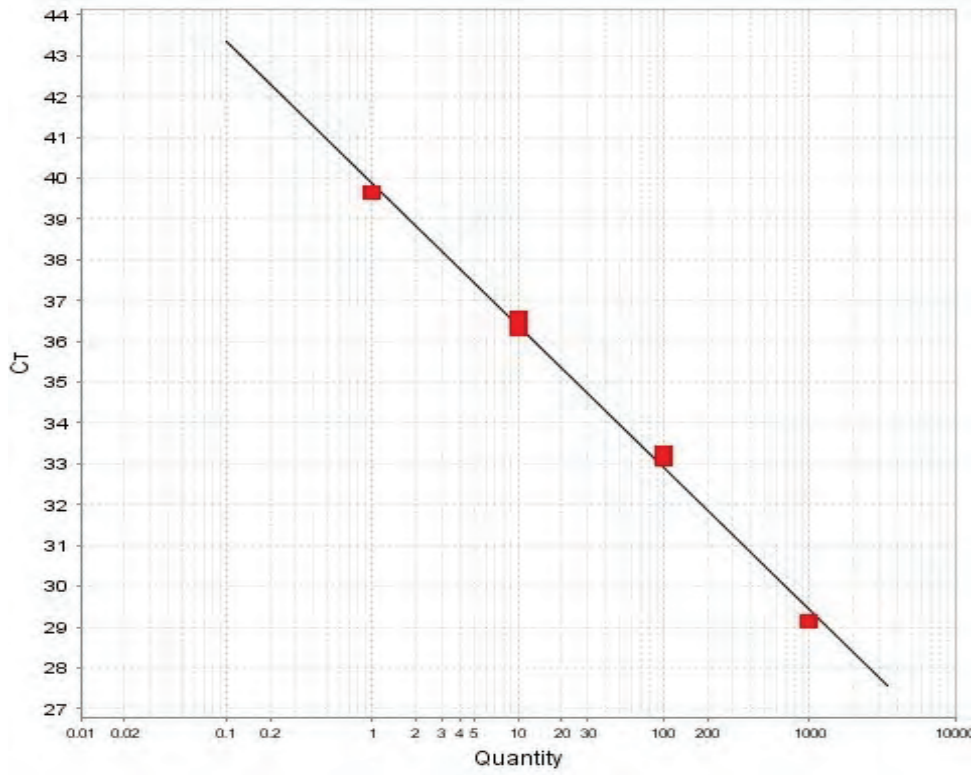

Figure 6.17: Standard curve for HAV quantitation in Buffalo River

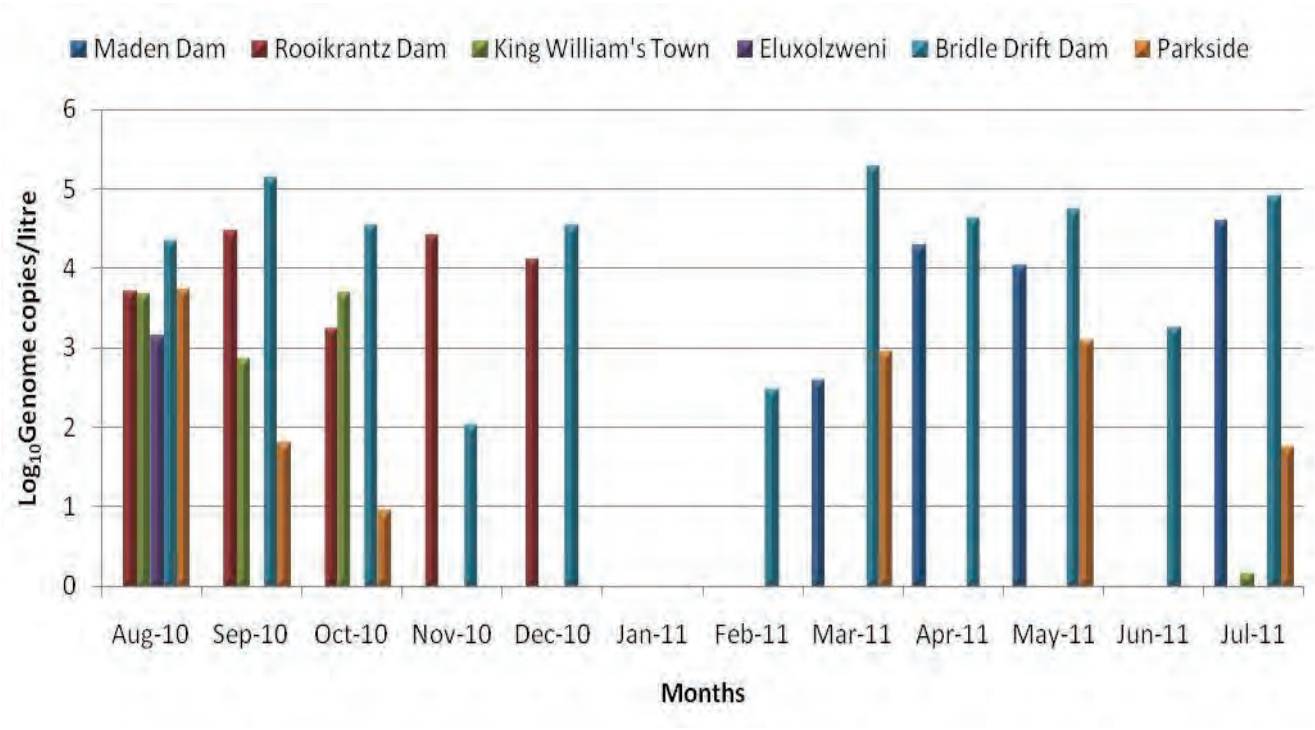

Figure 6.18: Quantitative detection of HAV at the sampling sites on Buffalo River using real-time RT-PCR

Enteroviruses (EnV)

Figures 6.19 and 6.20, respectively, show the amplification plot and standard curve for the quantitation of EnV in the Buffalo River samples. No EnV was detected during the hottest months (November 2010 to February 2011) (Figure 6.21). This is consistent with previous studies that reported significant correlation between low water temperatures and occurrence of enteroviruses in water (Chen et al., 2008). In this study we detected enteroviruses in 9.7% (7/72) of the samples by real-time RT-PCR and in 1.4% (1/72) by semi-nested reverse transcriptase PCR, thus reaffirming the greater sensitivity of qPCR in comparison with conventional PCR (De Paula et al., 2007). The detection rate of 1.4%, by semi-nested PCR, recorded in this study is similar to the 1.2% reported elsewhere (Schvoerer et al., 2000).

While about 57% (4/7) of the positive samples were from a combination of two freshwater sites (3 samples from King William's Town and 1 from Eluxolzweni), about 43% (3/7) of the EnV-positive samples were from the Buffalo River estuary. This observation is consistent with reports that EnVs are tolerant of a wide range of temperatures and salinities which facilitates their survival in environmental waters (Skraber et al., 2004; Gregory et al., 2006). However, our findings apparently differ from the results presented by Schvoerer et al. (2000), in which they studied 26 water samples in south-western France, and all 16 bathing freshwater samples were positive for 3 EnVs while no EnV was identified in seawater (10 samples).

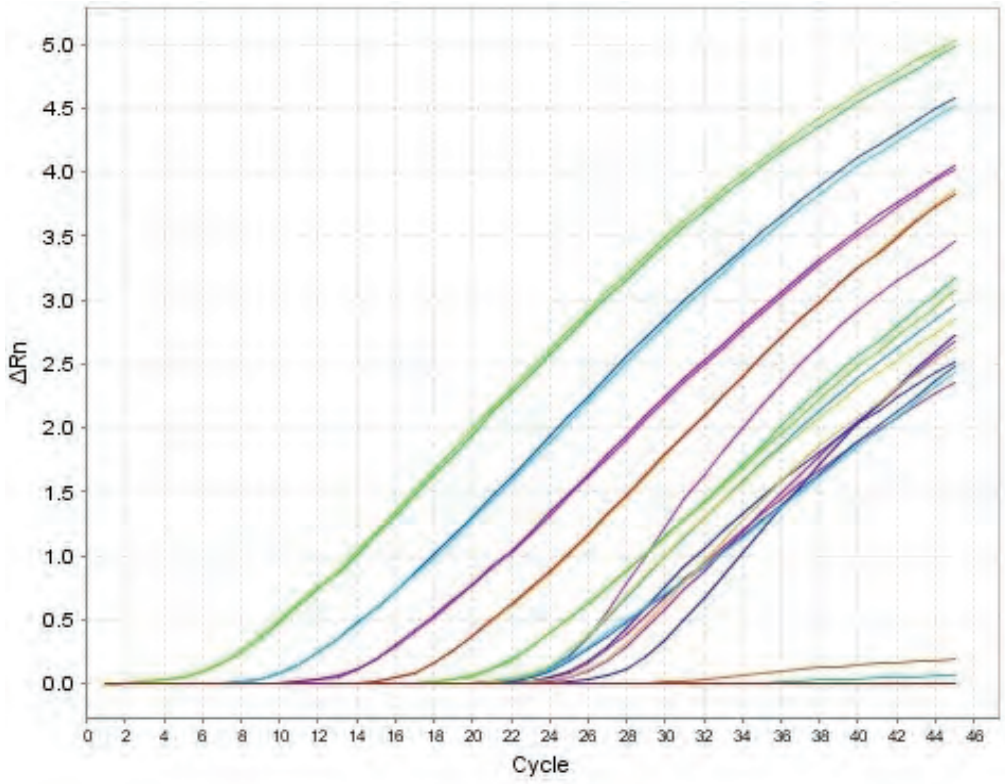

Figure 6.19: Amplification plot for enterovirus quantitation in Buffalo River

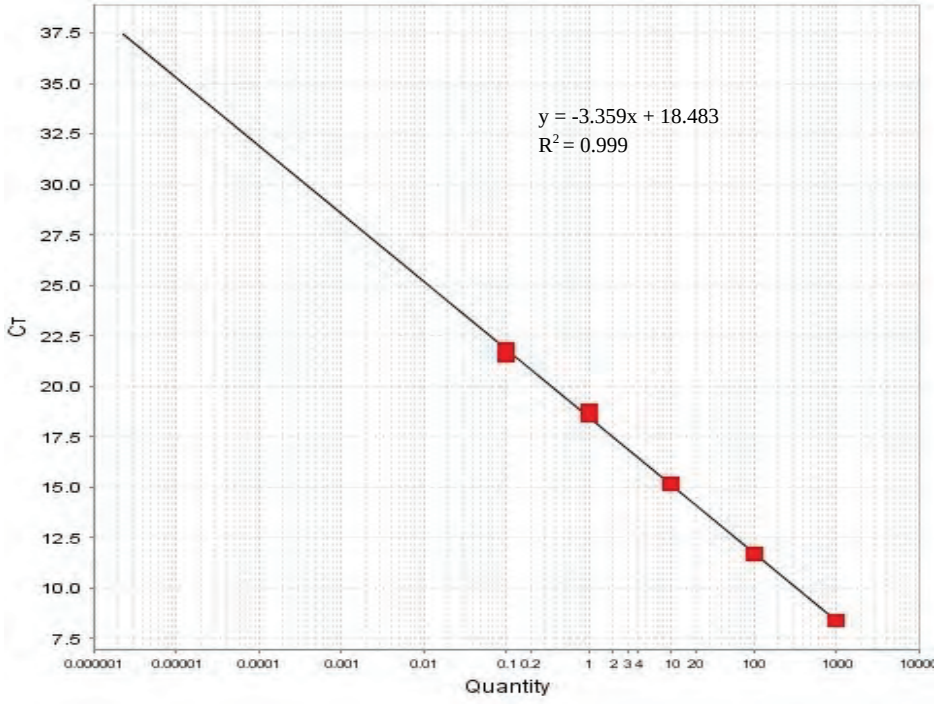

Figure 6.20: Standard curve for enterovirus (EnV) quantitation in Buffalo River

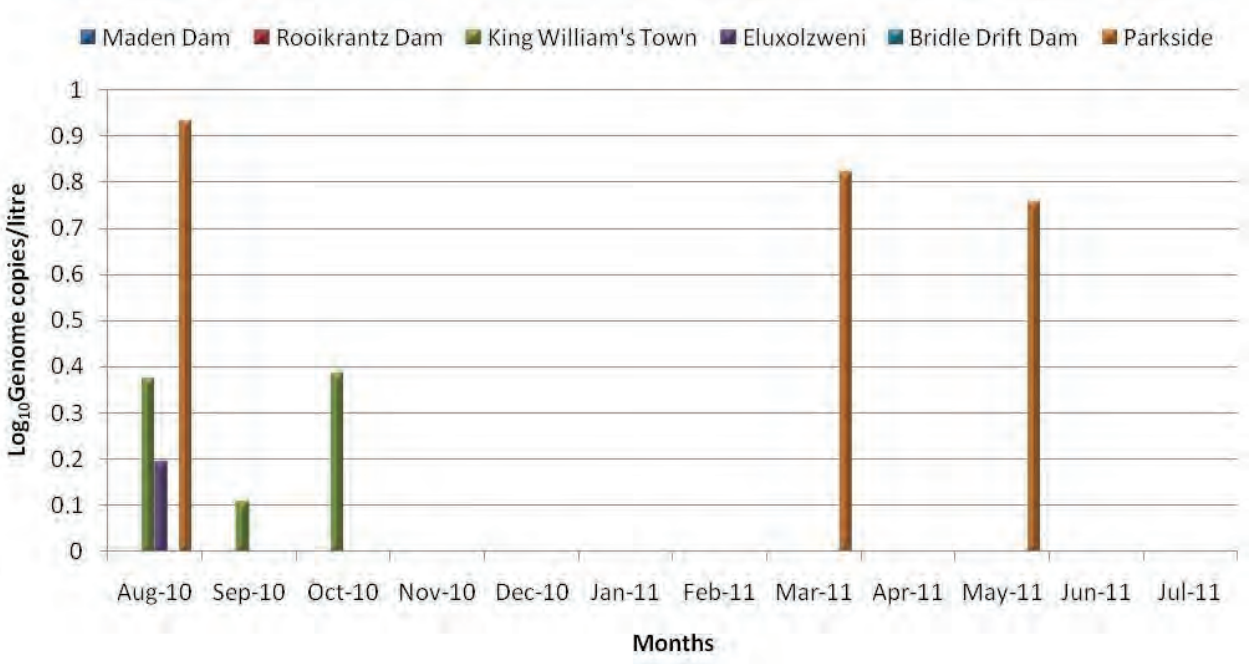

Figure 6.21: Quantitative detection of EnV at the sampling sites on Buffalo River using real-time RT-PCR

Rotavirus (RoV)

Figures 6.22 and 6.23 show the amplification plot and standard curve for the quantitation of RoV in Buffalo River water samples. RoV is the most common cause of severe diarrhoea worldwide and is believed to account for 25% of all the deaths due to diarrhoea among children less than 5 years old (Steele et al., 1999) and outbreaks have continued to occur in South Africa (Rinaldi et al., 2009). In this study, rotavirus concentrations ranging between 3.0 genome copies/ℓ and 2.1×10^2 genome copies/ℓ and RoV were detected in about 14% (10/72) of the samples. In a report by Van Zyl et al. (2006), on the molecular epidemiology of Group A rotaviruses in water sources and selected raw vegetables in Southern Africa, Group A RoVs were detected in 11.8% of partially treated and 1.7% of finally treated drinking-water samples; in 14% of irrigation-water samples; and 1.7% of corresponding raw vegetable samples. However, higher detection rates have been reported elsewhere. Lodder et al. (2010) reported the detection of RoVs in 48% of the surface-water samples in the Netherlands.

Figure 6.24 illustrates the quantitative detection of RoV at the 6 sampling sites on the Buffalo River. The frequency of virus detection varied greatly between the locations. Fifty percent of RoV detections occurred at Parkside, 40% at King William's Town, and 10% at Eluxolzweni. No RoV was evident at any of the 3 dams. This could be due to human population difference along the river course. The lower catchments are significantly more populated than the upper catchments. In a similar report from Kenya, Kiulia et al. (2010) detected Group A rotavirus in 10 (100%) of samples collected from a river located in an urban area and in 3 (25%) of the rural river-water samples.

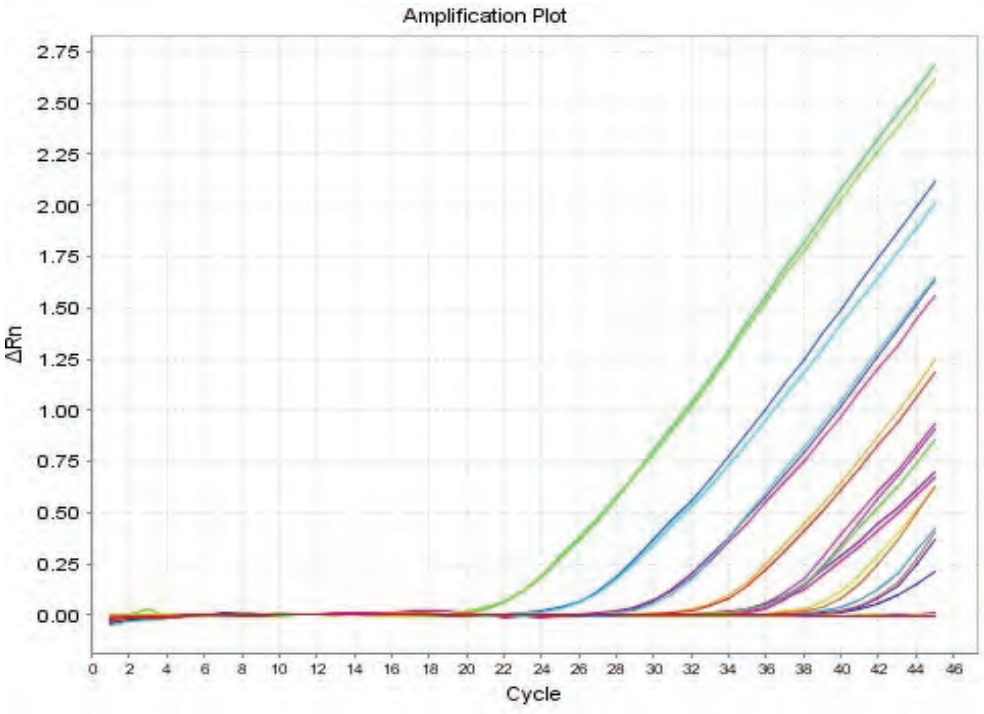

Figure 6.22: Amplification plot for rotavirus quantitation in Buffalo River

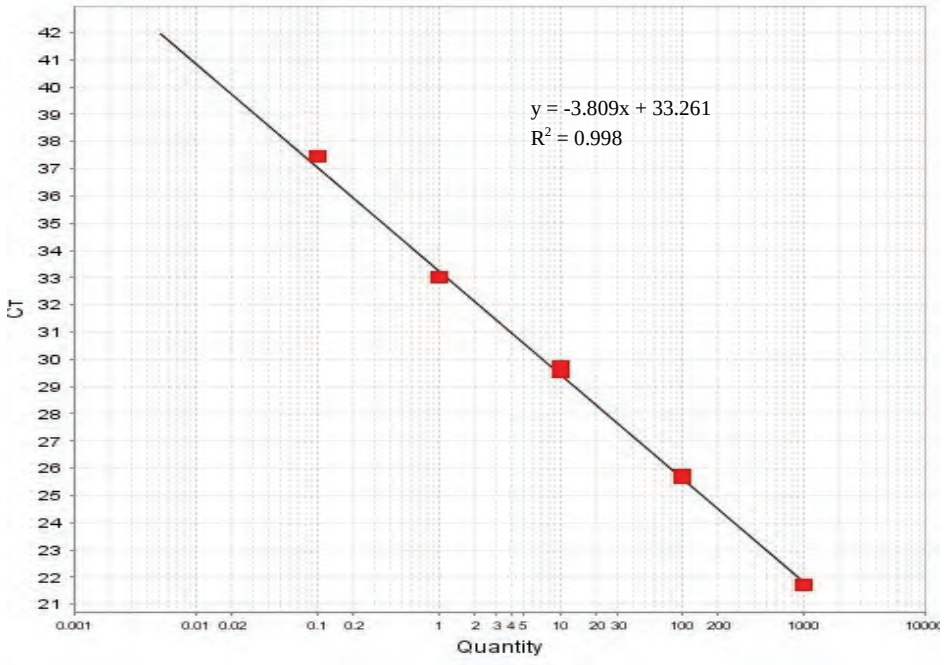

Figure 6.23: Standard curve for rotavirus quantitation in Buffalo River

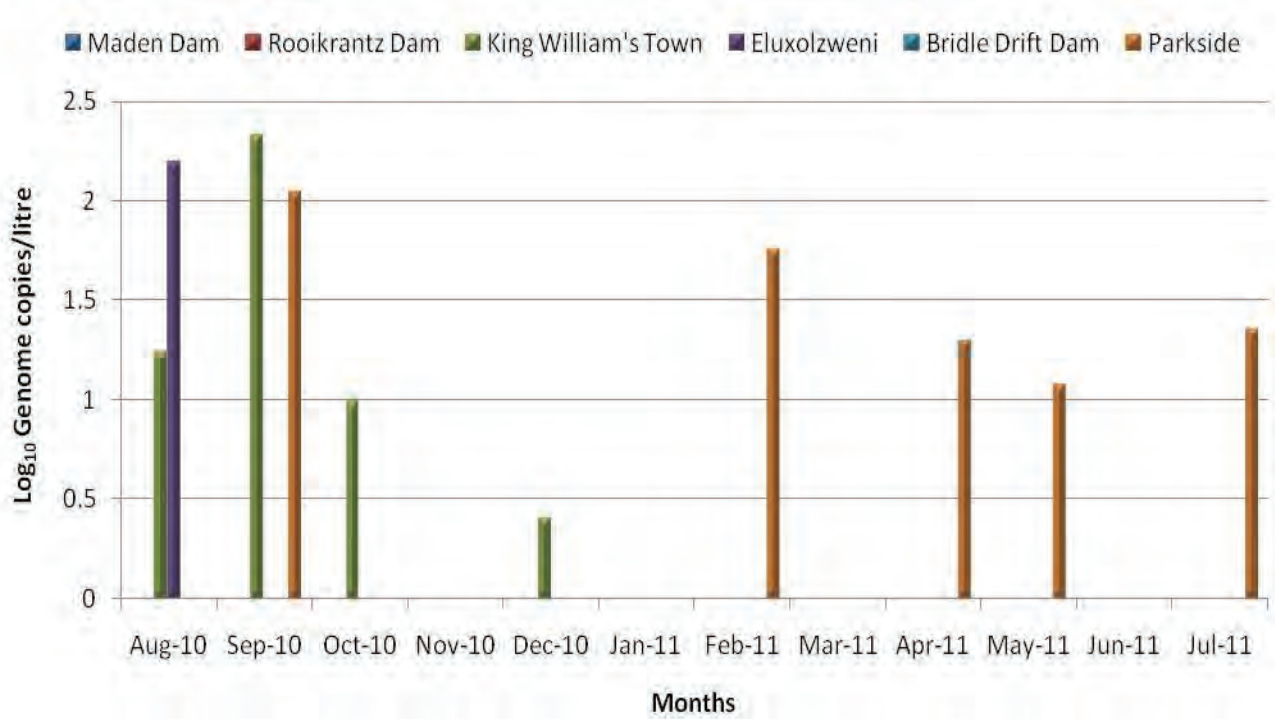

Figure 6.24: Quantitative detection of RoV at the sampling sites on Buffalo River using real time RT-PCR

Norovirus (NoV)

Of the 72 water samples screened for NoV only two were positive and both were GI; these were detected in samples collected from King William's Town in August and October 2010. Further studies on the prevalence of this virus, based on the real-time PCR technique are recommended to determine the true prevalence of this virus for which there are neither cell-culture systems nor small-animal models for its propagation (Kojima et al., 2002; Kageyama et al., 2003).

Detection rates

Figure 6.25 shows the detection rates for enteric viruses in Buffalo River at the 6 sampling sites. Only HAV was detected at all the sites, with Bridle Drift Dam recording significantly higher (P<0.05) concentration. AdV was detected in 5 of the 6 sites with significantly higher (P<0.05) titres detected at Parkside, East London and King William's Town. The mean concentration of HAV (2.45×10^4 genome copies/ℓ) detected was significantly higher than that of AdV (1.04×10^2 genome copies/ℓ), and both viruses were detected in significantly higher (P<0.05) concentrations than RoV (6.2×10^1 genome copies/ℓ) and enteroviruses (4.0 genome copies/ℓ). This may reflect both the epidemiological status of infections caused by these viruses and their survival in the water environments. While HAV is known to be hyper-endemic in South Africa, enteric adenoviruses have been shown to be substantially more stable than either polio 1 or HAV. They are also

reported to be more resistant to inactivation by UV than enteroviruses and are sometimes at higher concentrations in polluted waters (Crabtree et al., 1997).

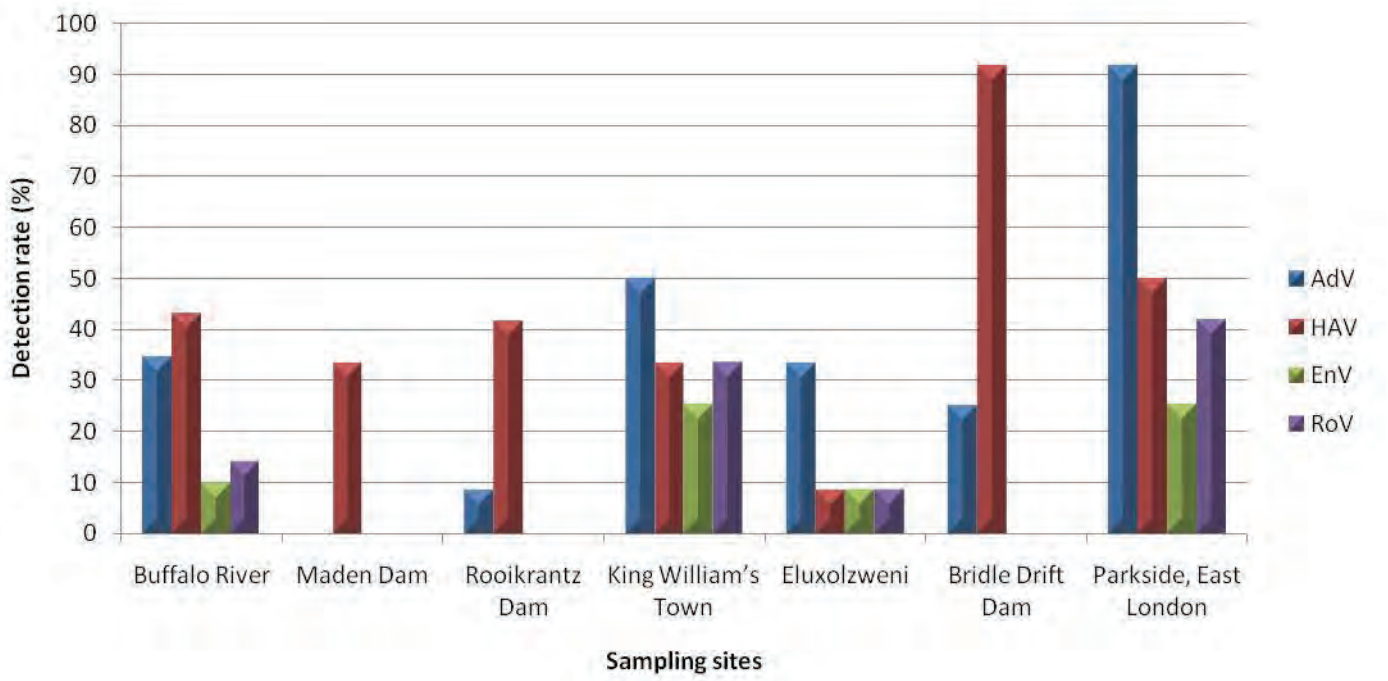

Figure 6.25: The detection rates for enteric viruses in Buffalo River at the 6 sampling sites

Correlations amongst water-quality parameters

Table 6.10 shows the correlation coefficients of physicochemical, bacteriological and virological parameters covered in this study. There was no apparent correlation between water temperature and bacterial counts. There was, however, a significant (P<0.01) positive correlation between water temperature and DO. It is worth pointing out that the significant correlation discernible between water temperature and DO validates the knowledge that the temperature of water determines the amount of oxygen held in solution in an inverse relationship - as temperature increases, dissolved oxygen decreases (Manasrah et al., 2006). Also, significant negative correlations existed between DO and each of nitrate and phosphate.

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There existed a significant (P<0.01) inverse correlation between enteric viruses and each of water temperature and pH. A review by Fong and Lipp (2005) revealed that in several studies, enteric viruses have been reported to survive longer and occur more frequently at lower temperatures in natural aquatic environments including seas, rivers, and aquifers. In their study on the incidence of adenoviruses in raw and treated water in South Africa, Van Heerden et al. (2003) reported that adenovirus detection peaked in winter, when up to 30% and 60% of water samples were positive for adenoviruses, for treated and river water, respectively. Thirty-three percent of all enteric virus detections in this study occurred in summer, while 64.4% occurred in the colder months of May through October.

Table 6.10: Correlation half-matrix of physicochemical and microbiological indicators for Buffalo River

Para-meters	DO	BOD	COD	pH	WT	TBD	EC	TDS	NO_2^-N	NO_3^-N	$PO_4^{3-}P$	TCC	FCC	EntC	EntVC
DO	**1**														
BOD	0.097	**1**													
COD	-0.106	**0.192****	**1**												
pH	**0.289****	0.075	0.060	**1**											
WT	**0.200****	0.057	0.101	0.085	**1**										
TBD	-0.057	**0.290****	-0.020	**0.171***	-0.050	**1**									
EC	**-0.172***	**0.331****	0.097	0.068	-0.028	0.032	**1**								
TDS	**-0.177****	-0.057	**0.375****	0.074	-0.012	**-0.294****	**0.191****	**1**							
NO_2^-N	-0.116	**0.154***	**-0.154***	**0.174***	-0.133	**0.494****	0.102	-0.113	**1**						
NO_3^-N	**-0.168***	0.088	-0.111	-0.037	**0.163***	**0.333****	0.076	**-0.218****	**0.261****	**1**					
$PO_4^{3-}P$	**-0.295****	0.045	0.102	0.020	**0.197****	0.123	**0.166***	-0.121	0.130	**0.323****	**1**				
TCC	-0.004	-0.007	0.064	0.059	-0.038	-0.075	-0.039	**0.284****	**0.225****	-0.133	-0.029	**1**			
FCC	**-0.149***	-0.014	0.115	0.083	-0.074	0.075	-0.029	-0.018	**0.181****	-0.037	-0.082	-0.019	**1**		
EntC	**-0.170***	-0.080	0.125	0.076	-0.102	0.073	-0.041	0.017	**0.181****	-0.084	-0.060	-0.018	**0.915****	**1**	
EntVC	-0.055	-0.048	-0.129	**-0.243****	**-0.192****	-0.110	-0.018	-0.043	-0.020	-0.029	-0.026	-0.023	-0.030	-0.027	**1**

** Correlation is significant at the 0.01 level (2-tailed)
* Correlation is significant at the 0.05 level (2-tailed)
Abbreviations: WT, water temperature; EC, electrical conductivity; TDS, total dissolved solids; TBD, turbidity; DO, dissolved oxygen; BOD, biochemical oxygen demand; COD, chemical oxygen demand; TCC, total coliform count; FCC faecal coliform count; EntC, enterococci count; EntVC, enteric virus concentration

Also, there existed a significant (P<0.01) inverse correlation between enteric viruses and pH. Virus attachment has been observed to be a function of pH and the isoelectric point of the virus, among other factors (Guan et al., 2003). That pH and temperature correlated negatively with enteric viruses implies that they both influence the stability of enteric viruses. In general, no correlation could be deduced between enteric viruses, all the tested chemical and bacteriological parameters. In a similar observation, Jurzik et al. (2010) reported that no strong correlation could be established for human AdV and other chemical parameters assayed, and concluded that neither chemical nor microbiological parameters could be used as a reliable indicator for the presence of enteric viruses in river water.

Enteric viruses showed no significant association with chemical parameters. The existence of a negative correlation (P<0.05) between DO and FC counts (r =-0.149) and enterococci counts (r =-0.170) agrees with previous reports on the existence of an inverse relationship between these parameters (Clark and Norris, 2000). Finally, across the sites, faecal coliform counts were generally higher than the enterococci concentrations except at Parkside. This higher enterococci count may be attributable to the ability of enterococci to survive harsh environments and the extremes of which salinity is characteristic at the Parkside site.

Conclusions

The presence of enteric viruses in water samples constitutes public health risks. Our results suggest that enteric viruses are consistently present in the Buffalo River. This consistent presence of these viruses, over a 12-month period, in some parts of the Buffalo River suggest the need for regular monitoring for viral contamination of water resources in the Province to help protect public health. To the best of our knowledge this is the first report on molecular detection and quantitation of enteric viruses from surface waters in the Eastern Cape.

6.4 RISK ASSESSMENT

The calculations for the microbial risk assessment were done to assess the fitness-of-use of the water for domestic and recreational purposes based on assuming accidental consumption of 10 mℓ and 100 mℓ of the river waters, respectively. A major limitation of the real-time PCR assay used in this study is its inability to determine the viability and infectivity of viruses detected, as the presence of viral nucleic acid does not necessarily indicate the presence of infectious viruses (Hamza et al., 2009; Bofill-Mas et al., 2010). To circumvent this limitation, ratios of infectious viruses to total virus particles based on outcomes of previous studies (Ward et al., 1984; Grabow et al., 1992; Rodríguez et al., 2009; Deng et al., 1994; Pinto et al., 2009) were used to estimate the infectious virus doses for the viruses in this work. In the case of rotavirus grown in the MA104 cell line, the ratio of infectious virus particles to total detected virus particles was 1:40 000 (Ward et al., 1984; Rodríguez et al., 2009). For adenovirus grown in the PLC/PRF/5 cell line, the ratio was 1:1 000 (Grabow et al., 1992; Rodríguez et al., 2009) while for hepatitis A virus the ratio was 1:60 (Deng et al., 1994; Pinto et al., 2009). Limitations of using these ratios will be:

- The interpretation of PCR results with those obtained by cell culture in the detection of viruses in water is difficult, because the ratio of infectious viruses to viral particles is variable (Rodríguez et al., 2009)
- This infectious viral particle/total particle ratio is largely dependent on the assay method and how long the virus has been passaged in the particular cell line. Thus, viruses from direct clinical or environmental samples have a much higher ratio than those viruses that have been adapted to cell culture (Reynolds et al., 1996).

While cell culture still remains the most viable method for determining virus infectivity, it is important to emphasise that even with cell culture, the detection of infectious viruses in environmental samples is difficult as each virus has different capabilities to propagate in any given cell line (Rodríguez et al., 2009).

The mean recovery efficiency of our filtration method was 56% (Haramoto et al., 2005). The average and corrected concentrations of the enteric viruses are shown in Table 6.11 below.

Table 6.11: Mean concentrations of enteric viruses in Tyume and Buffalo Rivers

	Genome copies/ℓ							
	HAdV		HAV		RoV		EnV	
River	Uncorrected	Corrected	Uncorrected	Corrected	Uncorrected	Corrected	Uncorrected	Corrected
Tyume	1.27×10^{4}	2.27×10^{4}	8.05×10^{3}	1.44×10^{4}	1.89×10^{3}	3.37×10^{3}	ND	ND
Buffalo	1.04×10^{2}	1.86×10^{2}	2.45×10^{4}	4.37×10^{4}	6.2×10^{1}	1.11×10^{2}	3.0	6.0

Uncorrected = viral concentration from qPCR machine
Corrected = (uncorrected × [1/recovery efficiency])
ND - not detected

Using the corrected virus concentrations (Table 6.11) and the ratio of infectious virus particles to total virus particles for each virus, the infectious dose of each virus was estimated and is shown in Table 6.12.

Table 6.12: Calculated infectious doses for the enteric viruses detected in Tyume and Buffalo Rivers

Enteric virus	Infectious:Total number ratio	Reference	River	Corrected	Calculated infectious dose
HAdV	1:1000	Grabow et al., 1992;	Tyume	2.27×10^{4}	2.27×10
		Rodríguez et al., 2009	Buffalo	1.86×10^{2}	1.86×10^{-1}
HAV	1:60	Deng et al., 1994; Pinto et	Tyume	1.44×10^{4}	2.4×10^{2}
		al., 2009	Buffalo	4.37×10^{4}	7.28×10^{2}
RoV	1:40 000	Ward et al., 1984;	Tyume	3.37×10^{3}	8.43×10^{-2}
		Rodríguez et al., 2009	Buffalo	1.11×10^{2}	2.78×10^{-3}

Estimates of risks of daily infection for the enteric viruses were determined using the models (Haas, 1996; WHO, 2001) shown below:

$$P_i = 1 - e^{(-rN)} \quad (1)$$

$$P_i = 1 - [1 + d/N_{50}(2^{1/\alpha} - 1)]^{-\alpha} \quad (2)$$

$$P_i = 1 - [1 + d/\beta]^{-\alpha} \quad (3)$$

Equations (1), (2) and (3) were used for HAdV, HAV and RoV, respectively. The parameters are described in Table 6.13.

Table 6.13: Parameters used in estimating the risks of daily infection using Equations (1), (2) and (3)

Parameter	Description	Reference
P_i	probability (risk) of infection	Haas, 1996
N or *d*	dose or exposure	
α and β	parameter characterised by dose-response relationship	Haas et al., 1999
r	parameter of exponential model	WHO, 2001
N_{50}	median infectious dose	

The exponential model is based on the following assumptions: microorganisms are distributed in water randomly and thus follow the Poisson distribution for infection to occur; at least one pathogen must survive within the host; and the probability of infection per ingested or inhaled organism is constant (Haas et al., 1999). The beta-Poisson model has the same assumptions as the exponential model except nonconstant survival and infection probabilities. Survival probabilities (α and β) are given by the beta distribution and the slope of the dose-response curve is more shallow than the exponential one (Haas et al., 1999).

The parameter values and estimated daily risks for the enteric viruses obtained using the calculated infectious doses are represented in Table 6.14 for both water bodies.

Table 6.14: Parameters and risk of infection values

Enteric virus	River	Dose		N_{50}	α	β	*r*	P_i	
		D	*R*					D	R
HAdV	Tyume	0.227	2.27				0.4172	0.09036	0.6121
	Buffalo	0.00186	0.0186					0.00078	0.0077
HAV	Tyume	2.4	24	1000	0.2			0.01425	0.1053
	Buffalo	7.28	72.8					0.03988	0.2103
RoV	Tyume	0.000843	0.00843		0.2531	0.4265		0.00051	0.005
	Buffalo	0.0000278	0.000278					1.66×10^{-5}	0.0002

D = domestic water use
R = recreational water use
P_i = probability of infection
Values of α and β, are, respectively, 0.2531 and 0.4265 for rotavirus (Haas et al., 1993)

The dose-response parameter, *r*, assumes a value of 0.4172 (Crabtree et al., 1997)

For both categories of water use, the information given in Figure 6.26 and Figure 6.27 shows that HAdV and HAV presented the highest risks of infection in the Tyume and Buffalo Rivers. For both rivers and all 3 viruses, recreational water use posed a higher risk of infection compared to domestic water use, most probably because of the larger volume (100 mℓ) involved which has the consequence of also increasing the dosage. Enteroviruses did not present any significant risk of infection in either of the rivers.

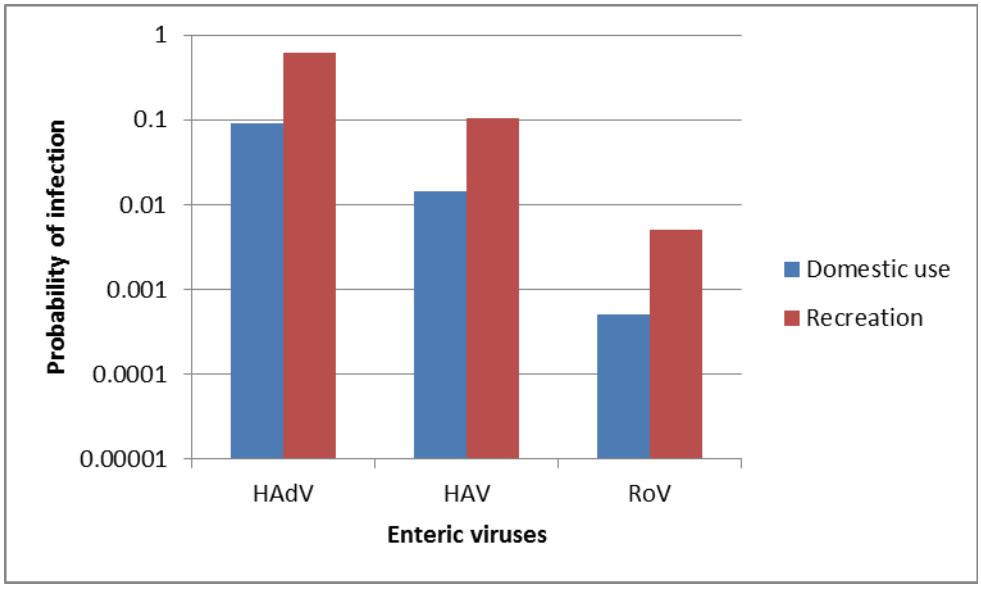

Figure 6.26: Risk of infection for enteric viruses in Tyume River

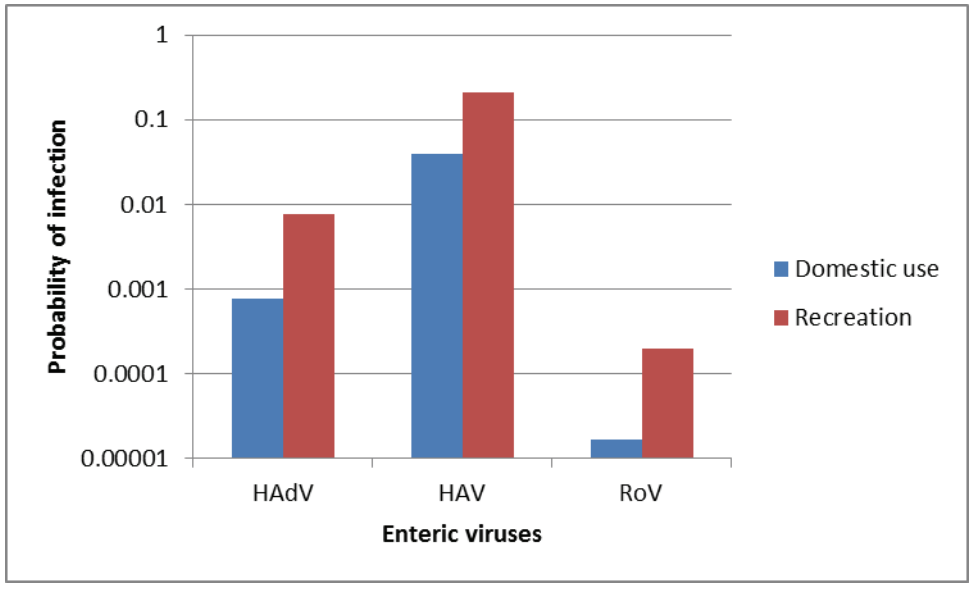

Figure 6.27: Risk of infection for enteric viruses in Buffalo River

While exposure to a minimal volume of 10 mℓ of Buffalo River water would lead to a 1:20 000 probability of infection from rotavirus, exposure to the same volume of Tyume River water would lead to a 1:2 000 probability of infections, which is 10 times higher. However, Buffalo River water meets the recommended 1:10 000 probability of infection with respect to rotavirus while the risk in Tyume River water is 5 times more than the acceptable risk level. Rotavirus is the leading cause of GI morbidity and mortality among young children and is of much greater public health concern to young children and immunocompromised persons

and populations than the general population (US.EPA, 2010). Both HAV and HAdV present a significantly higher risk of infection figures compared to RoV in the case of ingestion of 10 mℓ of water from both rivers. Serotyping of qPCR positive samples in this work showed the presence of HAdV serotypes 40 and 41 in addition to other serotypes. While HAdV types vary widely in their pathology, strains 40 and 41 are notable for causing enteric infections in young children (USEPA, 2010). The risk of infection with HAV in faecally polluted water has been found to increase with increased immersion in contaminated water (Taylor et al., 1995; Gammie and Wyn-Jones, 1997). This implies that water from both Tyume and Buffalo rivers may not be suitable for full-contact recreational activities. This risk is significantly higher in children under 10 years of age as well as in immunocompromised individuals (Venter et al., 2007). This calculation of the risk of infection presents a number of uncertainties. The volume of water that was used to assess the fitness-for-use of the water for domestic purposes is far below what an individual human being could consume per day. Even then, the amount of water that samples of individuals consume per day may differ between individuals depending on their levels of physical activity and the state of their health. In this regard, if a volume larger than 10 mℓ were used, the estimated risk could only be larger. However, since environmental samples usually yield a much higher ratio of infectious viruses to total PCR detectable viruses than those viruses that have been adapted to cell culture (Reynolds et al., 1996), the calculated risk of infection values in this study could still be an overestimation of the actual risk.

Water from both rivers also did not conform to the US Environmental Protection Agency (US EPA) bacterial criteria of 200 CFU/100 mℓ faecal coliforms and 33 CFU/100 mℓ enterococci for bathing waters (Wade et al., 2003; USEPA, 1986). Faecal indicator bacteria data therefore corroborate the risk of infection as determined by both the beta-Poisson and exponential models in this study. The susceptibility of various individuals to waterborne viral infections may differ depending on various factors, including the immune status and age of an individual as well as the virulence, serotype and route of infection of the virus (Regli et al., 1991). Immunocompromised patients, pregnant women, the elderly and children are more susceptible to viral infections than the average population (Crawford- Miksza and Schnurr, 1996).

Recommendations for future research

The presence of enteric viruses in surface waters located in Amathole District Municipality has been affirmed. Even though the proportion of infective viruses was estimated in this study, fact remains that there is considerable risk of infection posed by the use of raw surface water for either domestic or recreational use. Future research work in this field may include cell culture to verify the proportion of infectious viruses to total virus particles in environmental water samples. Questionnaire surveys may also be conducted in communities within river catchments in the Eastern Cape Province so that risk assessment profiling is aligned to water-use patterns specific for communities in those catchments. Capacity-building towards microbial source tracking (MST) may be a significant step towards pollution-mitigation measures in surface-water systems.

Conclusion

There are many uncertainties in this risk calculation; however, what is indicated is that the risks are significantly higher than the recommended 1 in 10 000 probability of infection that the US-EPA suggests is an acceptable risk. Since the faecal indicator bacteria data corroborates viral risk of infection data, we conclude that water in both Tyume and Buffalo rivers are not fit for domestic, recreational or fresh produce agricultural activities.

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APPENDIX A

Typical pollution sceneries on the Buffalo River catchment

Bridle Drift Dam showing extensive deposition of chemical pollutants

Eluxolzweni showing extensive growth of water weeds

Rooikrantz Dam showing the presence of considerable amounts of animal dung