ASSESSMENT OF THE PHYSICOCHEMICAL AND MICROBIOLOGICAL QUALITIES OF TYUME RIVER IN AMATHOLE DISTRICT IN THE EASTERN CAPE PROVINCE, SOUTH AFRICA

BY

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DECLARATION

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

Name: _____

Signature: _____

Date: _____

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I acknowledge my God the King of Kings by quoting Psalms 45v1 "My heart overfloweth with a goodly matter; I speak the things which I have made touching the king: My tongue is the pen of a ready writer." [American Standard Version]

DEDICATION

To the Lord God Almighty

To my wife Elisa Sibanda

To my son Tivadar Sibanda

LIST OF ACRONYMS

AEMREG	Applied and Environmental Microbiology Research Group
ANOVA	Analysis of variance
APHA	American Public Health Association
ATCC	American type culture collection
BOD	Biochemical oxygen demand
CFU	Colony forming unit
COD	Chemical oxygen demand
DEAT	Department of Environment Affairs and Tourism
DNA	Deoxyribonucleic acid
DO	Dissolved oxygen
DWAF	Department of Water Affairs and Forestry
EC	Electrical conductivity
EnV	Enterovirus
EPA	Environmental Protection Agency
EtBr	Ethidium bromide
FIB	Faecal indicator bacteria
HAdV	Human adenovirus
HAV	Hepatitis A Virus
HEV	Hepatitis E Virus
HIV	Human immunodeficiency virus
NoV	Norovirus
PCR	Polymerase chain reaction
NTU	Nephelometric turbidity units
qPCR	Quantitative real-time PCR
RHP	River Health Programme

RNA   Ribonucleic acid

RoV   Rotavirus

RT-PCR   Reverse transcription polymerase chain reaction

TDS   Total dissolved solids

USEPA   United States Environmental Protection Agency

WHO   World Health Organisation

WRC   Water Research Commission

TABLE OF CONTENTS

TABLE OF TABLES

TABLE OF FIGURES

GENERAL ABSTRACT

Tyume River water samples were collected monthly, over a 12-month period starting from August 2010 and ending in July 2011, and transported on ice to the Applied and Environmental Microbiology Research Group (AEMREG) Laboratory at the University of Fort Hare, Alice for analyses within 6 h of collection. 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 the membrane filtration method. 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.

Biochemical oxygen demand (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. DO concentrations generally ranged between 7.47 mg/ℓ and 10.42 mg/ℓ, well within the target water quality requirements. The temperature regime ranged between 6°C and 28°C and for most sampling sites, the temperature regimes were within the acceptable limit of no risk (≤ 25°C) for domestic water uses in South Africa.

EC ranged between 47 µS/cm and 408 µS/cm well within the South African target water quality EC guideline of 700 µS/cm though it was observed to increase as the river flowed through settlements. The pH 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. Unpolluted waters normally show a pH of between 6.5 and 8.5. Most of the pH values observed in this study lie between pH 8.5 and pH 10.8 levels which are not far off from the upper level guideline of pH 9.0 for domestic use. Turbidity ranged between 6 NTU and 281 and fell short of the target water quality range (0 NTU to 1 NTU) of no risk for domestic water uses in South Africa. Monthly TDS values and EC values showed direct proportionality. TDS concentrations at all sites fell within the acceptable guideline of 0 mg/ℓ to 450 mg/ℓ of TDS for domestic use. Nutrient profiles were as follows: nitrate (0.18 mg/ℓ to 4.21 mg/ℓ); nitrite (0.02 mg/ℓ to 2.35 mg/ℓ); and orthophosphate (0.06 mg/ℓ to 2.72 mg/ℓ).

The bacteriological qualities of the water were poor, exceeding the guideline of 200 CFU/100 mℓ and 33 CFU/100 mℓ for FC and enterococci respectively, for recreational water. FC counts also exceeded the 1 000 CFU/100 mℓ guideline for water used in fresh produce irrigation. Generally, higher counts of TC, FC and enterococci were recorded at the sampling sites located at the lower reaches of the river compared to the upper reaches.

Adenovirus was detected in 31% of the river samples in concentrations ranging between 1.0×10^0 genome copies/ℓ and 8.49×10^4 genome copies/ℓ. Serotyping showed the presence of species C adenovirus serotypes 1, 2, 6 and 7, and species F adenovirus serotype 41. The prevalence of norovirus was 4% while rotavirus was detected in 4% of river samples in concentrations ranging between 9×10^0 genome copies/ℓ and 5.64×10^3 genome copies/ℓ. Hepatitis A virus was detected in 13% of river samples in concentrations ranging between 1.67×10^3 and 1.64×10^4 genome copies/ℓ while enteroviruses were not detected.

Detection of enteric viruses was inversely correlated to temperature. Risk analysis showed that both hepatitis A virus and adenovirus presented significantly higher risk of infection values compared to rotavirus in the case of ingestion of 10 mℓ or 100 mℓ of water from Tyume River while enteroviruses did not present any significant risk of infection. Tyume River water samples 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. Whereas the physicochemical parameters showed that Tyume River water was relatively clean, the bacteriological water quality was poor. Most of the microbiological contamination observed in this study (especially FIBs) can be blamed on inadequate sanitary infrastructure as we observed that open defecation is commonplace in this catchment, which also serves as a conduit for effluent discharges from wastewater-treatment facilities. Enteric viruses were detected along the course of the river in a sporadic pattern, generally not related to natural hydrological cycles and so we conclude that the presence of enteric viruses in the river is suggestive of the dynamics of the same in the host population. 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. This study further confirmed 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.

Key words: Tyume River, microbiological, physicochemical, enteric virus, quantitative PCR, semi-nested PCR, risk of infection

CHAPTER ONE

GENERAL INTRODUCTION

1.0 Background

Rivers constitute the main water resources in inland areas, making it a prerequisite for effective and efficient water management to have reliable information on water quality (Vega *et al*., 1998). The history of human civilisation is inextricably linked to the world's rivers, with most ancient societies developing alongside major rivers, most notably the Nile, the Tigris and Euphrates, the Indus, and the Yellow River (WWF, 2006). Human and ecological use of in-stream water depends on its microbiological and physicochemical qualities, which are largely influenced by anthropogenic activities (Chang, 2008; WWF, 2006). While waterbodies like streams, rivers and oceans have amazing self-purification capabilities, continual disposal of wastewater and other pollutants into these waterways has greatly reduced their self-purification abilities (Tahir *et al*., 2011). For instance, nutrients in a river mainly come from industrial and municipal wastewater, runoff from urban and agricultural areas, mining practices and septic tanks (Wit and Bendoricchio, 2001). High nitrate concentrations in streams have been linked to agricultural land-use and the widespread application of fertilizers and manure to agricultural fields (Rock and Mayer, 2006). Stream bank deterioration has also been linked to high phosphorus sediment losses and poor overall water quality (Sekely *et al*., 2002). In particular, streams impacted by cattle grazing have been shown to exhibit poorer water quality than streams where cattle access is restricted

(Nagels *et al.*, 2002). Streams impacted by cattle grazing indicate higher levels of nitrate, suspended solids and faecal coliforms (Muenz *et al.*, 2006).

Excessive loading of nitrogen (N) and phosphorus (P) into water bodies is linked to eutrophication (Stow *et al.*, 2001), causing undesirable algal blooming, reduced water transparency, anaerobic hypolimnions, taste and odour problems, and increasing cost of water treatment (Drenner *et al.*, 1997). Human alteration of the landscape has an extensive influence on microbiological water quality (Chang, 2007) and its heat budget, which subsequently increases water temperature (Nelson and Palmer, 2007) and modifies in-stream physicochemical processes that drive oxygen, nutrient, pH and sediment cycling (Baker, 2003). Rivers and their catchments also are increasingly under threat from ill planned development schemes (Yousafzai *et al.*, 2010) where the establishments of settlements is not matched by the establishment of adequate sanitary infrastructure to cater for increased volumes of wastes.

Wastewater effluents usually contain loads of pathogenic microorganisms and a high content of organic matter (Howard *et al.*, 2004). Although wastewater treatment facilities and processes are effective in reducing the organic contents of wastewater, there is still evidence that wastewater effluents still contain pathogens, including hepatitis A virus, enteroviruses, and noroviruses (Espigares *et al.*, 1999; Griffin *et al.*, 1999). The presence of microbial pathogens in source waters poses a considerable health risk to the general public (Tahir *et al.*, 2011), and also serve as an indicator of faecal contamination of the said waterbodies (Tyagi *et al.*, 2006). Around 250 million people each year get infected from exposure to contaminated waters; resulting in about 10-20 million deaths, mostly in developing countries which suffer from lower levels of sanitation and poor socioeconomic conditions (Toze, 1999). The risk posed by the presence of microbial pathogens in water necessitates monitoring source waters for various types of microbial pathogens by determining the type of

microbial pathogen present and its relative numbers in relation to seasonal changes. More than several hundreds of enteric microbial pathogens are known to infect man (Abbaszadega, 2001) most of them belonging to the following groups: viruses, bacteria, pathogenic protozoa and pathogenic helminths.

Determination of the relative numbers of pathogens in a water sample is imperative for risk analysis since different microbial pathogens have different infectious doses (Bosch, 1998). Besides the infectious dose of each pathogen, the health risk is also dependent on the human sensibility to micro-organisms (age- or immunity-dependent). While some pathogens are highly dangerous for humans even at low concentrations (hepatitis A virus, *E. coli* O157:H7, *V. cholerae*), others need to be ingested in high concentrations to be harmful (*Vibrio parahaemolyticus*), or, although highly infectious, are not very dangerous (norovirus) (OSPAR Commission, 2009). Microbiological pollution and its potential impacts are also dependent on the weather, climate and environmental conditions such as turbidity and amount of organic matter. For instance, some bacterial pathogens are susceptible to sunlight inactivation and tend to die off much faster in warm clear water than in cold turbid water which is less well penetrated by sunshine (OSPAR Commission, 2009). This present study was done to evaluate the water quality of Tyume River in the Eastern Cape Province of South Africa. This river is of strategic importance to its host community for several reasons as follows:

- It has a perennial flow, having its source in the high altitude Hogsback mountains and is therefore an ever reliable source of water to its community
- It is the feeder stream to several drinking water treatment plants along its length
- It provides raw water for several irrigation schemes located midstream
- To communities lacking basic clean water provision, it is a source of water for drinking and other domestic uses

- It provides for fishing and recreation activities
- It is an integral part of the communities' social and cultural substratum, e.g. Christian groups use it for baptism events
- It is a conduit for the disposal of wastewater effluents from several wastewater treatment facilities along its length

1.1 Statement of the problem

WHO (2003) stipulates that access to safe drinking water is a fundamental human need and a basic right. Contaminated water therefore jeopardises both the physical and social health of all people. The fundamental requirement of an open water supply system is to provide safe water whose quality is agreeable for any water use category and in sufficient quantities. In contrast with the bacteriological water quality, virological water quality concerning either drinking, bathing or recreation is in South Africa and much of the developing world, not routinely analysed for the presence of enteric viruses. Consumption of both contaminated water and infected fish can cause serious health concerns especially in cases where enteric viruses are involved. Water-borne pathogens infect around 250 million people each year resulting in 10 to 20 million deaths world-wide. A 2004 report indicated that in South Africa alone, about 17% of the population did not have access to potable water supply and about 54% of the population lacked basic sanitation (Zamxaka *et al*., 2004). This highlights the potential of infection due to water-borne pathogens. In South Africa nearly 80% of the population rely on surface water as the main source of water (Venter, 2001). Many of these waterbodies are often impacted by inadequately treated effluents from municipal wastewater treatment plants (Fatoki *et al*., 2003). This relatively high percentage of the population that is without proper water supply services indicates that many of the people still utilize untreated

surface water for domestic purposes. Most of these people are poor and rely on State intervention for improved water supply.

1.2 Hypothesis

The working hypothesise of this study was that the water in Tyume River is of sub-standard quality in terms of its physicochemical and microbiological qualities; that judging from its physicochemical and/or microbiological qualities, the water is not fit for use either for domestic, irrigation, and/or recreational purposes.

1.3. Justification for the research

The Eastern Cape Province has a plethora of socio-economic problems whose effects cascade down to the environmental degradation, chiefly, pollution of surface water resources. It is the second largest and third most populated province in South Africa (NRA, 2007). The 2001 Census indicated that the unemployment rate in the Eastern Cape was 55%, against the national average of 41% (NRA, 2007). The Eastern Cape is also characterised by high levels of poverty, which is clearly a rural phenomenon, with the rural poverty rate estimated at 82.2% compared to 42.1% in urban areas (Pauw *et al*, 2005). HIV AIDS accounted for more than 20% of deaths in the Province during 2000 while HIV prevalence in the 15-49 age group was 15.2% in 2008 (Makiwane and Chimere-Dan, 2010). The percentage of the population relying on natural sources of water, such as untreated springs, streams, rivers and dams is on average 31% for the Province (Makiwane and Chimere-Dan, 2010). The situation is most severe in the OR Tambo District, where 64% of the population rely on natural sources, 52% in Alfred Nzo, 34% in Ukhahlamba, 32% in Chris Hani, and 24% in Amatole District

5

Municipalities respectively (Makiwane and Chimere-Dan, 2010). While clean water and sanitation are necessary for reducing mortality and poor health and for increasing the productive capacity of the poor, the provincial average for people with no sanitation facilities is 30% (PGDP, 2004). About 43% of Eastern Cape residents make use of their own refuse dump, while 38% have their rubbish removed once a week (NRA, 2007). An unusually high percentage of Eastern Cape residents also live in informal settlements, with about 43.5% of migrants in O.R. Tambo and 40.4% in Amathole Districts residing in informal dwellings (Bank and Kamman, 2010). The above statistics are testimony to the unparalleled establishment of informal settlements versus the provision of clean water and sanitary facilities. Pollution from human settlements lacking appropriate sanitary infrastructure, the discharge of partially treated or untreated wastewater from overburdened wastewater treatment facilities, leachates from refuse dumps and from land-use activities such as agriculture are therefore the major sources of pollution to the surface waterbodies in this province. This coupled with a high percentage of immunocompromised individuals in the province means a significant proportion of the population is vulnerable to infections, of which water-borne infections are critical. This study was therefore sought to address the following research questions:

➢ Is the level of water pollution uniform throughout the course of a river; and if so, what factors could be contributing to the variations?

➢ Do physicochemical and microbiological qualities of river show any seasonal trends

➢ Is there a correlation between the presence and prevalence of both the faecal indicator bacteria (faecal coliforms, total coliforms and enterococci) and enteric viruses and the physicochemical parameters of the river

> Is the level of pollution in Tyume River (as determined by its physicochemical and microbiological parameters) within the acceptable limits/guidelines for the water to be fit for human use?

In answering these questions therefore, this study aimed at assessing the quality indices of Tyume River in the Eastern Cape Province in terms of its microbiological and physicochemical qualities. The specific objectives of this study were as follows:

> Monthly collection of water samples for analysis from six sampling sites along Tyume River for a period of one year (August 2010 to July 2011)

> Determining the physicochemical qualities of the water samples

> Assessing the incidence and distribution of faecal indicator bacteria in the water samples

> Assessing the prevalence and distribution of human viral pathogens in Tyume River

> Correlating both the faecal indicator and viral genome densities with the physicochemical qualities of the water samples

> Assessing the fitness for use of the water for recreational, domestic and agricultural purposes.

1.4 Chapter outline

This thesis consists of seven chapters. Chapter 1 is the introduction to the study and outlines the research questions, the broader aim and specific objectives of this work. Chapter 2 is the literature review and is designed in such a way as to focus on the role played by water quality in public health, agriculture and economic growth. Chapter 3 is a research paper focussing on the physicochemical qualities of Tyume River with a bias towards public health significance. Chapter 4 is also a research paper zooming into the bacteriological qualities of Tyume River,

and looks at seasonal and spatiao-temporal distribution of faecal indicator bacteria in Tyume River. Chapter 5 is a research paper dealing with the prevalence of RNA viruses in Tyume River, and goes on to assess the risk of illness involved in the use of the raw river water for drinking and recreation. Chapter 6 is also a research paper looking at the prevalence of adenoviruses in Tyume River water. It also looks at the serotypes and their spatial distribution and outlines possible sources of such contamination. Chapter 7 gives the general overview of the whole work, the recommendations and the conclusion.

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CHAPTER TWO

WATER QUALITY DETERIORATION AND ITS SOCIO-ECONOMIC IMPLICATIONS

This chapter was submitted for publication to the Journal of Pure and Applied Microbiology

2.0 Abstract

From ancient times, people have chosen to live near water, settling in river valleys. Improved water supply and water resources management boosts countries' economic growth and contributes greatly to poverty eradication. An adequate supply of safe drinking water is one of the major prerequisites for a healthy life but because of surface water pollution, waterborne disease became, and still is, a major cause of death in many parts of the world, particularly in children. Rapid urbanisation has exacerbated surface water pollution by increasing point pollution and non-point source pollution entering surface waterbodies. Physico-chemical and microbiological properties of water are used to assess water quality as they give a good impression of the status, productivity and sustainability of such waterbody. Water pollution control has been a matter of public concern for more than a century. Currently, human beings and natural ecosystems in many river basins suffer from debilitating effects of water pollution. Hence, development of better water conservation practices and policies are critical to the sustenance of our water quantity and quality to ensure protection of public health.

Key words: freshwater, water quality, pollution, public health, physico-chemical, microbiological.

2.1 Introduction

From ancient times, people have chosen to live near water, settling in river valleys, beside lakes, or along coastlines (Carpenter *et al.*, 1998). Without freshwater of adequate quantity and quality, sustainable development will not be possible (Kumar, 1997). Water quality reflects the composition of water as affected by natural causes and man's cultural activities expressed in terms of measurable quantities and related to intended water use (Kumar, 1997). Worldwide, waterbodies are the primary dumpsites for disposal of waste, especially the effluents from industries located near them (Ewa *et al.*, 2011). Effluents from industries harbour toxic contents, capable of altering the physical, chemical and biological nature of the receiving waterbodies (Sangodoyin, 1991; Adekunle and Eniola, 2008). First to be degraded by such waste is the physical quality of the water, while the biological degradation becomes evident later in terms of number, variety and organization of the living organisms in the water (Gray, 1989). DWAF (2001) defined water pollution as the alteration of the properties of a water resource so as to make it, among others, "harmful or potentially harmful to the welfare, health or safety of human beings". Much of the current concern with regards to environmental quality is focused on water because of its importance in maintaining human health and health of the ecosystem (Mahananda *et al.*, 2010).

2.2 Surface water stress and vulnerability

Water pollution occurs when unwanted or toxic substances are accidentally or intentionally introduced into waterbodies in quantities which affect the resource in providing its services, which include domestic use, irrigation, navigation, recreational and life support (ecological) functions (The Herald, 2012). Water stress and vulnerability are linked, since

16

pollution reduces the volume of water available for human use (Carpenter *et al*., 1998). Thus, preventing pollution is among the most cost-effective means of increasing water supplies.

Rapid urbanisation has exacerbated surface water pollution by increasing point source pollution and non-point source pollution entering surface waterbodies (Oke, 2003). Both the withdrawal of surface water for human use and economic activities and its subsequent discharge back into surface water resources as effluents can affect the ability of aquatic ecosystems to survive (Oke, 2003). Such effluents have been reported to also include antibiotics and other pharmaceutical compounds (Metcalfe *et al*., 2003). The occurrence of antibiotics in aquatic environments is of ecotoxicological concern because of potential ecosystem alteration (Levy, 1997). Prolonged exposure to low doses of antibiotics leads to the selective proliferation of resistant bacteria, which could transfer the resistance genes to other bacterial species (Batt *et al*., 2006).

Water quality problems and their effects are different in type and magnitude in developed and developing countries, particularly those stemming from microbial and pathogen content (Kundzewicz *et al*., 2007). Currently, human beings and natural ecosystems in many river basins suffer from water scarcity. In global-scale assessments, basins with water stress are defined either as having a per capita water availability below $1,000 m^3/yr$ (based on long-term average runoff) or as having a ratio of withdrawals to long-term average annual runoff above 0.4 (Kundzewicz *et al*., 2007). Populations living in such severely stressed basins are estimated to range from 1.4 billion to 2.1 billion (Kundzewicz *et al*., 2007).

2.3 Indicators of surface water pollution

Reporting on the endemic water pollution problem in Zimbabwe, The Herald of Friday 13 July 2012 had this to say,

"The presence of pollutants in water is primarily perpetrated by human activities. These pollutants are either discharge directly into rivers or are carried into streams and rivers by surface runoff, leading to serious deterioration of water quality. Water pollution can be categorised into four broad categories viz: organic pollution which occurs when excess of organic matter, such as manure or sewage, enters the water; toxic pollution which occurs when a chemical pollutant that is not natural component of an aquatic ecosystem is introduced; thermal pollution which occurs when water is used as a coolant near a power or industrial plant and then is returned to the aquatic environment at a higher temperature than it was originally and ecological pollution which takes place when chemical pollution, organic pollution or thermal pollution are caused by nature rather than by human activity." The presence of pollutants belonging to any of the above categories can be ascertained by periodically assessing the physicochemical and microbiological properties of in-stream water (Kolawole *et al.*, 2011).

2.3.1 Physicochemical parameters

Dissolved constituents of waterbodies are often determined as a major component for baseline limnological studies (Arain *et al.*, 2008). Physico-chemical properties of water are used to assess water quality as they give a good impression of the status, productivity and sustainability of such waterbody (Mustapha, 2008). Physico-chemical determinants of pollution in rivers, lakes, and oceans include temperature, pH, total dissolved solids (TDS),

turbidity, electrical conductivity (EC), biological oxygen demand (BOD), chemical oxygen demand (COD), dissolved oxygen (DO), heavy metals and nutrients amongst others (Kolawole *et al*., 2011; Tahir *et al*., 2011). These pollutants originate either from point or non-point sources (Carpenter *et al*., 1998). DO is the most important factor in the assessment of water quality and is vital for aquatic life (Tahir *et al*., 2011) while temperature is the most important physical variable affecting the metabolic rate of aquatic microorganisms (Crawshaw, 1979; Kumar *et al*. 1996) as well as the chemical reactions in water and thereby determines the solubility of gases (including oxygen) and imparts taste and odour to the water (Kulkarni and Tapase, 2012). Healthy freshwater bodies are characterised by a DO concentration of at least 5 mg/L (WHO, 1971). Surface water temperature is directly affected by changes in ambient air temperatures and indirectly by the inflow of water of a different temperature (Yousafzai *et al*., 2010), characteristic of discharge of large volumes of water from industrial plants. The pH of natural waters range from less than 4 to greater than 12, but usually falls between 6 and 9 for unpolluted river systems (Zafar, 1984). pH values above and below this range are indicative of water pollution (Tahir *et al*., 2011); and could give rise to toxic effects, largely as a result of disturbances in internal ion homeostasis (Yousafzai *et al*., 2010). High concentrations of dissolved phosphate may lead to osmotic stress, as is the case with high nitrate concentrations (DWAF, 1996). Even though trace quantities of phosphorous are naturally present in surface waters (Kulkarni and Tapase, 2012), higher concentrations of phosphate could be indicative of pollution from domestic waste and agricultural runoff, and may lead to eutrophication, which has drastic economic, social and ecological consequences (Kulkarni and Tapase, 2012). High levels of organic pollution can also result in low DO, high BOD and COD concentrations (Manson, 1989). High turbidity is harmful to aquatic organisms since it can cause anaerobic conditions, interfere with respiration in aquatic fauna and also reduce light penetration, hindering photosynthesis and natural aquatic life (Davies,

1996). EC is directly related to TDS in water and its value becomes greater with increasing degree of pollution (Dhakal, 2006).

2.3.2 Faecal indicator bacteria

Faecal indicator bacteria (FIB) have been used for many years to determine the quality and safety of surface and ground waters (Ahmed *et al*., 2010; Ahmad *et al*., 2006). Bacterial groups classified as FIB include the total coliforms (TC), faecal coliforms (FC) and enterococci (synonymously used as faecal streptococci) (WHO, 2000). Faecal streptococci have been suggested as the recommended indicator for salt water while either faecal streptococci or *Escherichia coli* can be used for monitoring freshwaters (WHO, 2000). Faecal streptococci are widely accepted as useful indicators of faecal pollution in natural aquatic ecosystems because they show a close relationship with gastrointestinal symptoms associated with bathing in marine and freshwater environments (Kay *et al.,* 1994; WHO, 1998) while their persistence patterns are also similar to those of potential water-borne pathogenic bacteria (Richardson *et al.,* 1991).

Faecal coliforms (also known as thermotolerant coliforms) include strains of the genera *Klebsiella* and *Escherichia* (Dufour, 1977). It has been suggested that for the purpose of sanitary water testing, *E. coli* should be used as an indicator of faecal pollution since it possesses a more direct and closer relationship with homeothermic faecal pollution (Tyagi *et al*., 2006). However, *E. coli* has been detected in some pristine areas (Ashbolt *et al.,* 1997) and has also been associated with regrowth in drinking water distribution systems (Lechevallier, 1990). The presence of these bacteria in surface waters is thought to indicate that pathogenic organisms such as *Salmonella* spp., *Shigella* spp. and hepatitis A may also be present (Ahmed *et al*., 2006). Polo *et al*. (1999) reported incidences of serotypes of

Salmonella spp. isolated from freshwater sources in Spain which were identical to serotypes found in clinical samples, a case that underlines the connection between water quality and public health. However, epidemiological studies in warm tropical waters demonstrate the lack of a strong relationship between faecal indicators and health outcomes, in part, due to the inappropriate nature of *E. coli* or faecal streptococci as indicators of waterborne pathogens in these recreational waters (WHO, 2000). Alternatively, spores of *Clostridium perfringens* have been proposed as a useful indicator when fresh faecal contamination is being investigated (Leeming *et al.,* 1998) and more importantly, as suitable indicators for parasitic protozoa and viruses in sewage-impacted waters (Ferguson *et al.,* 1996). Bacteriophages have also been suggested as indicators specific for human sewage, and more specifically *Bacteroides fragilis* phages which appear to survive in a similar manner to that of human enteric viruses under a range of conditions (Lucena *et al.,* 1996).

Contamination of surface waters with faecally derived bacteria can occur through point sources like sewage effluents and non-point sources such as agricultural and urban run-off (Balzer *et al*., 2010). Sewage effluents contain a wide variety of pathogenic microorganisms that may pose a health hazard to the human population when discharged into recreational waters (WHO, 2000; Shakalisava *et al*., 2010). The density and variety of these pathogens are related to the size of the human population, the seasonal incidence of the illness, and dissemination of pathogens within the community (Pipes, 1982). Studies also prove that bathers can be a significant source of pathogenic micro-organisms, even in cases where there is no faecal pollution from the outside (WHO, 2000). Storm events and recreational activity also cause the re-suspension of FIBs resulting in a rapid increase in the load of pathogens in the water phase (Balzer *et al*., 2010). The U.S. Environmental Protection Agency (2002) reported that 35% of impaired rivers and streams were polluted by FIB which could indicate the presence of enteric pathogens.

2.3.3 Water-borne enteric viruses

Faecal matter of patients suffering from virally-induced gastroenteritis contains high concentrations of human enteric viruses which, if not inactivated during wastewater treatment processes may infect surface water sources for drinking water, recreational activities, aquaculture and irrigation (Wyn-Jones and Sellwood, 2001; Phanuwan *et al.*, 2006; Hot *et al.*, 2003; Lee and Kim, 2002; Pusch *et al.*, 2005). Human enteric viruses include the families *Picornaviridae* (these are enteroviruses like poliovirus, coxsackievirus, and echovirus), *Adenoviridae*, *Caliciviridae* (norovirus, calicivirus), *Astroviridae*, and *Reoviridae* (reovirus, rotavirus) (Griffin *et al.*, 2003). They cause a wide range of diseases which include epidemic gastroenteritis, meningitis, ocular and respiratory infection, paralysis, myocarditis and hepatitis (Bosch *et al.*, 1991).

Enteric viral pathogens have been shown to be present in environmental waters even when bacterial indicators are absent (Morris, 1993; Tree *et al.*, 1997, 2003). Studies focusing on non-enteric viruses like bacteriophages, viruses infecting algae, protozoa, fish and vascular plants (Wommack and Colwell, 2000) have found that the dynamics of these viruses are linked to the dynamics of potential host cells (primarily bacteria and algae) (Peduzzi and Schiemer, 2004; Filippini *et al.*, 2006). The abundance of these viruses has been shown to peak following an increase in host cell abundance (Hennes and Simon, 1995; Farnell-Jackson and Ward, 2003). However, enteric viruses are obligate intracellular host-specific parasites which can neither grow nor survive for extended periods in environmental waters outside their host. These viruses are very small, ranging from 20-70 nm in diameter and consist of a nucleic acid genome surrounded by a protein capsid and, in some cases, a lipoprotein envelope (Virological Compliance, 2005). Survival and/or persistence of enteric viruses in the environment is strongly linked to various environmental factors like exposure to ultra-

violet radiation, temperature (Yates *et al*., 1985; Gantzer *et al*., 1998; Garza and Suttle, 1998), adsorption to particulate material (Bitton and Mitchell, 1974; Suttle and Chen, 1992) and salinity (Stallknecht *et al*., 1990; Sinton *et al*., 2002; Cissoko *et al*., 2008). Their susceptibility to the different environmental factors may however vary profoundly within the viriosphere as viruses are capable of developing resistance mechanisms to survive in harsh habitats including hypersaline waters and hot springs or hydrothermal vents (Guixa-Boixareu *et al*., 1996; Bettarel *et al*., 2006; Geslin *et al*., 2003; Breitbart *et al*., 2004).

2.3.4 Viral persistence in tropical freshwater environment

While specific viruses or strains of viruses are not always present in a community at any one time, representatives of the large groups are however, generally present on most occasions (Virological compliance, 2005). Enteric viruses may be found in high numbers in domestic wastewater, their numbers generally varying with the level of virus infection in the community (Lewis *et al*., 1986). Wastewater treatment processes that do not include a disinfection step are often inefficient in removing viruses (Virological compliance, 2005).

In previous studies, levels of human viral contamination in sewage and wastewater treatment plants were analysed (Albinana-Gimenez *et al*., 2006; Bofill-Mas *et al*., 2000, 2006; He and Jiang, 2005; Katayama *et al*., 2008; Pina *et al*., 1998). All these studies reported high concentrations of viruses in sewage. Viruses outside a host are inert particles possessing no intrinsic metabolism and do not require any nutrients to persist (Rzezutka and Cook, 2004). They are however, resilient enough to remain in the environment for long periods of time and still retain their infectivity during the various conditions that they may encounter between one host and another (Lopman *et al*., 2003; Skraber *et al*., 2004; Espinosa *et al*., 2008). This is illustrated by the number of outbreaks of enteric viral diseases

attributable to waterborne transmission (Mead *et al.,* 1999). Rzezutka and Cook (2004) reviewed works previously done by other researchers on enteroviruses (polio-, echo- and coxsackieviruses). Summarizing the observations from these studies and grouping them into freshwater sources gave mean viral inactivation rates of: 0.576 $\log_{10}$ d^{-1} (tap water); 0.325 $\log_{10}$ d^{-1} (polluted river water); 0.25 $\log_{10}$ d^{-1} (unpolluted river water); 0.374 $\log_{10}$ d^{-1} (impounded water); and 0.174 $\log_{10}$ d^{-1} (ground water). These rates were all less than 1 $\log_{10}$ per day, and indicated that viruses could survive in freshwater sources for prolonged periods of time.

Viruses have been found to be inactivated by prolonged holding in reservoirs exposed to sunlight, elevated temperature and extremes of pH (Sobsey 1989). A study carried out by Phanuwan *et al*. (2006) in Jakarta, Indonesia showed a statistically significant correlation between the physicochemical parameters (including conductivity, turbidity, temperature and total dissolved solids [TDS]) with all viruses tested (enterovirus, hepatitis A virus, Norovirus GI & GII and adenovirus). Turbidity showed positive correlation with all the microbes tested while conductivity, temperature, TDS showed negative correlation. The same study also showed a high prevalence of enteric viruses in floodwater compared to river water. While the authors did not explain this phenomenon, possible explanation could be that floods may cause the overflow of sewage treatment plants carrying with them large amounts of untreated and partially treated faecal matter. The erosive power of a flood causes the flood waters to be very muddy (turbid) thereby shielding the viruses (especially the RNA viruses) from the damaging effects of UV-rays of the sun.

In a study done by de Cardona *et al*. (1988) in a tropical lagoon, they found that virus inactivation rates were significantly higher in that tropical lagoon than in temperate areas, probably due to higher temperature and salinity. Hurst *et al*. (1989) examined the long-term

survival of coxsackievirus B3, echovirus 7 and poliovirus 1 in samples of surface freshwater collected from five sites of physically different characteristics (artificial lake, small groundwater outlet pond, large- and a medium-sized river, small suburban creek). Survival was studied at temperatures of $-20°C$, $1°C$ and $22°C$. The average viral inactivation was 6.5–7.0 $\log_{10}$ units over 8 weeks at $22°C$, 4–5 $\log_{10}$ units over 12 weeks at $1°C$ and 0.4–0.8 $\log_{10}$ units over 12 weeks at $-20°C$. Several physical and chemical parameters (hardness and conductivity) appeared detrimental to virus survival. The turbidity of the water and suspended solids represented a beneficial influence for virus survival. These findings concurred to a large extent with the work of Phanuwan *et al*. (2006) which was done in Jakarta, Indonesia where turbidity was found to enhance viral survival rates while sunlight and high temperatures were detrimental to virus survival.

Two particularly notable factors that have been associated with the level of human enteric viruses in freshwaters are seasonal changes in water temperature (Geldenhuys and Pretorius, 1989) and a "rainy season" effect observed by Keswick *et al*. (1984). Water temperature exerts an extremely strong influence on viral stability, lower temperatures increasing the survival time (Hurst *et al*., 1989). The "rainy season" effect arises because of the very high turbidity that characterises freshwater bodies at that time of the year, which correlates in a statistically significant manner with the presence of indigenous viruses in water and with virus stability in water under laboratory conditions (Hurst *et al*., 1989). In another study by Espinosa *et al*. (2008) in Mexico City (tropical highland), they reported that the presence of enterovirus and rotavirus was significantly more frequent during the cold-dry season (0.75 and 0.35, respectively) with an average low temperature of $4°C$ and an average precipitation of less than 10 mm than in the warm-rainy season (0.10 and 0.05, respectively) whereas astrovirus showed no significant relationship with the environmental variables recorded (pH, temperature, conductivity and dissolved oxygen concentration). This study also

indicated that enteric viruses could be damaged by rising temperatures, as reported previously (Raphael *et al*., 1985; Hurst *et al*., 1989), when EV and RV were studied in freshwater at 22°C and 20°C.

2.3.5 Determination of virus infectivity

Virus infectivity is generally believed to provide more relevant estimates of virus decay than disappearance of viral particles (Noble and Fuhrman, 1997; Wilhelm *et al*., 1998; Weinbauer *et al*., 1999). Tropical phages may be presumed to be genetically adapted to protect DNA and capsids against UV damage because of their capsid structure (Jacquet and Bratbak, 2003), or the dimerization in DNA that may reduce the susceptibility of destructive enzymes (Weinbauer, 2004). Experiments on the persistence and infectivity of phage isolates have demonstrated that temperature is a strong determinant of both (Giladi *et al*., 1995). Suttle and Chen (Suttle and Chen, 1992) and Noble and Fuhrman (Noble and Fuhrman, 1997) also showed that temperature notably affects the decay of virus infectivity.

While molecular assays such as direct reverse transcription-polymerase chain reaction (RT-PCR) are sensitive, they provide inaccurate estimates of infectious viruses in the environment because they detect both inactivated and infectious virus particles (Greening *et al*., 2002). On the other hand, cell-culture based techniques reveals infectivity of viruses and consequently risk of illness to water consumers (Murrin and Slade 1997; Reynolds *et al*. 1997). In a study done by Greening and co-workers (Greening *et al*., 2002), whose aim was to establish C-PCR methods for detection of culturable enteroviruses and adenoviruses in a broad range of environmental samples, and calibrate these methods against direct RT-PCR, PCR and plaque assay methods for sensitivity, as well as speed and ability to provide virus infectivity data, observed that direct RT-PCR detected 0.05-0.2 pfu/RT-PCR and was 10–100

times more sensitive than other methods but did not provide information on infectivity. Results for Adenovirus also indicated that the direct PCR was 10 times more sensitive than C-PCR and detected 0.16 pfu/PCR, but did not give information on infectivity. They concluded that while direct RT-PCR or PCR methods are the most sensitive methods, their main disadvantage is the inability to provide information on infectivity. This limits their use in environmental virology applications where it is important to ascertain virus infectivity. On the contrary, they found that the C-PCR assay provided sensitive detection and confirmation of infectious enteroviruses and adenoviruses within 2–5 days of sampling. However, infectivity can be inferred for certain uncultivable RNA viruses (norovirus, enteroviruses, Hepatitis A and E) from molecular detection data where the viruses have been subjected to chemical but not UV disinfection (Greening *et al*., 2002). Griffin *et al*. (1999) also suggested that while the presence of viral DNA does not necessarily indicate the presence of infectious viruses, virus viability is inferred whenever virus nucleic acid is detected because the nucleic acids, single stranded RNAs in particular, are extremely susceptible to degradation in the environment.

2.3.6 Health concerns

Enteric viruses are important waterborne pathogens which are frequently isolated from faecally contaminated water and have been linked to numerous waterborne outbreaks (Tani *et al*., 1995; Lee and Kim, 2002; Craun, 1991). Discharge of effluents from wastewater treatment plants into rivers that are used as source water in drinking water treatment plants (DWTPs) could present a risk of infection in the population if efficient drinking-water treatment is not applied and properly controlled before tap water distribution and consumption (Albinana-Gimenez *et al*., 2009). The presence of viruses and other pathogens

in the environment is an indicator of faecal pollution that poses a potential risk to the exposed population, since such pathogens do not constitute normal gastrointestinal microbiota, and are only excreted by sick individuals (Abad *et al*., 1997). Human adenoviruses are present at a higher frequency in sewage compared to other enteric viruses (Pina *et al*., 1998) and are excreted in high concentrations of up to 10^{11} viral particles per gram of faeces from infected patients (Fong *et al*., 2009). Adenoviruses are second only to rotaviruses as major etiologic agents of infantile gastroenteritis (Basu *et al*., 2003; Cruz *et al*., 1990; Logan *et al*., 2006; Meqdam and Thwiny, 2007) causing a variety of clinical manifestations associated with the gastrointestinal, respiratory and urinary tracts, as well as the eyes (van Heerden *et al*., 2005). Adenoviruses are ubiquitous in water environments and these viruses are exceptionally resistant to purification and disinfection processes (EPA, 1998). Enteric human adenoviruses (HAds) have a double-strand DNA genome which is more resistant to UV-light than the single strand RNA of other enteric viruses such as polio and hepatitis A viruses (Maier *et al*. 2000). The occurrence of Ads in finished drinking water and tap water has been reported in South Korea and South Africa (Jiang, 2006). Health outcomes attributed to Ads infection include enteric related illnesses, respiratory system, eye infections and fatal outcome for immunocompromised patients and organ and bone marrow transplant recipients (Kojaoghlanian *et al*., 2003). The consumption of clams harvested from a sewage-polluted area (Pinto´ *et al*., 2009) also exposes people to risk of virus-related food poisoning, especially debilitating infectious hepatitis which may also lead to death. Numerous outbreaks of HAV infection have been reported worldwide (Conaty *et al*., 2000; Dismukes *et al*., 1969; Leoni *et al*., 1998; Mackowiak *et al*., 1976; Mele *et al*.,1989; Sanchez *et al*., 2002; Stroffolini *et al*.,1990) with the most severe occurring in Shanghai, China, in 1988 (Halliday *et al*., 1991). Viral contamination of wastewater, recreational water, drinking water, irrigation water, ground or subsurface water have been reported frequently as a primary source of

gastro-enteritis or hepatitis outbreaks (Beller *et al*., 1997; Gray *et al*., 1997; De Serres *et al*., 1999; Kukkula *et al*., 1999; Haefliger *et al*., 2000; Beuret *et al*., 2002; Parshionikar *et al*., 2003).

2.4 Water quality as an economic growth determinant

Improved water supply and sanitation and water resources management boosts countries' economic growth and contributes greatly to poverty eradication (WHO, 2012). Economic growth itself can also drive increasing investments in improved water management and services, initiating a virtuous cycle that improves the lives of the people across socio-economic boundaries (WHO, 2012). The South African Department of Water Affairs and Forestry (DWAF, 1996) defined the term *water quality* as the physical, chemical, biological and aesthetic properties of water that determine its fitness for a variety of uses and for the protection of aquatic ecosystems. Freshwater resources have important social and economic benefits as a result of tourism and recreation, and are culturally and aesthetically important for people throughout the world (Arain *et al*., 2008). According to CSIR (2010), a healthy society and productive workforce play an important role in long-term economic growth and sustainable development. Water pollution therefore causes not only the deterioration of water quality, but also threatens human health, the balance of aquatic ecosystems, economic development and social prosperity (Babović *et al*., 2011).

2.4.1 Water quality and agriculture: Irrigation water

Typical sources of agricultural water include surface water, groundwater, and municipal supplies (Simonne, 2010). ***DWAF (1996) defines irrigation water as water which***

is used to supply the water requirements of crops and plants which are not provided for by rain, and refers to all uses water may be put to in this, including water for the production of commercial crops; irrigation water application and distribution systems; home gardening; the production of commercial floricultural crops and potted plants. Sewage spills, runoff from concentrated animal production facilities, storm-related contamination of surface waters, illicit discharge of waste, and other sources of pathogens threaten the quality of both surface water and groundwater used for fruit and vegetable production and therefore the safety of the consumed product (Suslow *et al*., 2003). Whenever water comes in contact with produce, its source and quality are directly linked to the potential for contamination (Simonne, 2010). These potential contaminants are classified into microbiological (bacteria, virus, and protozoa); chemical, and physical agents (Simonne, 2010). Chemical and physical properties of irrigation water are of paramount importance because they affect crop yield and soil physical conditions; fertility needs; irrigation system performance and longevity (Bauder *et al*., 2008). Some of the chemical agents of concern in irrigation water are listed in Table 2.1.

Table 2.1: Guidelines for nutrient concentrations in irrigation water (mg/ℓ).

Macronutrient	Low	Normal	High	Very High
Nitrate	<5	5-50	50-100	>100
Ammonium	<2	2-75	75-100	>100
Phosphorous	<0.01	0.1-0.4	0.4-0.8	>0.8
Potassium	<5	5-20	20-30	>30
Calcium	<20	20-60	60-80	>80
Magnesium	<10	10-25	25-35	>35
Micronutrient	**Acceptable range**	**Suggested maximum concentration**		
Iron	2.4-4.0	5.0		
Manganese	<0.2	0.2		
Copper	<0.2	0.2		
Zinc	<0.3	2.0		
Boron	<2.0	2.0		

Adapted from Landschoot, (2012).

However, current data from the Centre for Disease Control and Prevention (CDC) shows that 90% of foodborne illnesses come from microbiological agents (Simonne, 2010). In the 1990s, the CDC estimated that up to 12% of reported foodborne illness outbreaks were linked to fresh produce (Simonne, 2010). Table 2.2 shows some of the pathogens that have been associated with fresh produce since the 1990s.

Table 2.2: Selected confirmed multiple outbreaks of foodborne pathogens associated
with fresh produce since the 1990s

Produce	Pathogens/chemical
Cantaloupe	*Salmonella* spp./ *E. coli* O157:H7
Raspberries	*Cyclospora cayatenensis*
Tomatoes	*Salmonella* spp.
Basil	*Cyclospora cayatenensis*
Parsley	*Shigella* spp.
Green onions/scallions	Hepatitis A virus, *Shigella* spp.
Various berries	*Cyclospora cayatenensis*
Lettuce	*E. coli* O157:H7
Cabbage	*L. monocytogenes*
Watermelon	*Salmonella* spp./ Aldicarb

Adapted from Simonne, (2010).

Most victims of foodborne illnesses contract the diseases either through the ingestion of contaminated water or by eating minimally processed or raw vegetables that were irrigated with contaminated water (Ait and Hassan, 1999). Apart from the concern for the safety of consumers, there is also concern over the safety of pickers, handlers, packers and farmers that participate in the production of vegetables during pre-harvest and post-harvest, especially young children from families of farming communities who tend to be most vulnerable to salmonella infection as a result of contaminated irrigation water (Ait and Hassan, 1999; United States Food and Drug Association, 2004). Table 2.3 shows the trigger values for faecal coliforms in irrigation waters used for food and non-food crops.

Table 2.3: Trigger values for faecal coliforms in irrigation waters used for food and non-food crops.

Intended use	Level of faecal coliforms
Raw human food crops in direct contact with irrigation water (e.g. via sprays, irrigation of salad vegetables)	<10 CFU/100 mL
Raw human food crops not in direct contact with irrigation water (edible product separated from contact with water, e.g. by peel, use of trickle irrigation); or crops sold to consumers cooked or processed	<1000 CFU/100 mL
Pasture and fodder for dairy animals (without withholding period)	<100 CFU/100 mL
Pasture and fodder for dairy animals (with withholding period of 5 days)	<1000 CFU/100 mL
Pasture and fodder (for grazing animals except pigs and dairy animals, i.e. cattle, sheep and goats)	<1000 CFU/100 mL
Silviculture, turf, cotton, etc. (restricted public access)	<10 000 CFU/100 mL

Adapted from ANZECC & ARMCANZ (2000).

For the protection of public health, WHO (1989) set a bacterial guideline of ≤ 1000 faecal coliforms (FC) per 100 ml for unrestricted irrigation. This figure was reached at after data on pathogen removal by efficient wastewater treatment plants showed that at an effluent concentration of 1000 FC/100 ml, which reflects >99.99% removal, bacterial pathogens would have been eliminated and viruses would be at very low levels (Bartone *et al*., 1985).

2.4.2 Water quality and public health

Most river stretches are used for various activities such as bathing, drinking, municipal water supply, navigation, irrigation, fishing and recreation (Barua and Hubacek, 2012). Simultaneously they are also used as recipients for discharge of industrial effluent,

municipal sewage and dumping of solid wastes, which can potentially contaminate surface- and ground water resources (Barua and Hubacek, 2012). An adequate supply of safe drinking water is one of the major prerequisites for a healthy life (United Nations Committee on Economic, Social and Cultural Rights, 2003), but waterborne disease is still a major cause of death in many parts of the world, particularly in children, and it is also a significant economic constraint in many subsistence economies (Fawell and Nieuwenhuijsen, 2003). There is a clear link between the state of the environment and human health and well-being (CSIR, 2010). For example, the run-off of nutrients to surface waters, often combined with sewage discharges, leads to significant growths of cyanobacteria which can produce a wide range of toxins (Chorus and Bartram, 1999) and, where drinking water treatment is limited or non-existent, there is a potential for undesirable concentrations to be present in drinking water (Fawell and Nieuwenhuijsen, 2003). The abundance of organic compounds, radionuclides, toxic chemicals, nitrites and nitrates in water may cause unfavourable effects on the human health especially cancer, other human body malfunctions and chronic illnesses (Arain *et al.*, 2008). The World Health Organisation (WHO, 2006) estimates that 23% of all deaths in Africa are the result of avoidable environmental hazards such as contaminated water, poor hygiene, inadequate sanitation and poor water resource management among others. According to DWAF (2001), WHO recognises diarrhoeal diseases as the leading cause of death in developing countries where many communities are still relying on untreated water from surface resources for their daily supply, with limited or no access to adequate sanitation facilities. The lack of access to safe water, basic sanitation and good hygiene is the third most significant risk factor for poor health in developing countries with high mortality rates (WHO, 2012). In South Africa alone, it had been estimated that as many as 43 000 people might die annually as a result of diarrhoeal diseases (DWAF, 2001). The microbiological quality of recreational water bodies is of utmost public health concern since some population

groups such as the very young, the elderly, the immunocompromised and tourists might be more susceptible to local endemic pathogens and, thus, may be at higher risk to swimming-associated disease (WHO, 2009). Children are clearly at higher risk because of their swimming behaviour and immature immune systems, while visiting populations may be at higher risk because they have not been previously exposed to local pathogens (WHO, 2009). For full body contact recreational waters, USEPA (2003) suggested that the geometric mean of bacterial densities should not exceed 126 CFU/100 ml for *E. coli* or 33 CFU/100 ml for Enterococci.

2.5 Pollution burden of surface water resources: South Africa as a case in view

South Africa is the 30th driest country in the world (Nkwonta and Ochieng, 2009) and its available freshwater resources are already almost fully-utilised and under stress (Oberholster and Ashton, 2008). At the projected population growth and economic development rates, it is unlikely that the projected demand on water resources in South Africa will be sustainable (Brulliard, 2009). Water is increasingly becoming the limiting resource in South Africa (Binns *et al*., 2001), and supply will become a major restriction to the future socio-economic development of the country, in terms of both the quantity and quality of available water (Walmsley *et al*., 1999). Predictions are that South Africa as a whole is likely to have a water deficit of approximately 1.7% by 2025 (National Committee on Climate Changes, 1998; Blignaut and van Heerden, 2009; RandWater, 2012). A 2010 report issued after experts sat down to roundtable talks with Business Leadership South Africa (BLSA) and the Centre for Development and Enterprise (CDE) (an independent policy research and advocacy organization that focuses on critical national development issues and their

relationship to economic growth and democratic consolidation) issued the following warning…

"On current trends, South Africans may one day have to make do with significantly less water per capita. For a country already using almost all its available water resources, this would be a dramatic change, with far-reaching implications for households, businesses, communities and government," (Marshal, 2010).

South Africa is located in a predominantly semi-arid part of the world whose climate varies from desert and semi-desert in the west to sub-humid along the eastern coastal area, with an average rainfall for the country of about 450 mm per year, well below the world average of about 860 mm per year (DWAF, 2002). Only 8.5% of this low average annual rainfall finds its way to rivers as runoff. The total annual surface runoff of South Africa is 150×10^6 m^3/a (DWAF, 1997) which is less than half that of the Zambezi River (Brulliard, 2009). South Africa's local geology of hard rocks also means there are few exploitable aquifers (Brulliard, 2009) while water which is naturally of poor quality also occurs in some areas, which limits its utilisation (DWAF, 2002). Because of the spatial variability of water resources and the scarcity of water throughout the country, the need for water far exceeds supply in many catchments (Walmsley *et al*., 1999). While the discrepancies in the water situation of different catchments have been managed through inter-basin water transfers, South Africa cannot afford to build more dams and water transfer schemes as they cost large amounts of money (RandWater, 2012). This situation is likely to worsen as the discrepancies between water requirements and availability in other water-scarce catchments increase. As of 2003, the available surface water resources in South Africa had been fully utilized, with an estimated water deficit of about 600 million m^3 per year (GOSA-DWAF 2003). Groundwater resources were said to account for about 350 million m^3 per year, reducing the overall water deficit to about 250 million m^3 per year (CSIR Environmentek, 2003).

South Africa's scarce freshwater resources are also decreasing in quality because of an increase in pollution and the destruction of river catchments (RandWater, 2012). Rivers play a major role in assimilating or carrying of industrial and municipal wastewater, manure discharges and runoff from agricultural fields, roadways and streets, which are responsible for river pollution (Vega *et al*., 1998). Typical pollutants of South Africa's freshwater environment include industrial effluents, domestic and commercial sewage, acid mine drainage, agricultural runoff, and litter. At one point, freshwater pollution (in the form of Chemical Oxygen Demand) was estimated to be 4.74 ton/km^3 while the average phosphorous concentrations (as orthophosphate) were estimated at 0.73 mg/L; values which indicated that South Africa's freshwater resources could be excessively enriched and may be considered to be moderately to highly eutrophic (Nationmaster.com, 2003). Access to water was one of the key needs identified by poor communities in 1994, as well as jobs, housing, health care and education (Schreiner, 1999). Between 1996 and 2009, the share of South Africa's households with access to clean water rose from 62 percent to 92 percent, inclusive of shared neighborhood taps (Marshal, 2010). However, population growth and economic growth are regarded as the primary determinants with respect to future water requirements, both scenarios for which deficits are generally projected to increase and surpluses to diminish (DWAF, 2002). This necessitates better understanding, management and mitigation of pollution issues to help the situation.

2.6 Global perspectives on water quality: legislative approach

Water pollution control has been a matter of public concern for more than a century (Löwgren *et al*., 1989). The World Health Organization (WHO) has been pro-active in this aspect (Figueras and Borrego, 2010); developing important guidelines of universal

application and has, in recent years, promoted a more preventive approach dubbed the Water Safety Plans (Bartram *et al*., 2009), which takes into account all factors that endanger the quality of drinking water from the source to the consumer (Figueras and Borrego, 2010). It has been proven that reactive measures to clean up polluted sites and water bodies are generally much more expensive than pro-active measures to prevent pollution from occurring (WHO/UNEP, 1997). It is for this reason that national governments the world-over have been formulating landmark pieces of legislation to safe-guard both the quality and quantity of water available to their citizens. Examples are shown in Box 2.1 to Box 2.4.

Box 2.1: Water quality governance in Zimbabwe

In Zimbabwe, the Environmental Management Act Chapter 20:27 and Statutory Instrument 6 of 2007 are used by the Environmental Management Agency to advocate for the application of the "Polluter Pays" principle in its entirety. Local authorities operating a sewerage system or owner or operator of any trade or industrial undertaking is required to obtain a licence from the Environmental Management Agency to discharge any effluents or other pollutants into the environment. The effluent is divided into four categories denoting risk as safe, low hazard, medium hazard and high hazard (Government of Zimbabwe, 2007). The scale of the charges for the licence are related to the quality and quantity of the effluent, the poorer the management or quality of the effluent, the higher the charge. In addition, the polluter pays for the policing of the regulations and for the monitoring of the effluent. Penalties for polluting that were moderately punitive under the Water Act, 1998 (imprisonment for a period not exceeding one year or/and a fine) are even more punitive under the Environmental Management Act (CAP 20:27) of 2002 (imprisonment not exceeding five years or/and a fine). The polluter also remedies damage caused either to the environment or to a third party. In practice, although this command and control approach is in force, the Water Quality Section is using a co-operative rather than confrontational approach, with fines being used as a last resort (The Herald, 2012).

Box 2.2: Water quality governance in Nepal

In Nepal, The Water Resource Act 1992 (2049 BS) contain provisions for the prevention and control of pollution of water resources. Section 19 provides that: No one shall pollute water resources by placing litter, industrial waste, poisons, chemicals or other toxicants to the effect that it exceeds the pollution tolerance limit. The "pollution tolerance limit" for water resources shall be prescribed by His Majesty's Government (HMG), by way of a public notice published in the Nepal Gazette. The prescribed officer (prescribed in the Nepal Gazette) may examine, or cause to examine, a water resource in order to determine whether or not the water resource has been polluted and if pollution tolerance limit has exceeded. Section 22 of the Water Resource Act 1992 (2049 BS) provides that any person or corporation who pollutes water resources will incur a fine of up to NRs.5000 and must pay compensation to any person sustaining a loss as a result of the pollution (WaterAid Nepal, 2005).

Box 2.3: Water quality governance in the USA

In the United States of America, The Clean Water Act (CWA) of 1972 establishes the basic structure for regulating discharges of pollutants into the waters of the United States and regulating quality standards for surface waters. Under the CWA, Environmental Protection Agency (EPA) has implemented pollution control programs such as setting wastewater standards for industry and water quality standards for all contaminants in surface waters. The CWA made it unlawful to discharge any pollutant from a point source into navigable waters, unless a permit was obtained. EPA's National Pollutant Discharge Elimination System (NPDES) permit program controls discharges. Individual homes that are connected to a municipal system, use a septic system, or do not have a surface discharge do not need an NPDES permit; however, industrial, municipal, and other facilities must obtain permits if their discharges go directly to surface waters (USEPA, 2012)

Box 2.4: Water quality governance in South Africa

In South Africa, the National Water Act (NWA) (Republic of South Africa National Water Act (NWA) No. 36 of 1998) provides for protection of the quality of water resources and for the integrated management of water resources. While also dealing with a number of diverse issues, the NWA oversees pollution prevention in South Africa's water resources by putting a responsibility on the person who owns, controls, occupies or uses the land from where the water is polluted to take measures to prevent pollution. If he or she does not take these measures the Catchment Management Agency (CMA) may do what is necessary to remedy the situation and recover the cost from the person that is responsible (Republic of South Africa, Government Gazette, 1998).

As more information is disseminated and public awareness of water quality issues increases, national governments continue to develop and enforce better programs aimed at the preservation of water resources. In the face of competing water uses like growing populations, energy production, and agriculture among others, all of which claim a share from increasingly limited water supplies, conservation of both the quality and quantity of water is now a predominant issue on a global scale. Hence, development of better water conservation practices and policies are critical to the sustenance of our water quantity and quality to ensure protection of public health.

2.7 Conclusion

Pollution of water resources remains a global concern, moreso with the on-going climatic changes that are negatively impacting on the rainfall patterns in every part of the world. Water quality deterioration will most likely see an escalation in the treatment costs for potable water production with a concomitant negative impact on the world's economies and general lifestyles of the world's citizens. Pollution can also have drastic ecological consequences and if not attended to as a matter of urgency, the world's aquatic ecosystems might face certain demise. Pollution of the world's water resources, coupled with the scarcity thereof is likely to see powerful tribes and/or nations besieging the weaker ones for their freshwater resources in the near future, as is already the situation of some parts of the world. The only way forward now is pollution reduction by all nations of the world, rich or poor; we all need to take responsibility over the state of our water resources.

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CHAPTER THREE

CHARACTERISATION OF THE PHYSICOCHEMICAL QUALITIES OF A TYPICAL RURAL-BASED RIVER: ECOLOGICAL AND PUBLIC HEALTH IMPLICATIONS

3.0 Abstract

The physicochemical qualities of a typical rural-based river were assessed over a 12-month period from August 2010 to July 2011. Water samples were analysed for total nitrogen, orthophosphate, biochemical oxygen demand, temperature, pH, dissolved oxygen, electrical conductivity, total dissolved solids and turbidity. Biochemical oxygen demand regimes did not differ significantly between seasons and between sampling points and ranged from 0.78 to 2.76 mg/L across seasons and sampling points, while temperature ranged significantly ($P < 0.05$) between 6 and 28˚C. Turbidity varied significantly ($P < 0.05$) from 6 to 281 nephelometric turbidity units while total dissolved solids (range: 24 to 209 ppm) and conductivity (range: 47.6 to 408 mg/L) also varied significantly ($P < 0.05$) across sampling points with a remarkable similarity in their trends. Orthophosphate concentrations varied from 0.06 to 2.72 mg/L across seasons and sampling points. Negative correlations were noted between temperature and the nutrients ($P < 0.01$), dissolved oxygen and temperature ($P < 0.01$) and, total dissolved solids and dissolved oxygen ($P < 0.01$). Positive correlations were noted between total dissolved solids and temperature ($P < 0.01$), electrical conductivity and temperature ($P < 0.05$) and, dissolved oxygen and pH ($P < 0.01$). All nutrients were positively correlated to each other ($P < 0.01$). Most measured parameters were within prescribed safety guidelines. However, the general trend was that water quality tended to deteriorate as the river flows through settlements, moreso in rainy seasons.

Key Words: environment; pollution; physicochemical qualities; seasons; river.

3.1 Introduction

Rivers are self-sustaining ecosystems which, without any human interference and natural disasters, could be able to indefinitely support both themselves and all life forms within them. They are vital but vulnerable freshwater systems that are critical for the sustenance of all life (Venkatesharaju *et al*., 2010). The declining quality of water in these systems threatens their sustainability and is therefore a cause for concern. Since most rural communities around the world traditionally take their water supply from rivers, dams, springs or from shallow dug wells (Sun *et al*., 2010; Aneck-Hahn *et al*., 2009; WHO and UNICEF, 2009), increasing pollution from urban, industrial and agricultural sources is making available resources unusable and dangerous to health (Contaminated surface water, 2008). Globally, about 1.6 billion children under the age of 5 years die annually due to unsafe drinking water, coupled with a lack of basic sanitation (WHO and UNICEF, 2009). In the developing world alone, almost 5 million deaths annually are due to water related diseases as water quality problems are affecting virtually all of the developing world's major rivers (Contaminated surface water, 2008). Even where pollution levels in the main stem of a river are in acceptable levels, serious problems are often seen in the tributaries; usually local streams which have become "urban drains". Unfortunately, these "urban drains" are also the main source of water for drinking and daily use for downstream poor communities along their banks (Contaminated surface water, 2008). Compared with assumed pristine conditions, rivers and streams worldwide have doubled their content of nitrogen and phosphorus as a consequence of human activities (Camargo *et al*., 2004). Severe pollution levels can cause rivers to become biologically dead and poisonous to drink from (Hutton *et al*., 2007).

Causes of surface water pollution are diverse but anthropogenic activities are the major contributors to freshwater pollution. For example, continued discharge of nutrient-rich wastewater effluent into surface water resources leads to eutrophication problems. While

statistics show Cambodia, Indonesia, the Philippines and Vietnam all have abundant internal freshwater resources per capita, they nevertheless suffer from significant freshwater pollution from human activities (Hutton *et al.*, 2007). In Vietnam for example, 13% of households dispose of solid waste to water courses (Hutton *et al.*, 2007). In agriculture, while application of organic manure and/or inorganic fertilisers will boost production of food crops and so make food affordable to even the low socio-economic class, nutrients application in excess of plant needs has a potential to pollute surface and groundwater (Bhumbla, 2011). Apart from wastewater effluents and agriculture, storm water runoff from the built-up environment is another source of nutrient enrichment in rivers (Bhumbla, 2011).

Surface water pollution also has economic consequences. A serious problem impacting on communities relying on polluted water sources for the production of potable water is the eventual costs of potable water. Treatment costs may become so excessive that water becomes available only to those who can afford it (Hutton *et al.*, 2007). Pollution of surface waters by agricultural run-offs like sediment, nutrients, pesticides, salts, and pathogens can impose costs on water users (Water quality impacts of agriculture, 2012). Pesticides are especially difficult to remove from freshwater and thus can be found in municipal or bottled water, even after conventional treatment (Maria, 2003). Eutrophication of surface waters may accelerate algal production, resulting in clogged pipelines, fish kills; which may result in loss of revenue, and reduced recreational opportunities (USEPA, 1998). Sediment is the largest contaminant of surface water by weight and volume (Koltun *et al.*, 1997). Besides increasing the cost of water treatment for municipal and industrial water uses, sediment can also destroy or degrade aquatic wildlife habitat, reducing diversity and damaging commercial and recreational fisheries. In addition, many toxic materials can be bound to silt and clay particles that are carried into water bodies, including nutrients, pesticides, industrial wastes, and metals (Osterkamp *et al.*, 1998).

In addition to anthropogenic activities, meteorological events are also major determinants of physico-chemical parameters such as temperature, pH, and turbidity of the water (Zamxaka *et al*., 2004). These parameters greatly influence the biochemical reactions that occur within the water and drastic changes in their levels may be indicative of changing conditions in the water. The imbalance between the construction of settlements (formal and informal) and sanitary infrastructure to carter for such increases in population growth poses a serious threat to the existing water resources, both through increased demand in terms of increased abstraction and storage, and through pollution by disposal, dilution and transportation of effluents. Hence, the need for a better understanding of the qualities of water resources becomes imperative in the management and mitigation of problems that may arise, such as pollution. Major pollutants in surface waters can be classified into two groups viz. physicochemical pollutants and the microbiological pollutants. In this paper, we report for the first time the physicochemical qualities of Tyume River in the Eastern Cape Province of South Africa.

3.2 Methods and Materials

3.2.1 Description of study site

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 Town and surrounding rural settlements. Tyume River water samples were collected from six sampling sites which include Hala, Khayalethu, Sinakanaka, Alice, Drayini and Manqulweni communities. Figure 3.1 depicts a map showing the Tyume River course while Table 3.1 shows the morphometric details of the sampling sites.

Figure 3.1: Map showing Tyume River course

Table 3.1: Morphometric details of the sampling sites along Tyume River

Sampling site	Description	Co-ordinates
Hala	Hala is a community immediately downstream the source of Tyume River in Hogsback. The river source at Hogsback is inaccessible. At this point the river is a source of drinking water for livestock and wild animals.	32°36'39"S and 26°54'34"E
Khayalethu	Located in a major rural community, upstream the Binfield Park Dam. The inhabitants of this settlement use the river for irrigation, recreation, stock watering and domestic purposes.	32°38'22"S and 26°56'10"E
Sinakanaka	Sinakanaka is a rural community on the banks of the Tyume River further downstream of Khayalethu, and comprises several densely populated settlements. The Tyume River is very important to the inhabitants of this community as it is used for drinking, fishing, irrigation, recreation and other domestic purposes.	32°45'37"S and 26°51'27"E
Alice	Alice is a semi-urban settlement comprising several suburbs which include 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. The combined population of Alice is over 48 000 according to UFH Interstudy (2012). The river is extensively used for irrigation, fishing and domestic purposes, as well as a source of drinking water for livestock.	32°47'17"S and 26°50'31"E
Drayini	Drayini is a rural town further downstream the banks of the Tyume River after Alice. The sampling site is located downstream of Fort Hare farmlands and Alice Town. Its water is perpetually turbid with green aquatic plants covering its surface. The river serves as drinking water for domestic animals.	32°48'37"S and 26°52'20"E
Manqulweni	Manqulweni is located further downstream the Tyume River after Drayini; just before the confluence of Tyume River with the Keiskamma River.	32°54'50"S and 26°56'13"E

3.2.2 Sampling and analytical procedures

Water samples from the six sampling points were collected over a 12-month period starting from August 2010 and ending in July 2011 to shed light on the effect of season on the parameters. During sampling, the bottles were triple-rinsed sample water before being filled with the sample. The actual samplings were done midstream by dipping sample bottles at approximately 20-30 cm below the water surface, projecting the mouth of the container against the flow direction. Samples were transported in cooler boxes to the Applied and Environmental Microbiology Research Group (AEMREG) Laboratory at the University of Fort Hare, Alice for analyses. Processing and analysis of samples was done within 6 h of

sample collection, following the procedure recommended by American Public Health Association (APHA, 2005).

A total of 10 recognized physicochemical parameters in water quality control and pollution studies were determined. Temperature (^{o}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) and nitrate (mg/ℓ NO_3-N) were determined using a spectrophotometer (Merck; Spectroquant NOVA 60). Dissolved oxygen (DO) (mg/ℓ) and five-day biochemical oxygen demand (BOD_5) were determined using a BOD meter (HACH; HQ 40d).

3.2.3 Statistical analysis

All data were subjected to descriptive statistical analysis (95 % confident limit). The generalised 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).

3.3 Results and Discussion

Physicochemical data were pooled according to the four climatic seasons of South Africa as follows: summer (November to January); autumn (February to April); winter (May to July); and spring (August to October). Table 3.2 shows the summary of the seasonal variations in physicochemical parameters of Tyume River. Collectively, the BOD levels

observed in this study ranged as follows: Hala (0.78-1.36 mg/ℓ), Khayalethu (1.03-2.73 mg/ℓ), Sinakanaka (0.98-1.46 mg/ℓ), Alice (1.33-2.14 mg/ℓ), Drayini (1.26-2.44 mg/ℓ) and Manqulweni (1.44-2.76 mg/ℓ) and fell within the stipulated BOD guideline of 10 mg/ℓ for surface waters where full contact use is allowed and $\leq$ 30 mg/ℓ where public access is prohibited, restricted, or infrequent (EPA, 2004).

Also, DO concentrations which generally ranged from 7.47 to 10.42 mg/ℓ were well within the criteria standard of 5 to 6 mg/ℓ for warm-water biota and 6.5 to 9.5 mg/ℓ for cold-water biota (Enderlein, 1996). BOD and DO results imply that Tyume River is clean with respect to organic pollution (Bhutiani and Khanna, 2007; Kannel et al., 2007). DO concentrations were not significantly different when compared by sampling point in autumn and winter probably because of high rainfall activity which resulted in high water volumes and thorough water mixing throughout the river course. In spring and winter when sampling coincided with extended dry weather conditions, significantly higher DO concentrations ($P < 0.05$) were observed at upstream sampling points (Hala and Khayalethu) where the stream gradient is steeper compared to the downstream points (Alice, Drayini and Sinakanaka). DO levels are important in determining the natural self-purification capacity of a river (Mukherjee et al., 1993). Good levels of DO in all the sampling sites of the river may also be indicative of high re-aeration rates and rapid aerobic oxidation of biological substances (Suthar et al., 2010).

Besides the use of temperature, BOD and/or COD to explain DO concentrations in surface waters, other determinants of DO concentrations include the stream gradient the nature of the river bed as well as the rate of primary productivity. When the stream gradient is steep, combined with a rocky outcrop on the river bed, mixing of water with atmospheric oxygen is highly encouraged resulting in high concentrations of DO in the water (Chang, 2008; DWAF, 1996c).

Table 3.2: Statistical comparisons of the mean values of physicochemical parameters in Tyume River water

Parameter	Season	Sampling sites						P value
		Hala	Khayalethu	Sinakanaka	Alice	Drayini	Manqulwen	
BOD (mg/L)	Summer	$1.25\pm0.91_a$	$2.73\pm0.9_a^A$	$0.98\pm0.0_a^A$	$1.39\pm0.3_a^A$	$1.83\pm0.5_a^A$	$2.33\pm1.1_a^A$	0.22
	Autumn	$1.36\pm0.8_a^A$	$1.28\pm0.6_a^A$	$1.44\pm0.6_a^A$	$2.14\pm1.3_a^A$	$2.44\pm1.2_a^A$	$2.76\pm1.0_a^A$	0.05
	Winter	$1.18\pm0.3_a^A$	$1.03\pm0.3_a^A$	$1.46\pm0.4_a^A$	$1.33\pm0.3_a^A$	$1.26\pm0.8_a^A$	$1.72\pm1.1_a^A$	0.47
	Spring	$0.78\pm0.4_a^A$	$1.72\pm1.1_a^A$	$1.32\pm0.7_a^A$	$1.47\pm0.6_a^A$	$1.95\pm0.6_a^A$	$1.44\pm0.3_a^A$	0.28
	P-value	0.59	0.33	0.28	0.26	0.15	0.15	
pH	Summer	$7.2\pm0.4_b^B$	$7.2\pm0.6_b^B$	$7.4\pm0.5_c^B$	$7.7\pm0.7_c^B$	$8.6\pm0.5_{bc}^A$	$8.8\pm0.7_b^A$	<0.00
	Autumn	$10\pm0.3_a^A$	$10.1\pm0.4_a^A$	$9.5\pm0.61_b^{AB}$	$9.1\pm0.9_b^B$	$9.8\pm0.1_a^{AB}$	$9.9\pm0.1_a^A$	0.000
	Winter	$9.6\pm1.0_a^{AB}$	$9.5\pm1.2_a^{AB}$	$10.4\pm0.8_a^A$	$10.6\pm0.4_a^A$	$8.8\pm1.2_b^B$	$8.95\pm1.2_{ab}^B$	0.001
	Spring	$7.7\pm0.5_b^{AB}$	$7.2\pm0.5_b^{AB}$	$7.1\pm0.2_c^B$	$7.3\pm0.4_c^{AB}$	$7.8\pm0.8_c^A$	$7.7\pm0.6_c^{AB}$	0.01
	P-value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	
Temperature (°C)	Summer	$15.6\pm1.7_a^B$	$17.8\pm2.0_{ab}^B$	$23.1\pm1.4_a^A$	$21.3\pm1.7_a^A$	$21.8\pm2.2_a^A$	$23.4\pm0.9_a^A$	<0.00
	Autumn	$16.6\pm2.8_a^B$	$18.5\pm4.0_a^B$	$22.8\pm4.5_a^A$	$20.9\pm3.7_a^{AB}$	$22.4\pm3.9_a^A$	$23.8\pm4.6_a^A$	0.002
	Winter	$9.2\pm2.9_b^B$	$10.1\pm2.7_c^{AB}$	$12.3\pm2.3_b^{AB}$	$12.2\pm2.5_c^{AB}$	$13.1\pm2.4_b^A$	$13.0\pm2.4_c^A$	0.006
	Spring	$11.0\pm4.3_b^B$	$13.8\pm4.8_{bc}^A$	$16.4\pm4.5_b^{AB}$	$16.3\pm3.8_b^{AB}$	$15.9\pm3.4_b^A$	$17.5\pm3.8_b^A$	0.02
	P-value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	
Conductivity (µS/cm)	Summer	$62.2\pm10_{ab}^C$	$51.2\pm4_a^{CD}$	$176\pm41_a^{BC}$	$246\pm70_a^B$	$368\pm99_a^A$	$374\pm157_a^A$	<0.00
	Autumn	$57\pm2_{ab}^D$	$47.6\pm8_a^D$	$144\pm11_b^C$	$263\pm57_a^B$	$396\pm56_a^A$	$417\pm86_a^A$	<0.00
	Winter	$50.6\pm3_b^D$	$50\pm5.4_a^D$	$144\pm19_b^C$	$253\pm38_a^B$	$345\pm51_b^B$	$237\pm41_b^A$	<0.00
	Spring	$69\pm8_a^E$	$64.8\pm7_b^E$	$128\pm2_b^D$	$222\pm7_a^C$	$351\pm10_a^B$	$408\pm15_a^A$	<0.00
	P-value	0.005	<0.0001	0.004	0.49	0.38	0.002	
TDS (mg/L)	Summer	$32.1\pm6.2_{ab}$	$25.1\pm2.0_b^C$	$108\pm56_a^B$	$117\pm37_a^B$	$188\pm59_a^A$	$187\pm78_a^A$	<0.00
	Autumn	$29.6\pm5.5_{bc}$	$23.7\pm4.2_b^D$	$72\pm5_{ab}^C$	$133\pm27_a^B$	$199\pm28_a^A$	$209\pm43_a^A$	<0.00
	Winter	$25.3\pm1.7_c^D$	$25.1\pm2.6_b^D$	$73\pm8.4_{ab}^C$	$143\pm48_a^{AB}$	$172\pm26_a^A$	$118\pm21_b^B$	<0.00
	Spring	$36.2\pm1.6_a^E$	$32\pm2.4_a^E$	$65\pm2_b^D$	$109\pm3_a^C$	$170\pm10_a^B$	$194\pm17_a^A$	<0.00
	P-value	<0.0001	<0.0001	0.02	0.15	0.27	0.001	
DO (mg/L)	Summer	$8.57\pm0.1_c^A$	$8.45\pm0.03_c^A$	$7.77\pm0.33_c^C$	$8.13\pm0.37_c^B$	$7.56\pm0.28_c$	$7.55\pm0.02_c^C$	<0.00
	Autumn	$9.69\pm0.7_b^B$	$9.54\pm0.75_b^B$	$9.17\pm1.02_b^B$	$9.3\pm0.98_b^B$	$8.78\pm0.63_b$	$8.9\pm0.91_b^B$	0.18
	Winter	$10.42\pm0.6_a$	$10.28\pm0.49_a$	$10.25\pm0.47_a$	$10.27\pm0.88_a$	$9.6\pm0.7_a^A$	$10\pm0.77_a^A$	0.15
	Spring	$8.73\pm0.2_c^A$	$8.55\pm0.11_c^A$	$7.74\pm0.21_c^B$	$7.98\pm0.5_c^B$	$7.54\pm0.13_c$	$7.47\pm0.06_c^C$	<0.00
	P-value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	
Turbidity (NTU)	Summer	ND	ND	ND	ND	ND	ND	ND
	Autumn	$24.16\pm1_a^A$	$20.39\pm18_a^A$	$61.2\pm61_a^A$	$99.9\pm115_a^A$	$158\pm21_a^A$	$112\pm123_a^A$	0.09
	Winter	$19.72\pm8_a^B$	$17.38\pm8_a^B$	$76.3\pm56_a^{AB}$	$77.1\pm58_a^{AB}$	$83.8\pm63_a^{AB}$	$281.1\pm36_a^A$	0.008
	Spring	$11.81\pm3_a^C$	$6.48\pm1_a^C$	$22.3\pm4_a^B$	$22.2\pm4_a^B$	$12.63\pm3_a^C$	$33\pm3_a^A$	<0.00
	P-value	0.07	0.11	0.16	0.20	0.15	0.13	

Along the rows, different upper case letters show significantly different readings ($P< 0.05$) of the parameters per given season in sampling sites while across the rows, different subscript letters show significantly different readings ($P< 0.05$) of the parameters per site in different seasons.

Table 3.3: Statistical comparisons of the mean values of physicochemical parameters in Tyume River wate

Parameter	Season	Sampling sites						P value
		Hala	Khayaleth	Sinakanak	Alice	Drayini	Manqulwen	
Nitrate (mg/L)	Summer	$2.27\pm0.9_a^A$	$2.02\pm0.4_a^A$	$2.10\pm1.0_a^A$	$2.29\pm0.8_a^A$	$2.29\pm0.7_{ab}$	$2.65\pm0.8_a^A$	0.67
	Autumn	$0.25\pm0.1_b^C$	$0.81\pm0.1_b^B$	$0.54\pm0.3_b^C$	$1.31\pm0.1_b^A$	$1.54\pm0.8_a^A$	$0.58\pm0.4_b^C$	<0.001
	Winter	$0.93\pm0.4_c^B$	$0.95\pm0.4_b^B$	$2.34\pm2.0_a^A$	$1.48\pm0.6_b^A$	$2.52\pm0.9_{bc}$	$1.96\pm1.0_a^{AB}$	0.006
	Spring	$3.23\pm0.1_d^B$	$3.64\pm0.1_c^A$	$3.67\pm0.2_a^A$	$3.52\pm0.4_c^A$	$3.46\pm0.3_c^A$	$3.91\pm0.3_c^A$	0.01
	P-value	<0.0001	<0.0001	0.0003	<0.0001	0.0006	<0.0001	
Nitrite (mg/L)	Summer	$0.64\pm0.5_a^A$	$0.59\pm0.5_a^A$	$1.54\pm1.2_a^A$	$1.53\pm1.3_a^A$	$1.22\pm0.7_a^A$	$0.83\pm0.5_a^A$	0.06
	Autumn	$0.03\pm0.0_b^B$	$0.02\pm0.0_a^B$	$0.05\pm0.0_b^B$	$0.13\pm0.1_b^A$	$0.17\pm0.2_b^A$	$0.09\pm0.0_b^{AB}$	0.001
	Winter	$0.47\pm0.5_{ab}$	$0.51\pm0.5_a^A$	$0.48\pm0.6_a^A$	$0.47\pm0.5_b^A$	$0.48\pm0.5_b^A$	$0.53\pm0.6_{ab}^A$	0.99
	Spring	$2.34\pm0.1_c^A$	$1.65\pm0.7_b^B$	$2.19\pm0.0_a^A$	$2.03\pm0.5_a^A$	$2.35\pm0.1_c^A$	$2.04\pm0.3_c^{AB}$	0.04
	P-value	<0.0001	<0.0001	<0.0001	0.0001	<0.0001	<0.0001	
Orthophosphate (mg/L)	Summer	$0.77\pm0.5_a^B$	$0.77\pm0.4_a^B$	$0.92\pm0.4_a^A$	$0.79\pm0.5_a^B$	$0.92\pm0.3_a^A$	$1.43\pm0.6_a^A$	0.02
	Autumn	$0.06\pm0.0_b^C$	$0.08\pm0.0_b^C$	$0.13\pm0.1_b^B$	$0.16\pm0.1_b^B$	$0.63\pm0.5_a^A$	$0.44\pm0.3_b^{AB}$	<0.001
	Winter	$0.15\pm0.1_b^B$	$0.15\pm0.1_b^B$	$0.32\pm0.2_b^A$	$0.26\pm0.1_b^B$	$0.61\pm0.5_a^A$	$0.25\pm0.2_b^B$	0.0003
	Spring	$1.51\pm0.3_c^C$	$1.48\pm0.3_c^C$	$2.72\pm0.1_c^A$	$1.07\pm0.1_a^D$	$2.13\pm0.1_b^B$	$1.60\pm0.4_c^C$	<0.001
	P-value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	

Along the rows, different upper case letters show significantly different readings (P< 0.05) of the parameters per given season in sampling sites while across the rows, different subscript letters show significantly different readings (P< 0.05) of the parameters per site in different seasons.

Low DO concentrations, when combined with the presence of toxic substances may lead to stress responses in aquatic ecosystems because the toxicity of certain elements, such as zinc, lead and copper (not covered in this study), is increased by low concentrations of dissolved oxygen (EPA, 1986). Low concentrations of dissolved oxygen, associated with high water temperature also increase the adverse effects on biota (Enderlein, 1996). The water quality criterion for dissolved oxygen, therefore, cannot be taken independently of other water quality determinants.

The temperature range for Hala was 6 to 20°C, while those for Khayalethu, Sinakanaka, Alice, Drayini and Manqulweni were 8 to 23°C, 10 to 28°C, 9 to 25°C, 10 to 27°C and 10 to 25°C respectively. Temperature regimes varied significantly ($P < 0.05$) by season and, whereas there were also significant differences in the temperature regimes by sampling point, it was mainly at Hala and, in summer and autumn, Khayalethu also, where temperature varied with that of the other sampling points. Water temperature at Hala, which is located at the foot of the Hogsback Mountains, was always lower than at the other points. This point receives the least insolation because of the high mountains casting shed on this part of the river till very late in the afternoon and even then, riparian vegetation still shields this part of the river from receiving much sunshine (DWAF, 1996c). As has been pointed above, temperature generally drives the chemical reactions in an aquatic system and warmer temperatures may influence the toxicity of some substances like ammonia which may be found dissolved in water. However, in this study the temperature regimes for most sampling sites were within the acceptable limit of no risk (≤ 25°C) for domestic water uses (DWAF & WRC 1995).

Electrical conductivity (EC) data which generally ranged from 47.6 to 408.2 µS/cm showed significant variability among sampling points but when compared by seasons, it was

observed that results for Alice and Drayini did not show significant variation at the 95 % confidence level.

These two sites are impacted by sewage disposal from Alice Town and University of Fort Hare respectively which could explain why their electrical conductance was more or less the same. Previous findings indicate that sewage disposal tends to increase the EC levels of the receiving water body because of the high concentrations of salts and ions in the sewage (Suthar *et al*., 2010). EC estimates the amount of total dissolved salts, or the total amount of dissolved ions in the water and is controlled by, among other factors; the geology of the catchment area which determines the chemistry of the watershed soil and ultimately the water (Chang, 2008; DWAF, 1996c). The size of the catchment relative to the size of the river also has a significant impact on the EC of the water since a bigger catchment area means relatively more water draining into the river, and more contact with soil before reaching the river only when stream flow is low, otherwise high flow volumes will result in dilution (Ouyang *et al*., 2006). A quick glance at the mean values for EC in Table 3.2 shows that the mean values become progressively bigger from Hala to Manqulweni (upstream to downstream), contrary to the most commonly held notion that pollute concentration becomes progressively lower as we go downstream due to the effect of dilution and self-purification capacity of the river (Osode and Okoh, 2009). The most logical explanation why points further downstream have higher EC values is the water's increased contact with soil with time and distance of flow and also the cumulative effect of ions, as more and more tributaries/rivulets discharge their loads into the main stream (Brainwood *et al*., 2004). Sewage disposal and urban surface runoff in the downstream stretch of the river may also have contributed to the observed pattern. Similar findings have been observed elsewhere (Igbinosa and Okoh, 2009). High electrical conductivity levels can be damaging to aquatic life because of increased salinity in the stream and possible smothering of the stream bottom,

especially if the stream is deep (DWAF, 1996c). The target water quality limit of no risk for domestic water uses with respect to EC is set at 700 μS/cm (DWAF, 1996b), and results obtained in this study suggests that with respect to EC, the river water could be suitable for domestic use.

The pH regimes in this study showed significant seasonal variability in each of the six sampling sites. The pH of river water is affected by a number of factors among them the geology and mineral content of the catchment area, acid mine drainage, agricultural runoff, carbon dioxide concentration in the atmosphere and, accumulation and decomposition of organic detritus in the water producing weak carbonic acids which impact on pH. However, there is no mining activity in the Tyume River catchment while agricultural activity and use of fossil fuels is limited. Again, the observed pH ranges (Table 3.2) were either in the neutral or alkaline range, ruling out the accumulation and decomposition of organic detritus in the river as possible causes of pH fluctuations since that would have resulted in pH values below 7 being observed. The most likely causes of the observed fluctuations in river water pH therefore could either be the bedrock and soil composition of the catchment which could be containing some limestone deposits or photosynthetic activity which removes carbon dioxide from the water causing the pH to rise. DWAF (1996b) set the target water quality for pH in water for domestic use between 6 and 9, same as that of the European Union tolerance limit for pH in water for the support of fisheries and aquatic life (Chapman, 1996). pH values in this study ranged from 7.2 to 10.6 across seasons and sampling points.

Turbidity was assessed for only three seasons viz. autumn, winter and spring, and results revealed that the turbidity of the river water did not differ significantly by season at all the sampling sites. However, when analysed by sampling point, the turbidities of upstream sampling points (Hala, and Khayalethu) were significantly lower ($P < 0.05$) than the turbidities of downstream sampling points in winter and spring. In autumn, turbidity did not

significantly differ from one sampling site to another. Suspended silt and clay, organic matter, and plankton can contribute to turbidity; hence turbidity in a stream will fluctuate before, during and after storm flow (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 amount of flocculants needed to clarify the water (Osode and Okoh, 2009). The overall turbidity range for this study was 6-281 NTU and fell short of the target water quality range (0-1 NTU) of no risk for domestic water uses as proposed by DWAF (1996b). Increased turbidities also interfere with the normal workings of sanitizers by forming a chemical "shield" around bacteria, algae, and other substances normally attacked by chlorine (Hoko, 2005) in addition to shielding enteric bacteria from photooxydation. Highly turbid water will therefore present a microbiological hazard to consumers of raw river water.

Total dissolved solids (TDS) levels ranged from 23.7 to 209 mg/ℓ across seasons and sampling points. TDS is the sum total of all of the dissolved substances in a given body of water, and includes hardness, alkalinity, chlorides, bromides, sulphates, silicates, and all manner of organic compounds. Although elevated TDS concentration may not mean that the water is a health hazard, it however does mean the water may have aesthetic problems or cause nuisance problems. These problems may be associated with staining, taste, or precipitation. With respect to trace metals, elevated TDS may suggest that toxic metals may be present at an elevated level. DWAF (1996b) has set the water quality guidelines for TDS at 0-450 mg/ℓ as applied to domestic uses. TDS values for this study fell within stipulated DWAF guidelines.

Nitrate, nitrite and orthophosphate concentrations are remarkably similar in that significantly higher values ($P < 0.05$) were obtained in summer and spring compared to those obtained in autumn and winter (Table 3.3). The higher rainfall in autumn and winter could

have significantly increased the flow volume and resulted in dilution effect. Low-flow situations increase the concentrations of water quality stressors. Concentrations of substances that are continuously added, but in low doses (e.g., from sewage treatment plants), will increase and might reach levels toxic to organisms, or concentrations might exceed bathing and recreational water directives (Nilsson and Renöfält, 2008). Agricultural activities can result in high levels of these nutrients due to runoff. However, there is little agricultural activity in the Tyume catchment. In this case, nutrient loadings typically arise from non-point source runoff from flash storms, particularly in urban areas (Brainwood *et al*., 2004). In summer and spring when there is less rainfall, and hence low flow, the effects of sewage discharge into the river were evident from the higher nutrient concentrations observed at Alice and Drayini sampling points. Similar trends have been observed elsewhere (Jarvie *et al*., 2006; Castillo *et al*., 2000; Ferrier *et al*., 2001).

All three nutrients are naturally present in the environment and natural nutrient cycling processes prevents 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 (Blanchard and Lerch, 2000). 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 methemoglobin 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). Water quality guidelines for nitrate concentration consider the effect of this compound on the health of infants and pregnant women and thus DWAF (1996b) has set the safety limit for water meant for human consumption at 6 mg NO^-_3 as N/ℓ. The nitrate concentrations in this study all fell within this target water quality range.

Nitrite levels ranged from 0.02 to 2.35 mg/ℓ across seasons and sampling points. However, in spring alone, mean values for nitrite levels were as follows; 2.34, 1.65, 2.19, 2.03, 2.35 and 2.04 mg/ℓ at Hala, Khayalethu, Sinakanaka, Alice, Drayini and Manqulweni respectively. These levels were clearly above the limit associated with oligotrophic conditions (<0.5 NO^-_2 as mg N/ℓ) (DWAF, 1996c) and also above the drinking water limits of 0.5 mg N/ℓ and 1 mg N/ℓ for the EU and USA respectively. However, 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 season were within the safety guideline of 6 mg NO^-_3 as N/ℓ (DWAF 1996b) set for water meant for human consumption.

Orthophosphate (as P) concentrations in this study varied from 0.06 to 2.72 mg PO^{3-}_4/ℓ across seasons and sampling points. However, like nitrite, in spring alone, mean values of orthophosphate concentration were as follows; 1.51, 1.48, 2.72, 1.07, 2.13 and 1.60 mg/ℓ at Hala, Khayalethu, Sinakanaka, Alice, Drayini and Manqulweni respectively. These values were clearly 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 spring. Because spring season was dry compared to other seasons, the dilution effect on the sewage disposed into the river could have been less in this season, hence the higher concentrations of nutrients in this season than in other seasons. In the presence of sufficient available phosphorus as was the case in the spring season 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).

Correlation analysis output is shown in Table 3.3. Correlation analysis revealed a significant negative correlation between temperature and the nutrients (NO^-_3, NO^-_2 and PO^{3-}_4) at the 99 % confidence level. This result concurs with the findings of Badran (2001) and Manasrah *et al*. (2006) which they explained to be the result of increased nutrient consumption by primary producers in favourable temperature conditions. DO was also negatively correlated to temperature ($P < 0.01$) while TDS and EC showed positive correlation to temperature at 99% and 95% confidence levels respectively. This trend is expected since high water temperatures result in less DO in the water while low water temperatures will also result in high DO concentration in the water (Papafilippaki *et al*., 2008; Rounds, 2002; Vega *et al*., 1998). Results also show that dissolution of solids in the water is temperature dependant while EC is also a function of TDS, hence its positive correlation to temperature (DWAF, 1996c). TDS assumed an inverse correlation to DO ($P < 0.01$). All nutrients were positively correlated to each other at the 99% confidence level 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 neutrality. This will suggest that as the pH is increased, more and more bacteria die out and the oxygen concentration is

81

maintained at a high level. Similar results have been reported elsewhere (Araoye, 2009; Swaminathan, 2005). The positive correlation between DO and nutrients ($P < 0.01$) observed in this study is in agreement with the findings of Morgan *et al.* (2006) and that of Arheimer and Liden (2000) who attributed this trend to the fact that nutrient input promotes primary productivity during day, largely contributing to the water's DO concentrations during day though this trend is likely to reverse in the night when these primary producers are respiring.

Table 3.4: Correlation half-matrix for the physicochemical parameters of Tyume River

P Parameters	pH	WT	TDS	TBD	EC	NO_3^-N	NO_2^-N	$PO_4^{3-}P$	BOD	DO
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		
BOD	-0.110	-0.016	**-0.481****	0.006	**-0.278****	0.032	0.086	-0.101	1	
DO	**0.550****	-0.562**	**-0.333****	-0.003	**-0.246****	0.088	**0.166***	**0.271****	0.088	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.

3.4 Conclusion

Physicochemical parameters are major determinants of water quality that directly or indirectly affect its use. All physicochemical parameters were within recognised 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 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. Water quality investigations are carried out to provide information on the health of water bodies and for developing strategies that help in better management of catchment and water resources. Though the levels of all physicochemical parameters were within certain prescribed levels, the general trend was that water quality tended to deteriorate as the river flows through settlements, moreso in rainy seasons.

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CHAPTER FOUR

SEASONAL AND SPATIAO-TEMPORAL DISTRIBUTION OF FAECAL INDICATOR BACTERIA IN TYUME RIVER IN THE EASTERN CAPE PROVINCE, SOUTH AFRICA

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4.0 Abstract

We assessed the incidence of faecal indicator bacteria in Tyume River over a 12-month period between August 2010 and July 2011. Total coliforms, faecal coliforms and enterococci were determined by the membrane filtration method. Total coliforms were detected in counts ranging from 2.1×10^2 to 3.4×10^4 CFU/100 mℓ; faecal coliforms 1×10^2 to 1.6×10^4 CFU/100 mℓ while enterococci were in the range of 3.3×10^1 to 5.1×10^3 CFU/100 mℓ. Indicator bacteria counts increased from upstream to downstream sampling sites. Counts of indicator bacteria at all sites were significantly affected by seasonal changes. The bacteriological qualities of the river water were poor, exceeding the guideline of 200 CFU/100 mℓ and 33 CFU/100 mℓ for faecal coliforms and enterococci, respectively, for recreational water. Faecal coliform counts also exceeded the 1000 CFU/100 mℓ guideline for water used in fresh produce irrigation. Microbial source tracking results showed that faecal pollution was predominantly of human origin during spring at all sampling sites. During other seasons, human faecal pollution was largely confined to midstream to downstream sampling sites. Generally, the presence of faecal indicator bacteria in the river water samples suggests faecal pollution of this freshwater resource, raising the possibility of the presence of pathogenic microorganisms in the water and a threat to public health.

Keywords: Total coliforms; faecal coliforms; enterococci; pollution; public health.

4.1 Introduction

Polluted river waters can contain a large variety of pathogenic micro-organisms including viruses, bacteria and protozoa (Servais *et al*., 2007). Indicator bacteria have been used for many years to determine the quality and safety of surface and ground waters (Ahmed *et al*., 2010; Frenzel and Couvillion, 2002). The presence of these bacteria in surface waters also indicates that pathogenic organisms such as *Salmonella* spp., *Shigella* spp. as well as enteric viruses may be present (Ahmed *et al*., 2006). Hence, contamination of groundwater and surface water by faecal coliforms is of major concern in relation to environmental quality and public health (Carroll *et al*., 2006).

In order to develop appropriate management strategies to minimise the potential public health risks, the various sources of faecal contamination in a river catchment need to be identified (Carroll *et al*., 2006). Sources of faecal bacteria in environmental waters include agricultural sources, wild and domesticated animals, urban development and effluent treatment facilities such as on-site wastewater treatment systems (Kelsey *et al*., 2004). Non-point sources such as defective septic systems, stormwater drainage systems, runoff from animal feedlots and/or point sources such as industrial effluent and municipal waste are known to be sources of faecal pollution (Ahmed *et al*., 2010).

The use of indicator bacteria in assessing the microbiological quality of surface and ground waters has been questioned: chiefly because these groups of bacteria can also derive from various sources other than warm blooded animals (Dombek *et al*., 2000; Hagedorn *et al*., 1999; Kreader, 1995). However, faecal coliforms are still the most widely used normative microbiological parameter as all guidelines for assessing the water quality required for different water uses are based on the concentration of faecal indicator bacteria (Servais *et al*., 2007). The most common indicators used today are total coliforms, faecal coliforms and enterococci, which are used as surrogates for human pathogens to assess the health risk and

quality of water (Evanson and Ambrose, 2006). While these faecal indicator bacteria may not be pathogenic, studies have shown them to correlate with impaired water quality and the incident of illness in swimmers (Haile *et al*., 1999). Faecal indicator bacteria (FIB) have shown an affinity for fine particle attachment, providing advantages in survivability and increasing the rate of deposition to sediment (Fries *et al*., 2008). Faecal contamination of water is considered a human health risk, and there has always been a great deal of concern regarding the level of coliform bacterial counts in water (Djuikom *et al*., 2006).

Microbiological examination of river water is obligatory for use-related purposes such as drinking water production, irrigation and recreation (Kolarević *et al*., 2011). The primary uses of water from Tyume River by the host population are multiple and include: laundry purposes, car washing, bathing, watering of crops and livestock, fishing, and in certain parts; raw consumption and swimming by youth. Therefore, an overview of the microbiological quality of the Tyume River water is a major public health issue, thus necessitating this study. Enterococci, faecal coliform and total coliform counts are used as indices for measuring the quality of the surface water (Holland *et al*., 2004).

4.2 Methods and Materials

4.2.1 Description of study site

For the description of the study area, please refer to section 3.2.1, page 68-70.

4.2.2 Sampling and analytical procedures

Water samples from the six sampling points were collected over a 12-month period starting from August 2010 and ending in July 2011 to shed light on the effect of season on the parameters. The actual samplings were done midstream by dipping sterile sample bottles at

approximately 20-30 cm below the water surface, projecting the mouth of the container against the flow direction. Samples were transported in cooler boxes to the Applied and Environmental Microbiology Research Group (AEMREG) Laboratory at the University of Fort Hare, Alice for analyses. Processing and analysis of samples was done within 6 h of sample collection, following the procedure recommended by American Public Health Association (*Standard Methods*, 2005).

Analyses of Total Coliforms (TC), Faecal Coliforms (FC) and Enterococci were carried out by membrane filtration method in accordance with Standard Methods (*Standard Methods*, 2005). 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 filters papers were placed on m-Endo agar and incubated at $37^{o}C$ for 24 h. Typical red colonies with a metallic sheen were enumerated and reported as CFU/100 mℓ surface water. 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 $44.5^{o}C$ for 24 h. Colonies that exhibiting any shades of blue were counted and reported as CFU/100 mℓ surface water. 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 $37^{o}C$ 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.

4.2.3 Microbial source tracking

The ratio of faecal coliforms to enterococci (also referred to as faecal streptococci) counts in water samples from each of the sampling sites was used to distinguish between

faecal pollution of human and animal origin as previously described (Cabral, 2010; Pitt, 1998; Afshin and Saeid, 2011; Baudišová, 2009; Young and Thackston, 1999).

4.2.4 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 seasons and sampling sites.

4.3 Results and discussion

The average total coliform counts ranged as follows: Hala (2.1×10^2 to 9.5×10^3 CFU/100 mℓ), Khayalethu (3.5×10^2 to 1.2×10^4 CFU/100 mℓ), Sinakanaka (1.7×10^3 to 1.3×10^4 CFU/100 mℓ), Alice (2.0×10^4 to 5.3×10^4 CFU/100 mℓ), Drayini (1.4×10^4 to 3.4×10^4 CFU/100 mℓ) and Manqulweni (2.6×10^3 to 1.8×10^4 CFU/100 mℓ). Total coliform counts significantly differed from season to season at every site ($P<0.05$), as well as from site to site within the same season ($P<0.05$). Fig 4.1 shows the boxplot depicting the seasonal distribution of total coliform bacteria in Tyume River.
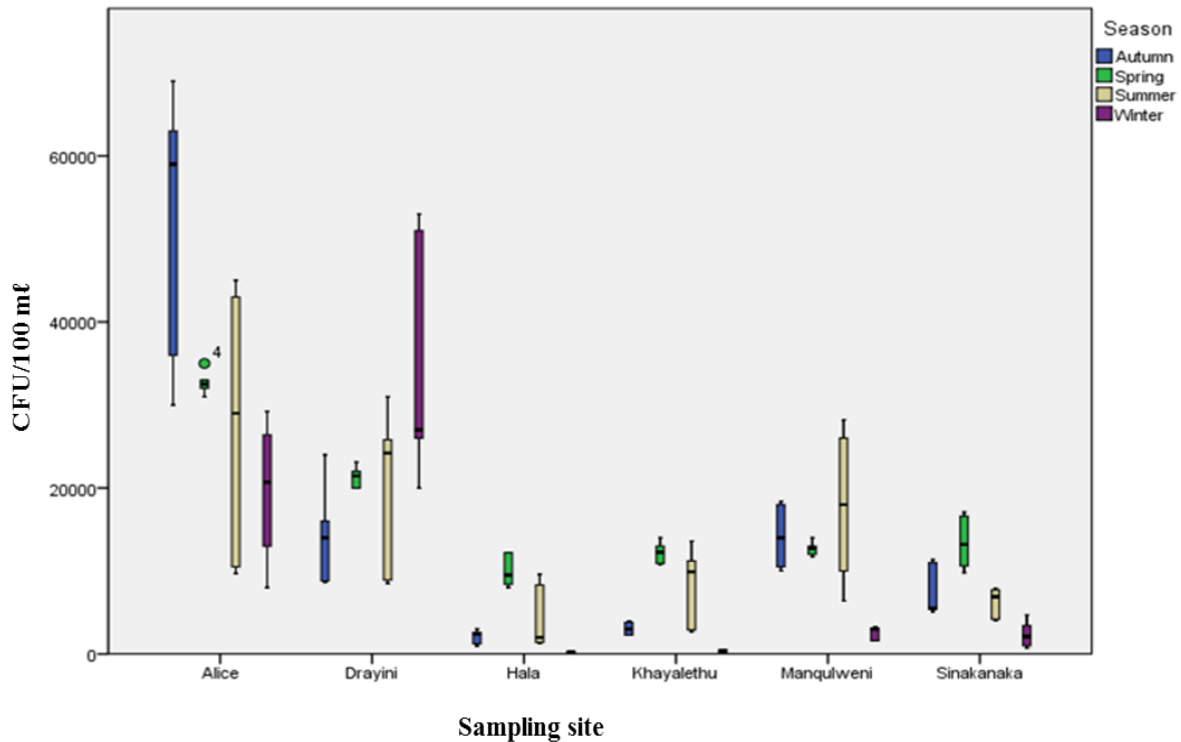
Figure 4.1: **Seasonal distribution of total coliform bacteria along Tyume River**

Total coliform counts were always highest at Alice site for the duration of the study. Over the same period, the second highest total coliform counts per season were obtained at Drayini. The same trend characterised both the faecal coliform and enterococci counts. Average faecal coliform counts ranged as follows; Hala (1×10^2 to 7.2×10^2 CFU/100 mℓ), Khayalethu (1.3×10^2 to 7.4×10^2 CFU/100 mℓ), Sinakanaka (1.9×10^2 to 8.7×10^2 CFU/100 mℓ), Alice (3.2×10^3 to 1.2×10^4 CFU/100 mℓ), Drayini (1.3×10^3 to 1.6×10^4 CFU/100 mℓ) and Manqulweni (2.3×10^2 to 2.0×10^3 CFU/100 mℓ). The seasonal distribution of faecal coliforms in Tyume River is depicted by a boxplot in Fig 4.2.
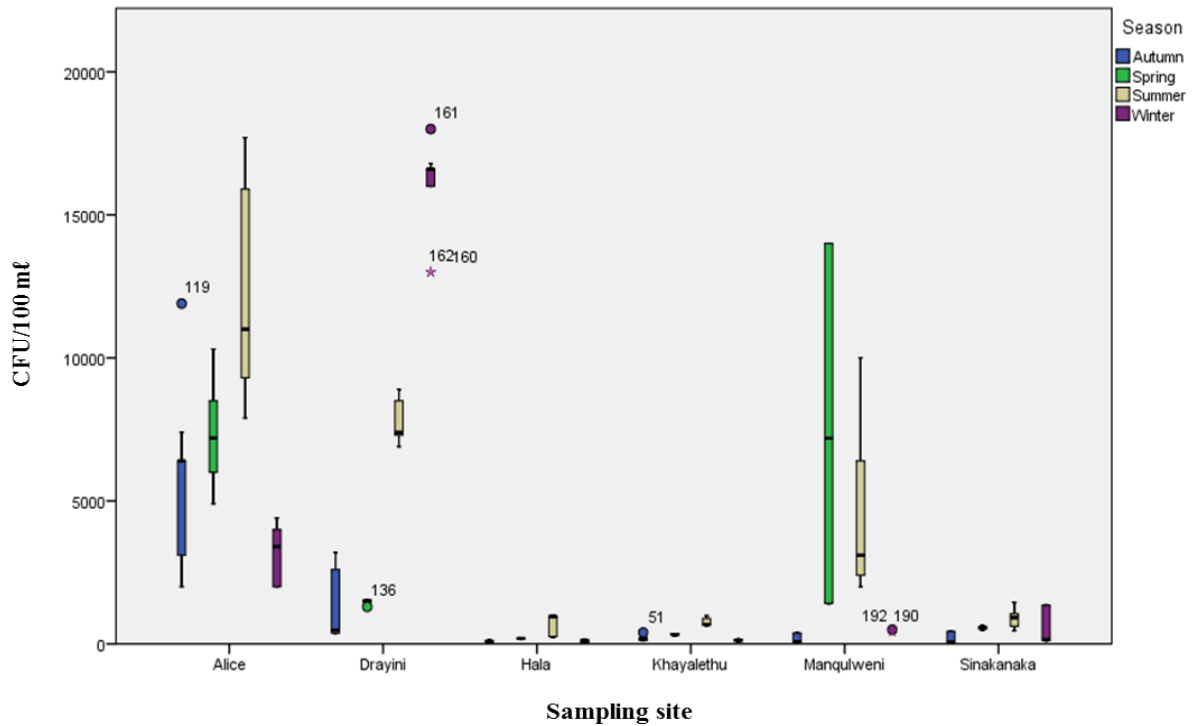
Figure 4.2: Seasonal distribution of faecal coliform bacteria along Tyume River

For enterococci, average counts ranged as follows; Hala (3.3×10 to 5.2×10^2 CFU/100 mℓ), Khayalethu (4.3×10 to 1.3×10^2 CFU/100 mℓ), Sinakanaka (8.2×10 to 2.0×10^2 CFU/100 mℓ), Alice (2.1×10^2 to 5.1×10^3 CFU/100 mℓ), Drayini (1.4×10^2 to 3.0×10^3 CFU/100 mℓ) and Sinakanaka (9.2×10 to 1.2×10^3 CFU/100 mℓ). The seasonal distribution of enterococci is depicted in a boxplot in Fig 4.3
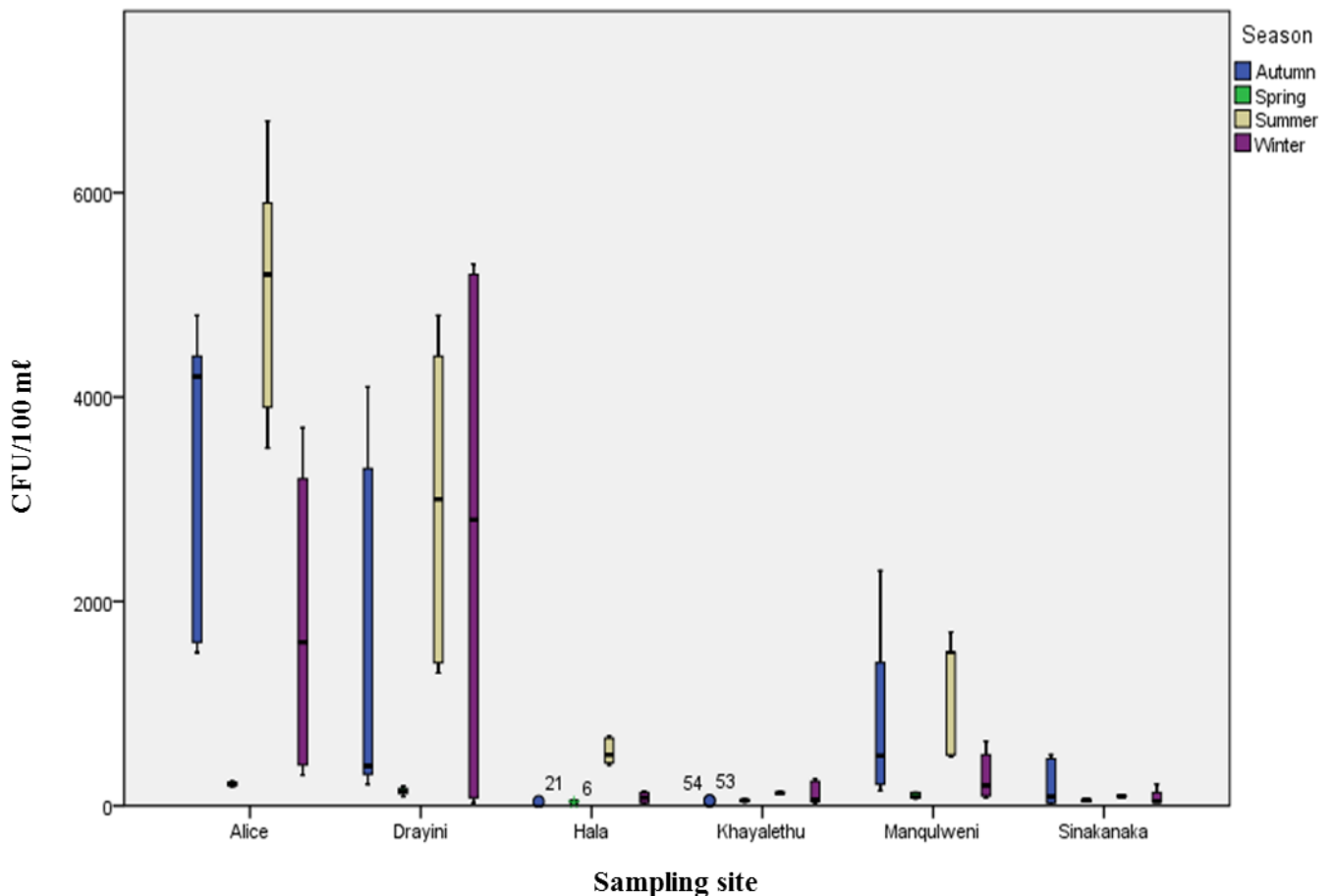
Figure 4.3: Seasonal distribution of enterococci along Tyume River

Total coliforms bacteria are widespread in nature. All members of the total coliform group can occur in human faeces, but some can also be present in animal manure, soil, and submerged wood and in other places outside the human body (Shilklomanov, 2000). Thus, the usefulness of total coliforms as an indicator of faecal contamination depends on the extent to which the bacteria species found are faecal and human in origin (USEPA, 2012). While total coliforms are no longer recommended as an indicator for recreational waters, they are still the standard test for drinking water because their presence indicates contamination of a water supply by an outside source (USEPA, 2012). The primary risk of consuming untreated river water is the transmission of communicable diseases by pathogenic organisms. Total coliform counts in unpolluted, natural surface waters may range between 1 and 10 CFU/100 mℓ while in polluted surface water the count may range between 100 and 100 000 CFU/100

mℓ (eWISA, 2012). Studies have shown that while faecal indicator bacteria may not be pathogenic, their presence correlates with impaired water quality and may actually increase the risk of gastrointestinal illness to full contact users (Bhandaram *et al*., 2011; Haile *et al*., 1999). The higher the concentration of indicator bacteria in water therefore, the higher the risk of illness especially to domestic users and swimmers who are more likely to consume substantial amounts of such water.

Both Alice and Drayini sites are located downstream of effluent discharge points from 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. According to Adewoye (2010), streams and rivers running through areas of significant human influence such as farms, cities and industrial locations are prone to pollution. The obtained results may therefore be a direct reflection of the human population distribution along which the river channel passes. Manqulweni which is located downstream of both Alice and Drayini sites had higher coliform and enterococci counts compared to each of the three upstream sampling sites (Hala, Khayalethu and Sinakanaka) possibly because these indicator bacteria were carried downstream by the river currents. However, over the course of time and distance, discounting additional sources of faecal pollution, the concentrations of faecal indicator bacteria tends to diminish by a combined effect of dilution and degradation while being transported downstream (Bhandaram *et al*., 2011), hence the lower concentrations observed at Manqulweni compared to both Drayini and Alice.

A significant number of faecal coliforms survive treatment processes at sewage treatment plants and thrive for a certain time in the aquatic environment (Kavka *et al*., 2002). Enterococci are a subgroup within the faecal streptococcus group and are distinguished by

their ability to survive in salt water: in this respect they more closely mimic many pathogens than do the other indicators. Because enterococci are typically more human-specific than the larger faecal streptococcus group, EPA recommends enterococci as the best indicator of health risk in salt water used for recreation and as a useful indicator in fresh water as well as marine water (USEPA, 2012).

At Drayini, an interesting trend was observed where the counts of all indicator bacteria assessed were significantly higher in winter than in any other season. Two reasons could be attributed to this observation. First is the fact that Drayini lies downstream of both the University of Fort Hare wastewater treatment plant effluent discharge point and the University of Fort Hare farmlands. According to Adewoye (2010), even practicable waste treatment processes, however efficient, may not remove all the pathogens. This means that the wastewater effluent discharge could be a veritable source of indicator bacteria isolated in the river samples. Secondly, winter was a relatively wetter season compared to the other seasons during sampling. Therefore, a combination of surface runoff from the farmlands and effluent discharge from the afore-mentioned wastewater treatment plant could have contributed to the observed trend. The low bacterial counts observed at the upstream sampling sites (Hala, Khayalethu and Sinakanaka) in the same winter season could be explained in terms of dilution, especially considering the fact that there is minimal agricultural activity in the upper catchment of Tyume River, meaning that incoming runoff had a dilution rather than an additive effect in as far as bacterial counts were concerned per unit volume of river water. According to the National Health and Medical Research Council (2008), the microbial quality of surface water may be strongly influenced by factors such as rainfall in the river catchment, leading to relatively short periods of elevated faecal pollution arising from washing of animal wastes from forests, pastures and urban land as well as the re-

suspension of sediment-trapped pathogens; a particular problem in freshwater river catchments.

It is unusual to obtain higher microbial load in the dry season than in the wet season as obtained at the upstream sampling sites in spring and summer in this study. This suggests that pollutants were not necessarily flushed into the river by stormwater/surface runoff but may have been directly deposited into the river either as a consequence of point discharge (which is highly unlike in this case as those communities use either oxidation ponds or septic tanks for waste disposal) or livestock defecating into the water during watering (a highly probable explanation). Absence of-/or low inflows could also have minimised dilution and caused the concentration of pollutants, hence indicator bacteria, per unit volume of the river water.

For sanitary quality assessment, total coliforms can indicate severe water pollution, though this does not have to be directly correlated with an anthropogenic source of pollution, while faecal coliforms are used to indicate faecal pollution (DWAF, 1996). The presence of faecal coliform 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 faecal coliform 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, 1996), may be considered to be poor. In South Africa in general, a large proportion of the population lives in informal settlements and low cost housing which lack proper and/or adequate sanitary infrastructure (Drechsel, 2006), leading to water sources becoming polluted

with untreated or partially treated wastewater (Nevondo, 1999; Obi, 2002). In the Eastern Cape Province and indeed in other South African Provinces as well, rivers are used as a source of water both for crop irrigation as well as for domestic personal use such as washing, drinking and cooking without prior treatment (Raschid-Sally *et al*., 2005). Outbreaks of food borne infections associated with the consumption of raw vegetables or fruits are frequently reported world-wide (CDC, 2009; Gillespie, 2004; Sivapalasingam, 2004). A study by Gemmell and Schmidt (2010) in KwaZulu Natal in South Africa revealed the presence of *E. coli* in both the irrigation water and on fresh produce samples. The elderly or immunocompromised people and young children may be at a higher risk of health damage from bodily contact with and/or consumption of contaminated water because they are more susceptible to pathogenic organisms (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 (Ahmed *et al*., 2006; Toze, 1998; Wilhelm and Maluk, 1998). Presence of faecal indicator bacteria also indicates contamination with sewage effluent or with run-off from soil and other land surfaces (Ahmed *et al*., 2010; Toze, 2005). Bathing water with more than 126 *E. coli* or 33 enterococci per 100 mℓ over a 30-day period has been found to present a health risk (Alm *et al*., 2003). An emerging issue on the bacterial pathogen scene is various haemorrhagic *E. coli* from domestic farm animal faecal matter, 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. 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 spotted at some spots along the river during sampling visits. Bernier *et al*. (2009) postulates that rivers can provide a constant supply of oxygen, organic and inorganic compounds as well as nutrients essential for bacterial growth and survival, resulting in persistence especially of faecal bacteria.

Also, enterococci concentrations were always lower than faecal coliform concentrations, probably because enterococci survive less easily in river water, in part because of their sensitivity to photooxydation (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 may indicate that recent contamination has occurred by humans or other warm-blooded animals (Ahmed *et al*., 2006). Rivers impacted by wastewater release are characterised by poor microbiological water quality with faecal coliform numbers higher than 2000 CFU/100 mℓ (Servais *et al*., 2007).

In rural areas such as Hala, Khayalethu and Manqulweni, faecal pollution can also be brought to rivers through non-point sources such as surface runoff and soil leaching its likely origin being wild life and grazing livestock faeces. Variations among the different sampling sites with respect to the overall mean values of the indicator bacteria measured might point to spatial variability in the human impact to the bacteriological quality of the water. This is substantiated by the fact that the catchment basin of the Tyume River is diversely inhabited, with settlements occasionally located close to the river and therefore discharging effluents from wastewater treatment plants directly into the river. Changes in concentrations in the

downstream direction reflect processes that result in faecal and coliform bacteria inputs as well as attenuation through sedimentation and/or dilution and die-off. These two latter processes are likely important at Manqulweni where concentrations decrease from upstream to downstream. Fig 4.4, 4.5 and 4.6 show the spatial distribution of total coliforms, faecal coliforms and enterococci at each of the selected sampling sites along Tyume River.

The values for all investigated groups of bacteria in the river water showed a great variability, which, at Alice, Drayini and, to a lesser extent, Manqulweni can be attributed to unequal loading with wastewater during the sampling seasons. The distribution patterns shown in Fig 4.4-6 reflect the population distribution along Tyume River catchment. Our results are in agreement with the findings of Frenzel and Couvillion (2002) whose research showed that river stretches passing through areas with high population densities were characterised by higher concentrations of faecal-coliform, *E. coli* and enterococci bacteria. The same study also showed that areas served by sewer systems had significantly higher faecal-indicator bacteria concentrations than did areas served by septic systems.
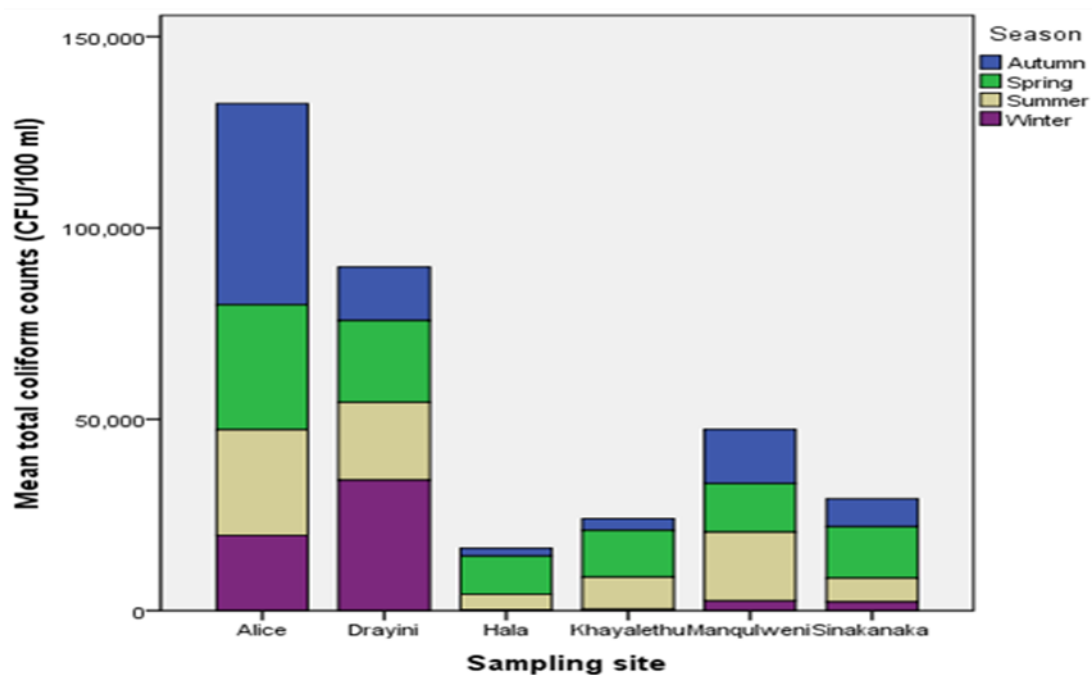
Figure 4.4: Spatial distribution of total coliforms at selected sites along Tyume River
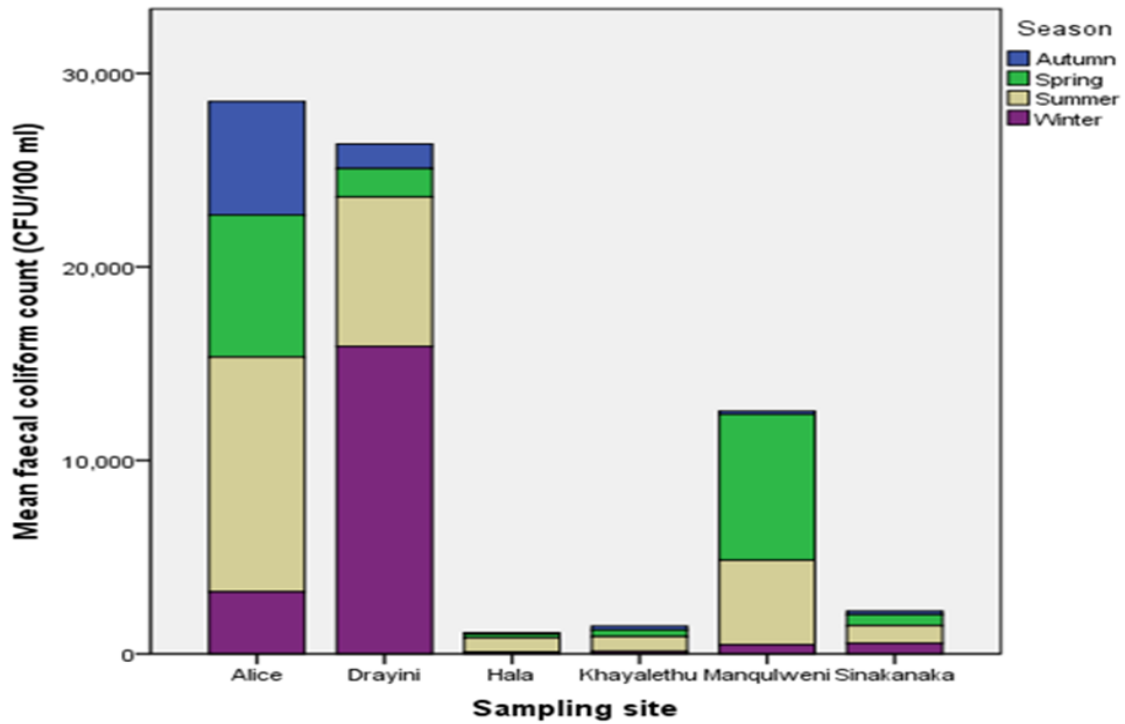
Figure 4.5: Spatial distribution of faecal coliforms at selected sites along Tyume River
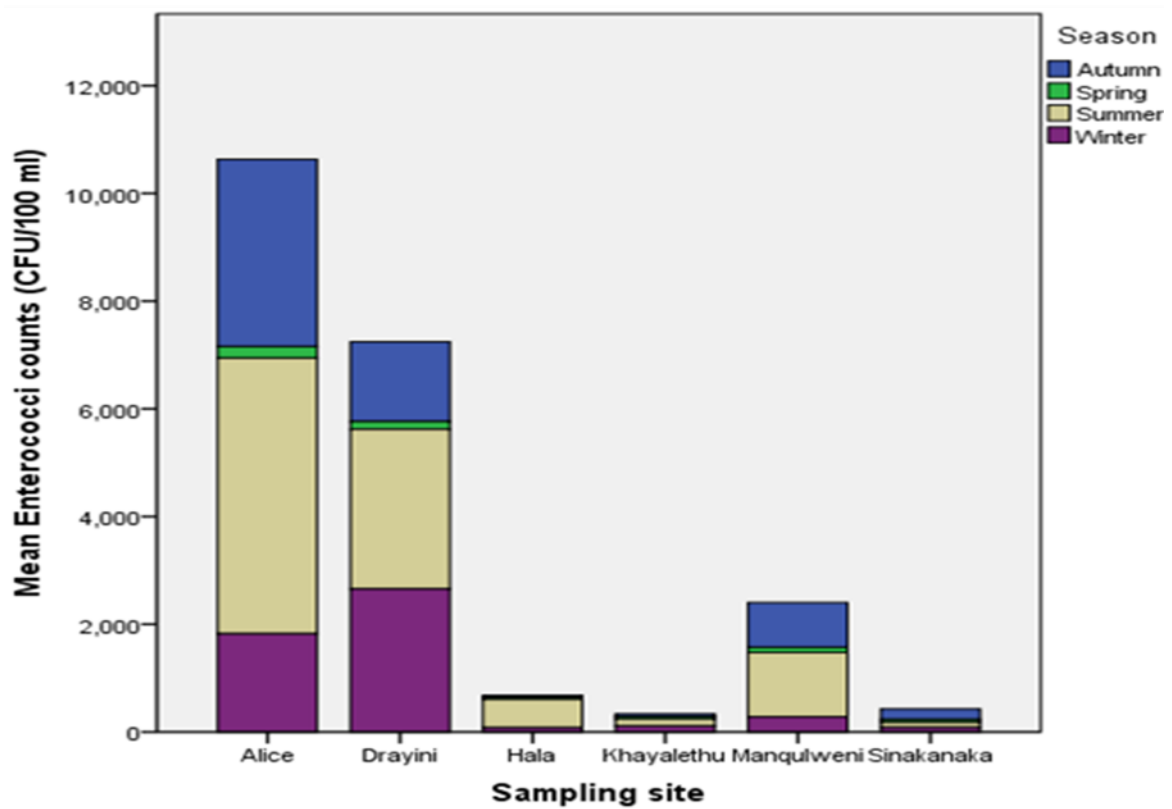
Figure 4.6: Spatial distribution of enterococci at selected sites along Tyume River

Sewer systems are convenient for areas of high population densities but they dispose their effluent (excess wastewater) directly into rivers and streams resulting in the contamination of surface water with faecal matter, an indication of which will be high faecal indicator bacteria counts. The link between poor microbiological water quality and infectious diarrhoea is well established (Cronin *et al*., 2008; Do *et al*., 2007; Obi *et al*., 2003; Oswald *et al*., 2007). Water from streams with poor sanitary quality can transmit diseases such as cholera, typhoid fever, and bacillary and amoebic dysentery. Faecal coliform bacteria have been correlated with the incidence of gastrointestinal disorders resulting from bodily contact with contaminated freshwater sources (Morace and McKenzie, 2002). It has also been suggested that the combination of unsafe drinking water, absence of acquired immunity and lack of hygienic facilities increase the risk of infection, with children, the immunocompromised (including HIV/AIDS patients) as well as the elderly being especially vulnerable (Copeland *et al*., 2009). Over the entire sampling period, the levels of these bacteria increased as the river flowed from the rural to the urban areas. Use of the faecal coliform to enterococci (FC:FS) ratios in distinguishing human from animal faecal pollution produced interesting results (Fig 4.7).

Taking an FC:FS ratio >4 to suggest human faecal pollution of water (Afshin and Saeid, 2011; Baudišová, 2009; Cabral, 2010), it can be observed that human faecal pollution at all sampling sites peaked during the spring. As has already been alluded to, spring is comparatively the driest of South Africa's four seasons. Minimal inflows into the river were therefore experienced during this period, the consequences of which could have been two-fold. Firstly, absence of inflows (hence dilution) could have resulted in the concentration of pollutants from point sources of pollution like wastewater final effluents which are expected to have higher numbers of faecal coliforms than enterococci since it largely contains

derivatives of human stool (Cabral, 2010; Young and Thackston, 1999), hence the FC:FS

ratio becomes >4.
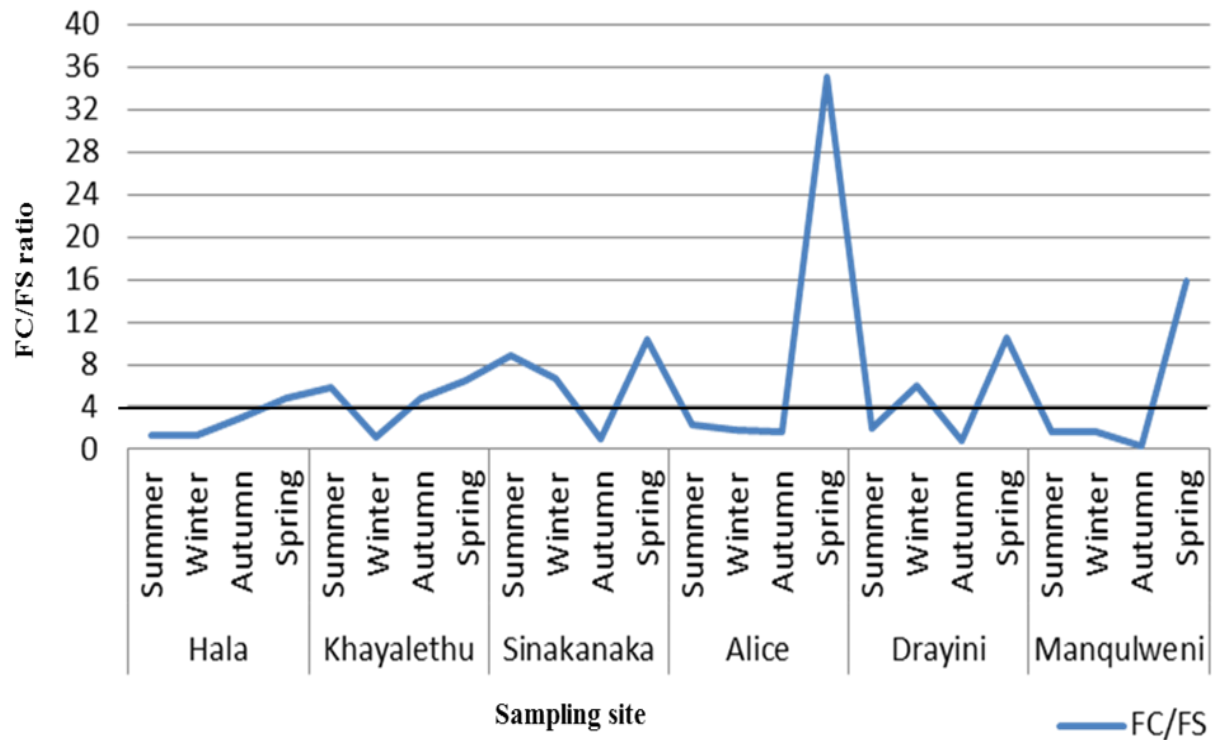
Figure 4.7: FC/FS ratios for tracking sources of faecal pollution at selected sites along Tyume River.

Secondly, the effect of non-point sources of pollution which in wetter seasons could result in

the offsetting of the FC:FS ratio to values <4 by bringing in larger quantities of non-human

faecal matter (which contains more enterococci than faecal coliforms) into the stream, is

suppressed during this season since it is more dependent on precipitation events. It is also

evident from Fig 4.8 that most of the human faecal pollution (FC:FS >4) was recorded in

midstream to downstream sampling sites (between Sinakanaka and Drayini), which paints a

rough picture of the human population distribution in Tyume River catchment. However, this

method of distinguishing between human and animal faecal pollution is not without

shortcomings. It is clear that in times when heavy rainfalls are experienced which could result

in the influx of non-human faecal matter say from pastures and arable land, the FC:FS ratio is

likely to be reduced to values <4 without necessarily having eliminated the human faecal pollution. Sinton *et al*. (1998) have also expressed reservations over the use and interpretation of FC:FS ratio which they say has the tendency to decrease without a change in the nature of the pollution source. Having said that however, it is still our view that the FC:FS ratio can be used to indicate the "predominant" pollution source.

4.4 Conclusions and Recommendations

Moving forward, since faecal contamination of surfaces 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 specific bacterial pathogens. Pathogens from human hosts are more likely to cause illness in humans compared to those from animal hosts. Efficient monitoring of drinking and/or recreational water requires tools that can help track the source(s) of contamination. Library dependent microbial source tracking (MST) is one such tool that may help ascertain the origin of 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 directly rely on surface waters for domestic uses. Recreational water managers may take steps to identify periods when water quality is poor, issue advisory notices warning the public of increased risk protecting public health action may be to permanently discourage its recreational use, for example by fencing or signposting.

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CHAPTER FIVE

REAL-TIME PCR QUANTITATIVE ASSESSMENT OF HEPATITIS A VIRUS, ROTAVIRUSES AND ENTEROVIRUSES IN THE TYUME RIVER LOCATED IN THE EASTERN CAPE PROVINCE, SOUTH AFRICA

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5.0 Abstract

We applied Real-Time PCR (polymerase chain reaction) to assess the incidence of hepatitis A virus, rotavirus and enteroviruses in Tyume River, an important water resource in the impoverished Eastern Cape Province of South Africa. Norovirus detection was done using conventional PCR. Water samples were collected once monthly from six sampling sites over a 12 month period starting in August 2010 and ending in July 2011. Hepatitis A virus was detected in 13% of the samples in concentrations ranging between 1.67×10^3 genome copies/ℓ and 1.64×10^4 genome copies/ℓ while rotavirus was detected in 4% of the samples with concentrations ranging from 9×10^0 genome copies/ℓ to 5.64×10^3 genome copies/ℓ. Enteroviruses were not detected in all the samples, while norovirus was detected in 4% of the samples. All hepatitis A and rotavirus positive samples were from the upstream sampling sites while norovirus was detected in samples from downstream sites only. Statistical analysis showed that occurrence of the viruses in Tyume River was spontaneous. Risk analysis showed that hepatitis A virus posed greater risk than rotavirus for both recreational and domestic water uses while enterovirus did not pose any risk. Because of the low infectious dose of enteric viruses, the detection of even low concentrations of hepatitis A virus, rotavirus and norovirus in surface water poses a significant risk to public health.

Keywords: Hepatitis A virus, Rotavirus, Norovirus, TaqMan real-time PCR, reverse transcription, Tyume River, public health

5.1 Introduction

Ground and surface water sources may be subjected to faecal contamination from a variety of sources, including sewage treatment plant effluents, on-site septic waste treatment discharges, land runoff from urban, agricultural and natural areas, and leachates from sanitary landfills (Abbaszadega, 2001). Consequently, millions of people throughout the world do not have access to microbiologically safe water for domestic, recreational and other general purpose uses (Gibson *et al*., 2011). Health risks associated with surface water use, either raw or treated, include infectious diseases predominantly caused by human and animal enteric pathogens, most notably viruses. RNA viruses constitute the most abundant group of pathogens in man, animals and plants (Bustin and Mueller, 2005). Surveillance of source waters for viral pathogens is therefore necessary to protect public health. The culture-propagation procedure is still the best method to enumerate viruses and demonstrate their infectivity. However, for the detection of norovirus (NoV), hepatitis A virus (HAV) and other enteric viruses like enterovirus (EV), for which appropriate cell cultures are not available, slow or limited (Hong *et al*., 2011; Schvoerer *et al*., 2000), molecular techniques become the most viable option. Molecular techniques have been successfully applied on environmental samples, allowing a rapid and specific detection of human enteric viruses (Bosch *et al*., 2008; Costafreda *et al*., 2006; De Paula *et al*., 2007). The ability of real-time reverse transcription polymerase chain reaction (qRT-PCR) to generate accurate quantitative data has had a huge impact on the study of viral agents of infectious disease (Schutten and Niesters, 2001).

HAV has a worldwide distribution and is the aetiological agent of hepatitis, an acute, usually self-limiting infection of the liver. On average, about 1.5 million cases of clinical hepatitis are recorded world-wide each year (WHO, 2003). The virus is a 27-nm-diameter

nonenveloped RNA virus belonging to the family Picornaviridae and the genus *Hepatovirus* (Feinstone, 1996). Its genome is a positive-sense RNA strand which is 7474 nucleotides long and encodes a polyprotein with structural and nonstructural components (Murray *et al*., 2005). HAV transmission occurs via the faecal-oral route with primary transmission occurring by close personal contact with an infected person, and is closely associated with poor sanitary conditions (Adhami and Carey, 2010; Kittigul *et al*., 2005a). Consumption of raw or partially cooked shellfish raised in contaminated waterways is another possible source of hepatitis A (Koff, 1995). The virus is shed in the faeces of persons with both asymptomatic and symptomatic infection and under favourable conditions HAV may survive in the environment for months (CDC, 1999). Hepatitis A disease is rarely fatal but may represent a substantial economic burden, particularly in countries with low and intermediate incidence rates where a larger portion of the adult population do not have immunity against the disease (Grabow, 1997). The WHO (2003) estimated that annual medical and work-loss costs of approximately US$ 200 million were incurred by the United States of America due to HAV infections. Infected persons are infective from 14 to 21 days before the onset of jaundice and up to 7 to 8 days after jaundice has resolved (Tong *et al*., 1995). Food and water have been identified as the main vehicles for HAV transmission (Koopmans *et al*., 2002). Contaminated drinking water (treated and untreated) and recreational exposure to faecally polluted surface water has been linked to outbreaks of hepatitis A (Hunter, 1997; Mahoney *et al*., 1992).

On the other hand rotaviruses are responsible for the majority of acute gastroenteritis infections occurring in young children world-wide (Jothikumar *et al*., 2009; Iturriza-Gomara *et al*., 2004). The genus *Rotavirus* belongs to the family Reoviridae and can be divided into seven groups A-G, with the majority of human infections being caused by viruses of group A (Adlhoch *et al*., 2011). Rotaviruses are non-enveloped RNA viruses (Fritzinger *et al*., 2011) with a segmented dsRNA genome, together with an RNA-dependent RNA polymerase that

transcribes each of the dsRNA molecules into an mRNA (Bustin and Mueller, 2005). Parashar *et al*. (1998, 2006) estimated that infection by group A rotaviruses results in about 600 000 fatalities annually, most of them in developing countries, while Freeman *et al*. (2008) postulated that by the age of 5 years, nearly all children in both industrialized and developing countries would have been infected with group A rotavirus. While rotavirus (RV) infections in young children can be severe, with life threatening diarrhoea; Kang *et al*. (2004) report that infections in older individuals may be asymptomatic or be associated with mild enteric symptoms, which the authors speculated to be due to increasing cross-protective immunity as a result of repeated infections.

The genus norovirus (NoV) is in the family Caliciviridae which comprise of non-enveloped, single-stranded RNA viruses, 27 nm to 32 nm in diameter and have been found in humans, pigs, cattle, sheep and mice (Hardy, 2005; Wolf *et al*., 2010). 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 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*., 2010). 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 90% of samples collected from urban rivers and streams. Also, in 12 samples collected from a rural river, they detected NoV GI in

8.3% and GII in 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.

Enteroviruses (EVs) are RNA viruses belonging to the *Picornaviridae* family and are classified in five human pathogenic species including poliovirus, human enterovirus (HEV) A, B, C and D (Dierssen *et al*., 2008). The EVs are approximately 27 nm in diameter and have a positive polarity, single stranded RNA genome of approximately 7400 bp (Abbaszadega, 2001). EVs, particularly echoviruses and coxsackieviruses, are the most commonly identified causes of viral meningitis in the pediatric population (Archimbaud *et al*., 2004) and in adults (Hong *et al*., 2010). EVs are associated with diverse clinical syndromes, ranging from mild febrile illness to severe central nervous system diseases, such as aseptic meningitis and encephalitis, potentially leading to paralysis (Hong *et al*., 2011). These viruses are common, faecally shed in extremely high numbers from infected individuals, highly tolerant to salinity and temperature fluctuations, and stable in the environment for extended time periods (Connell *et al*., 2012). EVs have a worldwide distribution and previously been detected in surface water in the USA (Donaldson *et al*., 2002), Switzerland (Gilgen *et al*., 1995), Japan (Haramoto *et al*., 2005) and in South Africa; in treated drinking water (Vivier *et al*., 2004). Contamination of surface waters with enteric viruses is a concern for public health, especially if these surface waters are used for recreational purposes and production of drinking water (Rutjes *et al*., 2005).

Pathogen monitoring should be a part of the future characterization of microbiological hazards in water in order to address risk and effect prevention strategies (Rose and Molloy, 2007). Many areas in developing countries lack access to safe drinking water with around

70% of the global population without improved drinking water sources residing in rural areas (WHO and UNICEF, 2010). Settlements in rural areas are far spaced and divided by rough terrain, dramatically reducing the capacity to provide a centralized drinking water system. Thus, rural populations commonly obtain water on an individual or household basis from nearby surface and groundwater sources where the microbial quality is often unknown (Ashbolt, 2004; Peter-Varbanets *et al*., 2009). Tyume River water is used for the production of drinking water, irrigation and recreational purposes. Faecal contamination of Tyume River has previously been linked to the presence of bacterial pathogens (Momba *et al*., 2006a). However, traditional bacterial indicators and/or pathogens often fail to predict or correlate with the occurrence of pathogenic waterborne viruses of public health concern (Brooks *et al*., 2005; Jiang *et al*., 2001; Noble and Fuhrman, 2001). To the best of our knowledge, no research has previously been done to detect the presence of viral pathogens in Tyume River. There is therefore a need to cover this knowledge gap, hence, in this paper we report on incidences of some RNA viruses in Tyume River as well as the risk associated with accidental consumption of water contaminated with the viruses.

5.2 Materials and methods

5.2.1 Description of study site

For the description of the study area, please refer to section 3.2.1, page 68-70.

5.2.2 Sampling

Water samples were collected once monthly at each sampling site over a 12 month period starting in August 2010 and ending in July 2011. In all, a total of 12 samples per site

were collected giving a total of 72 samples for the duration of the study. Samples were transported in cooler boxes to the Applied and Environmental Microbiology Research Group (AEMREG) Laboratory at the University of Fort Hare, Alice for analysis within 6 h of sample collection following the procedure recommended by the American Public Health Association (*Standard Methods,* 2005).

5.2.3 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. 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. The sample concentrates were stored at -80°C until ready for use. Storage of viruses at temperatures 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).

5.2.4 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 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 of 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°C to inactivate the DNase. Purified viral RNA was eluted in 60 µℓ of RNase-free water.

5.2.5 Quantification of HAV, RV and Enterovirus genomes by real-time PCR

HAV and RV were quantified in a two-step protocol where RNA was first reverse 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. For RV, prior to the reverse transcription reaction the RNA was denatured by heating at 95°C for 5 min followed by incubation in ice for 2 min to separate its double stranded RNA.

To quantify the viruses, the resultant cDNA was used as template in a quantitative TaqMan real-time PCR (StepOnePlus PCR system; OPTIPLEX 755, Applied Biosystems) with TaqMan probes using a 96-well plate. The wells were loaded 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). Subsequently, 5 µℓ aliquots of sample cDNA were added with mixing to give 25-µℓ total reaction mixtures. The plate was sealed and loaded in the thermocycler. The assays were performed under the following cycling conditions: 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); RVs (*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) and EVs (*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). Fluorescence data were collected at the end of the annealing step. The probes and primer pairs used for quantification are shown in Table 5.1.

To determine the qRT-PCR limit of detection, ten-fold serial dilutions of cDNA (for each virus) with quantities ranging from 100 000 genomic equivalents to 0.1 genomic equivalents were run under the same conditions as those of the individual viruses. Primer specificity and possible cross-reactivity was determined by substituting target cDNA with non-target DNA. The standard curve for each virus was formulated as described by Brooks et

al. (2005) and Haramoto et al. (2008). Briefly, RNA was extracted from positive ATCC strains (HAV: ATCC VR-1357; strain PA21 and RVs: ATCC VR-2274; strain 248) using commercially available extraction kits (Quick-RNA^{TM} MiniPrep (Zymo Research, USA)). The RNA extracts were then reverse transcribed into cDNA using random primers. The resultant cDNA was subsequently quantified using a Qubit$^{®}$ fluorometer (probes.invitrogen.com/qubit) and diluted by serial tenfold dilution. The sample extracts and standards were then subjected to real-time PCR simultaneously, followed by analysis using SDS software (Applied Biosystems^{TM}) to obtain quantitative data on the titre of viral cDNA in a well. Two wells were used for each of the standards, negative controls (no template controls) and samples, 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.

Table 5.1: Probes and primer pairs for HAV and RV quantification.

Enteric Virus	Primers and labelled TaqMan Probe	Reference
HAV	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
RV	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
EV	EV1 (F): 5'-CCCTGAATGCGGCTAAT-3' EV1 (R): 5'-TGTCACCATA AGCAGCCA-3' EV (P): 5'–FAM–ACGGACACCCAAAGTAGTCGGTTC–MGBFQ-3'	Gregory *et al*., 2006; Noble *et al*., 2006

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

5.2.6 Detection of NV genogroups by conventional semi-nested PCR

Norovirus genogroups GI and GII were detected by semi-nested PCR as described by Victoria *et al*. (2010). NoV genogroup GI and GII specific primer sets which target the viral RNA-dependent RNA polymerase gene (Boxman *et al*., 2006) were used. These primer sets have been used in previous studies for detecting NoV GI and GII in environmental samples (Victoria *et al*., 2010). The primer sets are shown in Table 5.2.

Table 5.2: Primers for detection of norovirus genogroups.

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

PCR cycling conditions for both norovirus GI and GII were as follows: 1st round PCR; 3 min at 94°C to activate the *Taq* DNA 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. The composition of the reaction mixtures was as follows: 12.5 µℓ PCR Master Mix (Fermentas), 1 µM of each of the forward and reverse primers, 5 µℓ of cDNA (and for 2nd round PCR, 2.5 µℓ of 1st round PCR amplicon), and nuclease free water to give a total reaction volume of 25 µℓ. Amplified products were analysed on ethidium bromide-stained 2% agarose gels.

5.2.7 Risk Assessment

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) while for hepatitis A virus the ratio was 1:60 (Deng *et al*., 1994; Pinto *et al*., 2009). 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 water respectively. The total number of infectious viruses was estimated first by "correcting" the virus concentrations obtained directly from the qRT-PCR assay. "Correcting" was done by multiplying the virus concentrations by the inverse of the mean recovery efficiency of the filtration method, which in this case was 56% (Haramoto *et al*., 2005). The corrected virus concentrations were then multiplied by the ratio of infectious virus particles to total virus particles for each virus in order to obtain the infectious dose of each virus. Estimates of risks of daily infection for the enteric viruses were determined using the models (Haas, 1996; WHO, 2001) shown below:

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

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

Equations (1) and (2) were used for HAV and RV, respectively. The parameters are described in Table 5.3.

Table 5.3: Parameters used in estimating the risks of daily infection using equations (1) and (2)

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

Values of α and β were 0.2531 and 0.4265 respectively for rotavirus (Haas *et al*., 1993) while for HAV, N_{50} and α assumed values of 100 and 0.2 respectively.

5.2.8 Statistical analysis

Results were analysed using the Statistical Package for the Social Sciences (IBM SPSS Statistics release 19; IBM, USA). To test if viral detection depended on season or sampling site, a one-way ANOVA and Tukey's Studentized Range (HSD) Test were used. All tests were carried out at a 5% level of significance.

5.3 Results

The detection limit of the real-time PCR assays was determined to be 10 genomic equivalents achieved, with a C_t value ≤ 35. Samples with C_t values ≥ 35 were therefore considered negative. When non-target cDNA was used, there was no amplification, confirming the specificity of the primer-probe combinations for the target cDNA. HAV was

detected in 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, 56% were collected between October 2010 and January 211 while the other 44% were collected between June and July 2011. HAV detection was not affected by season ($P > 0.05$). The amplification plot and standard curve and the $\log_{10}$genome copies of HAV per litre obtained after the real-time PCR assay are displayed in Figures 5.1 and 5.2.
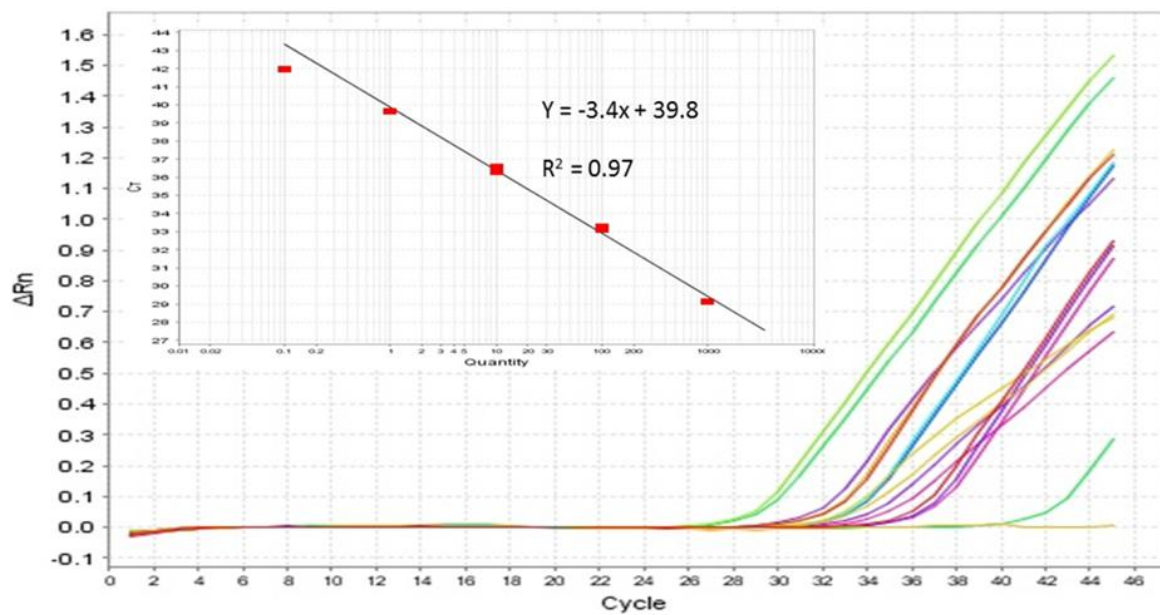
Figure 5.1: Standard curve and amplification plot for HAV quantitation in Tyume River.
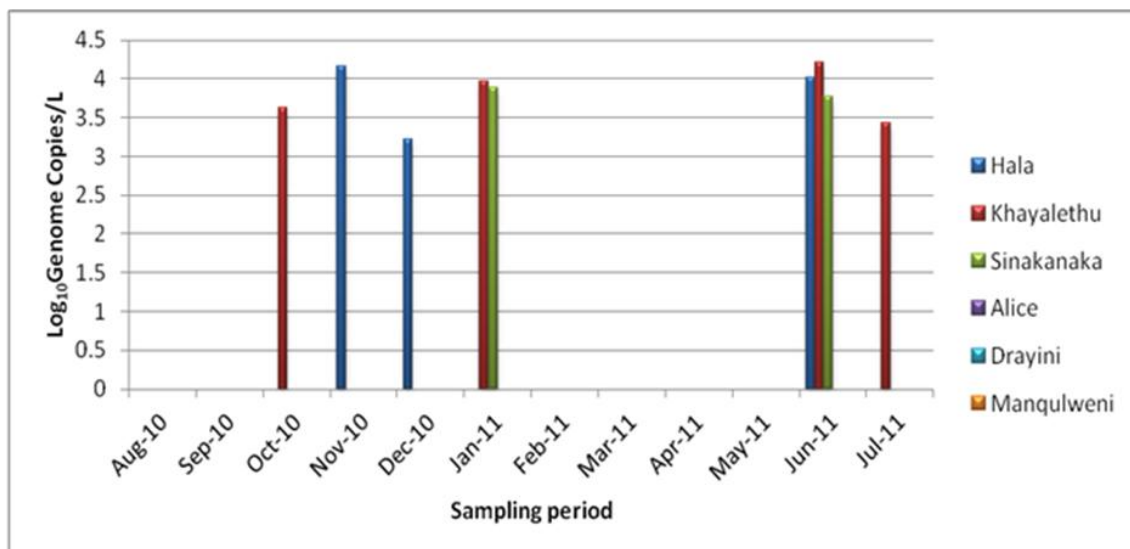
Figure 5.2: $\log_{10}$genome copies/ℓ of HAV at selected sites along Tyume River

Rotavirus RNA was detected in 4% of the samples in concentrations ranging between 9×10^0 genome copies/ℓ and 5.64×10^3 genome copies/ℓ. Of the positive samples about 67% were collected during the winter months (June and July 2011) while 33% were collected in September 2010 (spring). Rotavirus detection did not follow any seasonal pattern ($P<0.05$). Figure 5.3 and 5.4 show the standard curve and amplification plot and $\log_{10}$genome copies per litre of RV after qRT-PCR amplification.
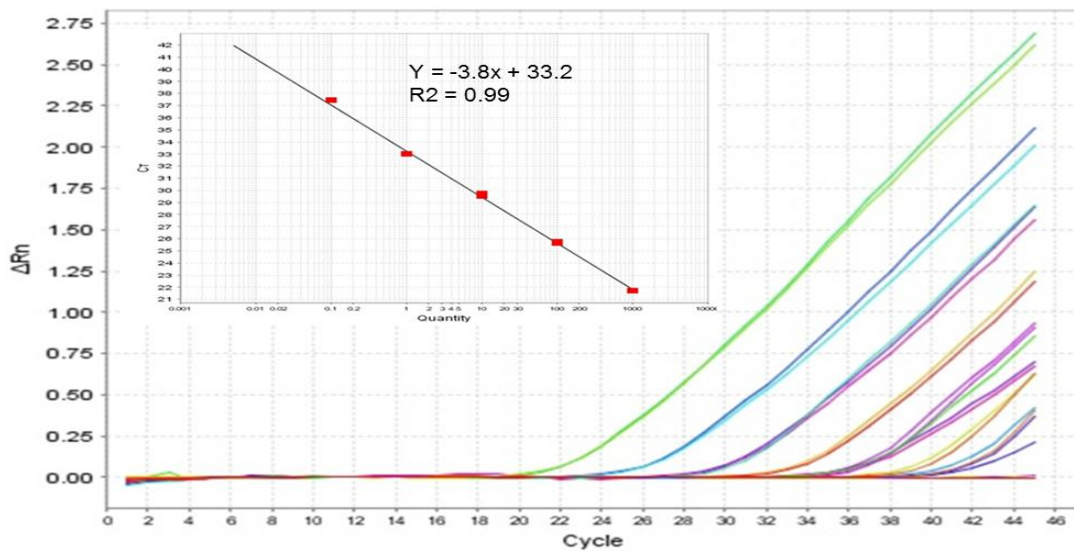
Figure 5.3: Standard curve and amplification plot for RV quantitation in Tyume River
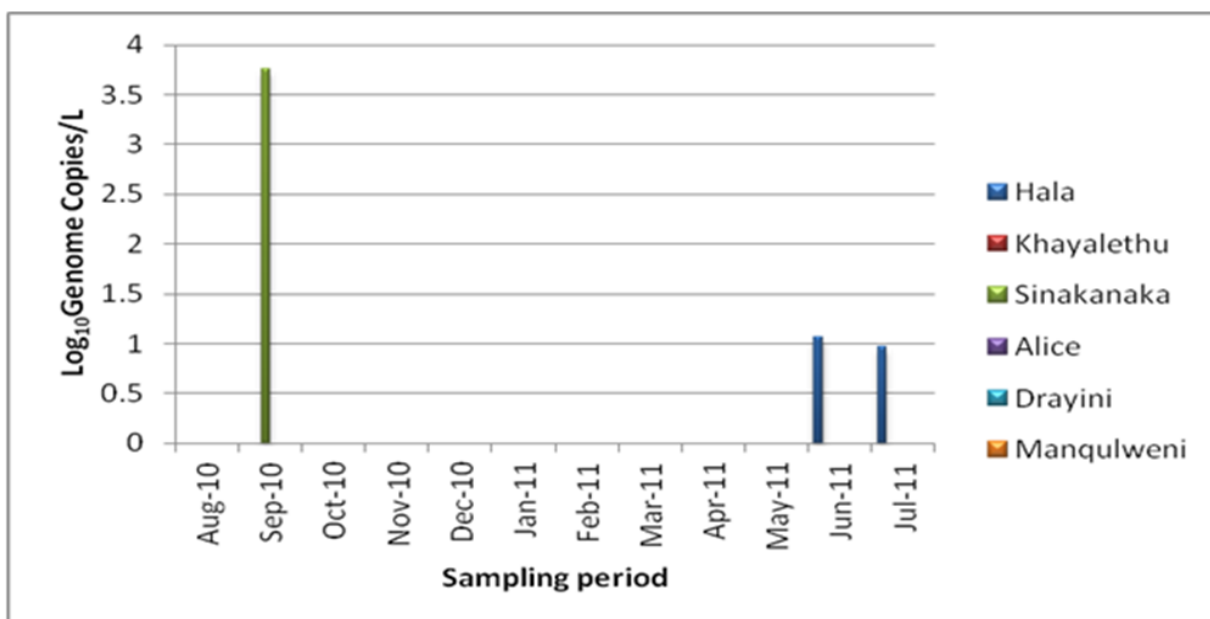
Figure 5.4: log$_{10}$genome copies/ℓ of RV at selected sites along Tyume River

Enterovirus was not detected in Tyume River water samples in all the months of sampling while NoV was detected in 4% of the samples tested (gel picture not shown). All NoV positive samples were collected from two downstream sites. NoV GI was detected in samples from Alice and Drayini sampling sites in August and September 2010 respectively while NoV GII was detected in a sample from the Alice sampling site in October 2010. NoV detection was spontaneous ($P<0.05$).

5.3.1 Risk assessment

The uncorrected and corrected mean concentrations of viruses as well as the calculated mean concentration of infectious viruses in Tyume River are shown in Table 5.4.

Table 5.4: Calculated mean concentration of infectious viruses in Tyume River.

Enteric virus	Infectious:Total number ratio	Reference	Uncorrected concentration (genome copies/ℓ)	Corrected concentration (genome copies/ℓ)	Calculated concentration of infectious viruses (genome copies/ℓ)
HAV	1:60	Deng *et al*., 1994; Pinto *et al*., 2009	8.05×10^3	1.44×10^4	2.4×10^2
RV	1:40 000	Ward *et al*., 1984; Rodríguez *et al*., 2009	1.89×10^3	3.37×10^3	8.43×10^{-2}

Assuming accidental ingestion of 10 mℓ and 100 mℓ for domestic and recreational water usage respectively, the calculated doses were 2.4×10^0 and 2.4×10^1 for HAV and 8.43×10^{-4} and 8.43×10^{-3} for RV respectively. The daily risk of infection results are displayed in Figure 5.5.
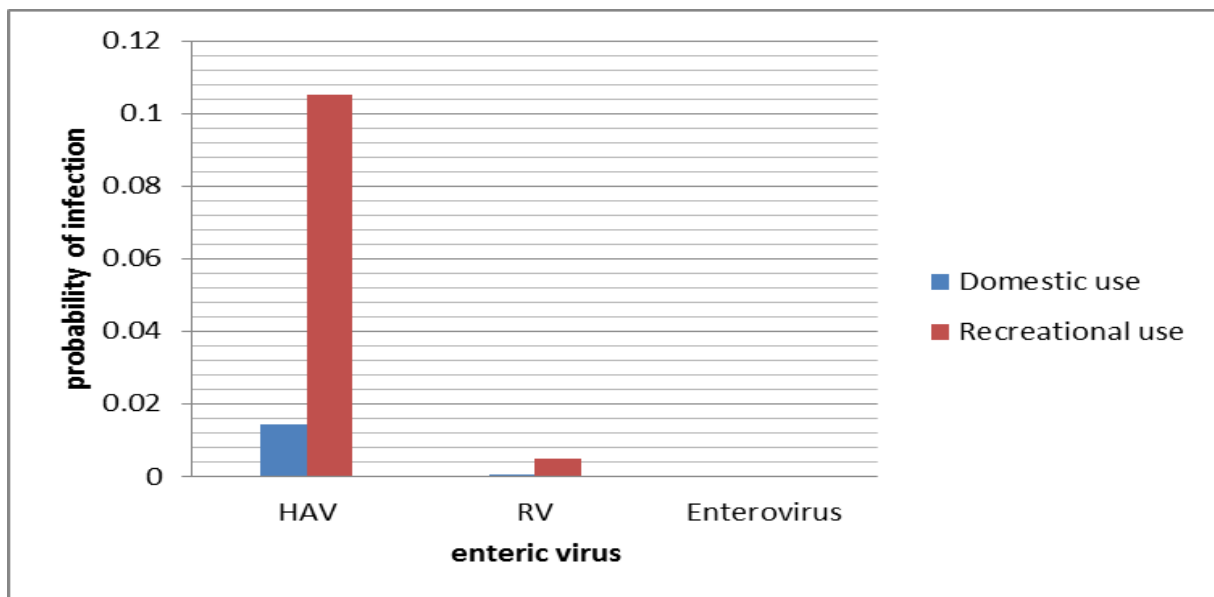
Figure 5.5: Risk of infection from enteric viruses in Tyume River

5.6 Discussion

Hepatitis A infection is the leading cause of acute viral hepatitis throughout the world (Costafreda *et al*., 2006) while RV gastroenteritis continues to be the single most important cause of dehydration in young children (Kang *et al*., 2004). In this study, both viruses were detected only in samples collected from the upstream sampling sites where population pressure is less compared to the downstream stretch of Tyume River. This observation is in tandem with documented findings that the distribution patterns of HAV and RVs in different geographical areas of the world are closely related to socioeconomic development, with endemicity being high in less developed regions (Jothikumar *et al*., 2009; Fernandez-Molina *et al*., 2004). Additionally and most crucially, Kang *et al*. (2004) suggested that exposure to an environment contaminated with human and animal faeces, and close contact with animals in the domestic environment are factors that are likely to promote mixed RV infections and inter-species transmission of RV strains, enabling viral reassortment and the emergence of new strains. Such scenarios are most commonly observed in rural areas, especially of developing countries. In this study, communities along the upstream stretch of Tyume River are rural, with cattle pens situated close to houses, maybe for the security of the animals. Homes are also situated further apart unlike in the downstream stretch which is characterised by urban to semi-urban settlements. The sanitary infrastructure in the upper Tyume catchment may therefore not be as established as it is in the lower Tyume with the possible consequence that the majority of the population still use the "bush-toilet" system. In the event of flash storms, raw human waste is washed down into the rivers possibly contaminating surface waters with enteric pathogens. Also, the use of pools found in the river for recreational activities like swimming is common practice among rural children, which practice raises both the chances of surface water contamination with enteric viruses and also the risk of swimmers getting infected. Waterborne RVs have been detected elsewhere

(Kittigul *et al*., 2005b) and the first large waterborne epidemic in an adult population was caused by group B rotaviruses in China (Logan *et al*., 2006). Faecally polluted natural surface water used for recreational activity could therefore pose a potential health risk to the public (Venter *et al*., 2007). Such swimming activities are also likely to cause localised distributions of enteric pathogens along stretches of the river course. It is a widely acknowledged fact that by the age of 10, nearly 100% of children in low socio-economic groupings would have suffered from and hence acquired immunity against, HA infections (Taylor *et al*., 2001). Such statistics could be attributed to a tendency by the children in these low socio-economic groupings to "play-in-the-water" thereby exposing themselves to HAV infection at that early age.

Untreated or insufficiently treated wastewater has been noted to play an important role in the transmission of both HAV and RVs (Lodder and de Roda Husman, 2005; Mara, 2000). In a study by Lodder and de Roda Husman (2005) they found that the concentrations of RV RNA in naturally contaminated raw and treated sewage were similar, suggesting inefficient removal of rotaviruses by the treatment processes used in the involved sewage treatment plant. Viral contamination of water sources has been frequently reported as a primary source of gastroenteritis 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). Consequently, HAV can survive in water for long periods of time (Soule *et al*., 1999). Several studies (Espinosa *et al*., 2008; Rutjes *et al*., 2005; Caballero *et al*., 2004) have also demonstrated the stability of RVs in water, and therefore the potential risk for the human population to acquire RV infections associated with environmental contamination by waterborne transmission. No detection of both HAV and RVs in samples from middle to downstream Tyume may have a two-fold interpretation. It could mean that these viruses were not in circulation in the host population hence their absence also from the

environment. Alternatively, these findings may also be an indication of the higher concentrations of PCR inhibitors from downstream samples which could also have caused false negative results to be obtained. However, no dilution of samples was carried out in order to ascertain if indeed the negative results were a consequence of inhibition.

The detection of HAV in this study is in tandem with the findings of Taylor *et al.* (2001) who also detected HAV in river and dam water used for recreational and domestic purposes in South Africa. Because only a few viral particles are needed to cause disease (De Paula *et al.*, 2007), detection of low concentrations of the virus in water becomes significant. The occurrence of HAV in raw water sources has been tied to epidemiological features such as an outbreak in a particular community, and it is thought that the minimal infectious dose is extremely low, possibly as low as a single infectious particle (Zhi-Yi *et al.*, 1992). HAV has also been estimated to impose a large economic burden throughout the world with adult sufferers estimated to miss 30 days of work per annum (Berge *et al.*, 2000). Berge *et al.* (2000) also estimated that medical treatment and work loss account for an estimated ZAR4 billion annually in the United States. While epidemiological studies have shown that HAV is endemic in South Africa (Venter *et al.*, 2007) the burden of HAV infection in South Africa is however, still unknown. This is because HAV can cause asymptomatic infections that go unrecognised until secondary person-to-person spread finally leads to overt disease in hardly traceable pockets of the population (Bosch, 1998).

Occurrence of infectious enteric viruses in environmental samples is mostly determined using cell culture techniques (Fong and Lipp, 2005). However, cell culture detection of HAV tends to be lengthy, with reduced specificity (Li *et al.*, 2002) and sensitivity (Kittigul *et al.*, 2005a), especially when applied to environmental samples where the virus titre could be low. The real-time PCR technique is an efficient tool in detecting HAV in environmental samples because it combines PCR amplification with the use of a

probe to confirm the identity of the PCR product (De Paula *et al*., 2007). Results of a study carried out by Villar *et al*., (2006) to evaluate methods used to concentrate and detect HAV in water samples also confirmed that compared to qualitative PCR, real-time PCR detects low concentrations of genome per millilitre and is more suitable than qualitative PCR for the detection of HAV RNA in environmental samples.

In the case of RVs, combinations of techniques have been used for its detection which includes cell culture (Rutjes *et al*., 2009), electron microscopy (EM) (Bishop *et al*., 1973), latex agglutination (LA) and enzyme immunoassay (EIA) (Pang *et al*., 2004). However, molecular techniques have also proven to be a technological advancement in the detection of RVs, both in clinical and environmental samples. Gunson *et al*. (2003) reported a 48% increase in the detection rate of rotaviruses A by reverse transcription-PCR compared to EIA or EM. Logan *et al*. (2006) however, reported a 110% and 186% increase in the detection of RVs by real-time PCR compared to LA and EM respectively. Interestingly also, Pang *et al*. (2004) observed that using the same primers, the real-time RT-PCR assay for RVs detection in clinical samples was 1000 times more sensitive than conventional RT-PCR. However, PCR methods are susceptible to inhibitors widely existing in water samples and may consequently yield false negative results (Yang *et al*., 2011).

Group A rotaviruses have been detected in untreated and treated drinking-water samples in Southern Africa (Van Zyl *et al*., 2006). While detection of both HAV and RVs was observed to be confined to the upstream stretch of the river compared to the downstream ($P<0.05$), occurrence of both viruses tended to be sporadic ($P>0.05$).

In general, seasonality of virus infections is difficult to detect in surface water samples because, whereas circulation within the human population is mainly during the summer season, their detection in environmental water samples is mainly during winter

(Rutjes *et al*., 2009) chiefly because of the low temperatures which significantly reduce the deactivation rates. Previous findings note that whereas RV infections are common all-year round in tropical climates (Cook *et al*., 1990), RV 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).

Unlike other RNA viruses detected in this study, all NoV detections were in samples collected from the downstream stretch of Tyume River which is more impacted by wastewater effluents than the upper stretch of the river. Noroviruses have previously been detected in wastewater and surface water (Haramoto *et al*., 2005; Lodder and De Roda Husman, 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 effluents (Arnone and Walling, 2007). Norovirus detection in samples from the downstream sampling sites might have been influenced by their relatively close proximities to sewage outfall points. The Alice sampling site is in the immediate downstream of the effluent discharge points of the Victoria Hospital and Alice Town wastewater treatment plants. The Drayini sampling site, while located downstream of the Alice sampling site, is also located in the immediate downstream of the University of Fort Hare wastewater treatment plant effluent discharge point. 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 concentrations of viruses in environmental waters (Aw *et al*., 2009), necessitating the concentration of NoVs from larger volumes of water. Noroviruses, like most other enteric viruses, are able to survive treatment

processes if there is inadequate chlorination. While it is reported that NoVs can be destroyed by 'adequate chlorination' (Shin and Sobsey, 2008), their physicochemical stability helps them to pass through sewage treatment without inactivation and reach many kinds of environmental waters (Victoria *et al*., 2010).

Naked RNA has been found to have limited survival in the environment (Tsai *et al*., 1995). Also, viral capsids and not naked RNA has been observed to bind to the membrane used for the selective recovery of viruses (Katayama *et al*., 2002). This may imply that the RNA found in the water is most likely accompanied by virus particles and would most probably cause infection. Viruses have also been reported to survive and remain infective for up to 130 days in seawater, and for up to 120 days in freshwater and sewage (Fong and Lipp, 2005). Rotaviruses in particular have been found to be stable in environmental conditions. Fischer *et al*. (2002) have reported that in tropical temperatures (30°C), RV particles can survive for more than 2 months and can maintain infectivity for more than 32 months at $\leq 10^{o}$C. While molecular detection methods cannot differentiate between infective and damaged viruses, both these classes of viruses are nevertheless detected by PCR and any such detections therefore could pose a public health risk to the consumer. In a rural setting with no potable water sources, the risk is made all the more real not only by the dependence (of rural people) on surface water for domestic and general purpose uses, but also by the possibility that all contaminating faecal matter, in which viruses could be present in large quantities, would have undergone little or no treatment at all.

5.6.1 Risk assessment

For both HAV and RVs, the calculated risk of infection was higher for recreational water use compared to domestic water use, most probably because of the larger volume (100

mℓ) involved which has the consequence of increasing the dosage also. Even though EVs were not detected in this study, they are nevertheless very widespread in environmental waters and the risk they pose to public health can therefore not be underestimated. It is possible that the results could have been different had sample volumes larger than 1ℓ been used considering that these enteric viruses are found in low concentrations in natural water environments and also the possibility of PCR inhibition which could have led to false negative results being obtained.

Exposure to a minimal volume of 10 mℓ Tyume River water would lead to a 1:2 000 risk of infection from RVs which is 5 times more than the acceptable risk level of 1:10 000. Still, this is to assume accidental consumption in a domestic set-up as opposed to complete reliance on such water for all domestic uses including drinking. Rotaviruses are the leading cause of gastrointestinal morbidity and mortality among young children and are of much greater public health concern to young children and immunocompromised persons and populations than the general population (US.EPA, 2010). Hepatitis A virus presented significantly higher risk of infection figures compared to RVs in the case of ingestion of 10 mℓ of river water. The risk of infection with HAV in faecally polluted water has been found to increase with increased immersion in contaminated water (Gammie and Wyn-Jones, 1997; Taylor *et al*., 1995). This implies that water from Tyume River 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 risk of infection calculation 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. A combination of characteristics cause human enteric viruses to be of great public health concern and these are: increased stability in the environment when compared with faecal bacteria (Fujioka and Yoneyama, 2002), resistance to some water treatment processes (WHO, 2008) and ability to cause infection at low doses (Fong and Lipp, 2005). Jiang *et al*. (2001) established a link between urban stormwater run-off and the virological contamination of receiving waters making it needful to study the virological water quality of streams and rivers, especially those relied upon as source waters for drinking and recreation. Since the detection of NoVs and HAV on the basis of infectivity is complicated by the absence of reliable cell culture methods (Blaise-Boisseau *et al*., 2010), this real-time PCR detection of these and other viruses in a river of strategic importance to its host community as Tyume River is, is enough to constitute public health risk. Because access to potable water in some pockets of the population within the Eastern Cape Province remains a pipe-dream (Momba *et al*., 2006b) the findings of this study point to a possible public health concern which needs urgent interventions from the responsible authorities.

5.7 Conclusion

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 purposes. The findings of this study also suggest that the

presence of enteric viruses in the environmental surface waters is not related more to general faecal pollution than it is to the presence of infected individuals in the host population. 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 wider Eastern Cape Province so that risk assessment profiling is aligned to water-use patterns specific for communities in those catchments.

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CHAPTER SIX

ASSESSMENT OF THE INCIDENCE OF ENTERIC ADENOVIRUS SPECIES AND SEROTYPES IN SURFACE WATERS IN THE EASTERN CAPE PROVINCE OF SOUTH AFRICA: TYUME RIVER AS A CASE STUDY

6.0 Abstract

River water samples were collected for 12 months and assessed for adenovirus by TaqMan real-time PCR. Of the 72 samples analysed, 22 samples were positive for adenovirus giving a detection rate of 31%. Of these samples, 82% were collected from downstream sites. Among the downstream sampling points, adenovirus detection rate increased with distance downstream, being 28%, 33% and 39% for Alice, Drayini and Manqulweni, respectively. The Alice sampling site had the highest concentrations of adenovirus ranging between 6.54×10^3 genome copies/ℓ and 8.49×10^4 genome copies/ℓ. The observed trend could have been 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 wastewater treatment plants of institutions and semi-urban areas. Adenovirus detection was sporadic at most sampling sites. Multiplex conventional PCR was used for the detection of clinically important adenovirus species B, C and F and their serotypes. The detection rate for Species C adenoviruses was 77% while 18% of the samples were positive for adenovirus Species F. Results obtained seem to correlate adenovirus occurrence in river water to increased population densities in river catchments.

Key words: real-time PCR, adenovirus, enteric virus, serotype, standard curve

6.1 Introduction

Enteric viruses are present in high concentrations in faeces of infected persons (Fong *et al*., 2009; Haramoto *et al*., 2010). In areas lacking adequate sanitary infrastructure these viruses eventually find their way into the environment with minimal or no reduction in their numbers and/or infectivity. Adenovirus is the most prevalent of enteric viruses in water environments worldwide (Pina *et al*., 1998). It is also the only DNA viral pathogen in the enteric virus family and hence tends to outlast other enteric viruses in environmental waters as a result of its thermostability (Jiang and Chu, 2004). 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; Jothikumar *et al*., 2005). Adenoviruses are members of the Adenoviridae family and include 70 nm to 100 nm non-enveloped icosahedral viruses. At present, there are 51 serotypes of adenoviruses, about 30% of which 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). The risk posed by adenovirus species F serotypes 40 and 41 as leading causes of childhood diarrhea lead the Environmental Protection Agency (EPA) to enact the Information Collection Rule in 1996, which required all water utilities serving more than 100,000 households to monitor their source water for viruses (USEPA, 2009). Serotypes of species A, B and C have been, to a lesser extent, linked to acute gastroenteritis in infants (Brown, 1990) with species B and C adenoviruses linked to outbreaks of pharyngoconjunctivitis in recreational waters (Papapetropoulou and Vantarakis, 1998). Adenovirus infections have been observed to occur throughout the year with little or no seasonal variation in shedding (Allard *et al*., 1990).

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). While faecal contamination of the water environment is currently being monitored only with bacterial indicators, it is vital to point out that bacterial and viral contaminations are not necessarily associated and linked with each other (Bosch, 1998; Plummer and Long, 2007). Monitoring specific virus pathogens and their relative numbers in water samples would provide more reliable information for risk assessments of waterborne viral infections (Toze, 1999). The presence of enteric viruses in sewage and hence in environmental surface waters reflects the infectious status of the population (Myrmel *et al*., 2006) and constitutes a public health risk (Silva *et al*., 2009). Despite large advances in water and wastewater treatment, water-borne diseases still pose a major world-wide threat to public health (Toze, 1999), moreso in developing countries where a substantive portion of the human population still rely on untreated surface waters for domestic purposes. Infectious adenoviruses have previously been detected in high frequencies in surface waters used for drinking water supplies in South Korea and South Africa (Jiang, 2006), and elsewhere (Haramoto *et al*., 2007; Muscillo *et al*., 2008). Inadequate chlorination during conventional drinking water treatment may fail to remove all viral pathogens, especially adenoviruses (owing to their increased resistance compared to other enteric viruses), from water; moreso when the source water is heavily polluted.

The sparsely populated upstream and the presence of a hospital, town and university midstream of Tyume River catchment make it an interesting study site. The student population at the University of Fort Hare comprise people from different geographic regions and the wastewater effluent discharged from the university's wastewater treatment plant is likely to be contaminated with a range of viral pathogens. Effluent from Victoria Hospital is equally likely to contain a variety of pathogens as well. Effluents from the University of Fort

Hare, Alice Town and Victoria Hospital wastewater treatment plants are discharged directly into Tyume River. So far, no studies have indicated the occurrence of adenoviruses in surface waters in the Eastern Cape Province of South Africa. Therefore, the purpose of this study was to use Real-Time PCR for the detection and quantitation of adenoviruses in Tyume River since it serves as a public water supply to the Nkonkobe Local Municipality. Because low levels of adenoviruses in drinking water could result in significant risks of infection and mortality in sensitive sub-populations (Reynolds, 2004), the presence of adenoviruses may confirm the risk of its transmission to the human population.

6.2 Methods and materials

6.2.1 Sampling

One litre water samples were collected once monthly for 12 months (August 2010 to July 2011) from six sampling sites along Tyume River. Samples were transported in cooler boxes to the Applied and Environmental Microbiology Research Group (AEMREG) Laboratory at the University of Fort Hare, Alice for processing and analyses. Processing of samples was done within 6 h of sample collection. In all, a total of 12 samples per site were collected giving a total of 72 samples for the 12 month sampling period.

6.2.2 Study site

For the description of the study area, please refer to section 3.2.1, page 68-70.

6.2.3 Concentration of viruses in water samples

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% $\pm$ 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. The concentrates were 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).

6.2.4 Extraction of adenovirus DNA

Two sample aliquots (200 µℓ each) of concentrated virus samples were prepared; one set of which was spiked with the specific virus control for quality assurance. Both sets were used for the extraction of viral DNA and purification with commercially available kits

(Quick-gDNA^{TM} MiniPrep; Zymo Research, USA) following the manufacturer's protocol. Purified viral DNA was eluted in 60 µℓ of DNase-free water.

6.2.5 Quantification of adenovirus genome by Real-Time PCR Assay

The concentrations of human adenovirus in the river water samples were estimated by using quantitative PCR (qPCR) with a TaqMan probe. Quantitative detection was performed using a StepOnePlus PCR System (OPTIPLEX 755, Applied Biosystems), forward primer JTVX(F) 5'-GGACGCCTCGGAGTACCTGAG-3', reverse primer JTVX(R) 5'-ACIGTGGGGTTTCTGAACTTGTT-3' and TaqMan probe JTVX(P) 5'-FAM-CTGGTGCAGTTCGCCCGTGCCA-MGBFQ-3' (Fong *et al*., 2009; Jothikumar *et al*., 2005; Xagoraraki *et al*., 2007). [FAM, 6-carboxyfluorescein (reporter dye); MGBNFQ, minor groove binder/non-fluorescent].

Quantification of AdV by qPCR was done following a one-step reaction in a 96-well plate. The wells were loaded 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). Subsequently, 5 µℓ aliquots of sample DNA were added with mixing to give 25-µℓ total reaction mixtures. The plate was sealed and loaded into the thermocycler under the following cycling conditions; 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. Fluorescence data was collected at the end of each cycle. The primers were tested for cross reactivity by substituting our target DNA with non-target DNA but no cross reactivity was observed.

The standard curve was formulated as described by Haramoto *et al*. (2008). Briefly, DNA was extracted from an adenovirus ATCC positive strain (ATCC VR-930) using commercially available extraction kits. The DNA 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. By using control viral samples, sensitivity of detection was demonstrated to be fewer than 10 copies of viral genome per reaction and quantitative linearity was demonstrated to be from 10 to 10^6 copies of input viral DNA.

6.2.6 Detection of 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 6.1 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 and 58 bp while the AdV41 product remains 152 bp. For quality assurances, the specific virus strains were used as controls.

Table 6.1: 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 (Metzgar *et al*., 2005; Tiemessen and Nel, 1996)

6.2.7 Controls

Each test included two controls; a positive control consisting of a spiked sample containing pre-determined concentrations of viral DNA (standard) and a negative control consisting of PCR-grade water and MasterMix formulation. The entire control virus strains (Table 6.2) used were obtained from ATCC and preserved at -80°C.

Table 6.2: **ATTC viral control strains**

Virus	Reference number	Strain
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	

6.2.8 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

The concentrations of adenovirus detected in this study ranged between 1.0×10^{0} genome copies/ℓ and 8.49×10^{4} genome copies/ℓ. 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 sites (Alice, Drayini and Manqulweni). Statistical analysis showed that adenovirus detection was significantly higher among the downstream sampling sites ($P<0.05$) compared to the upstream sites (Hala, Khayalethu and Sinakanaka). Figure 6.1 shows the amplification plot (with standard curve)

while Figure 6.2 shows the $\log_{10}$ genome copies/ℓ obtained after real-time PCR assay for adenovirus.
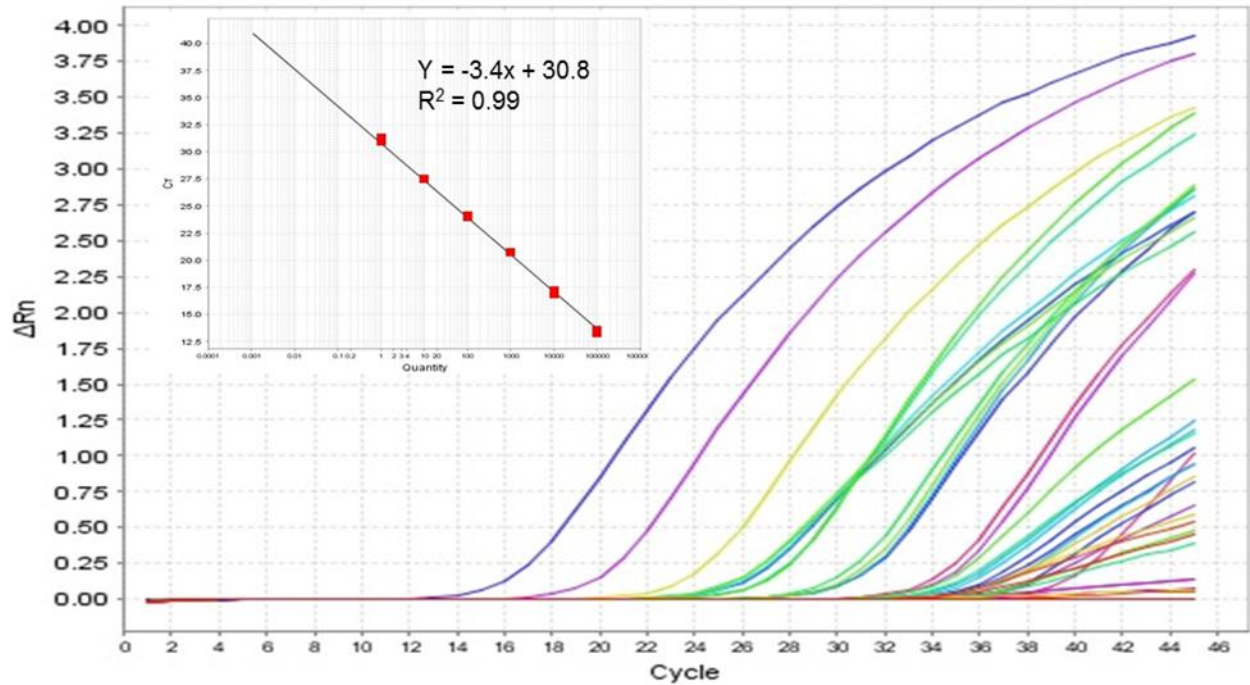
Figure 6.1: Amplification plot for adenovirus quantitation in Tyume River
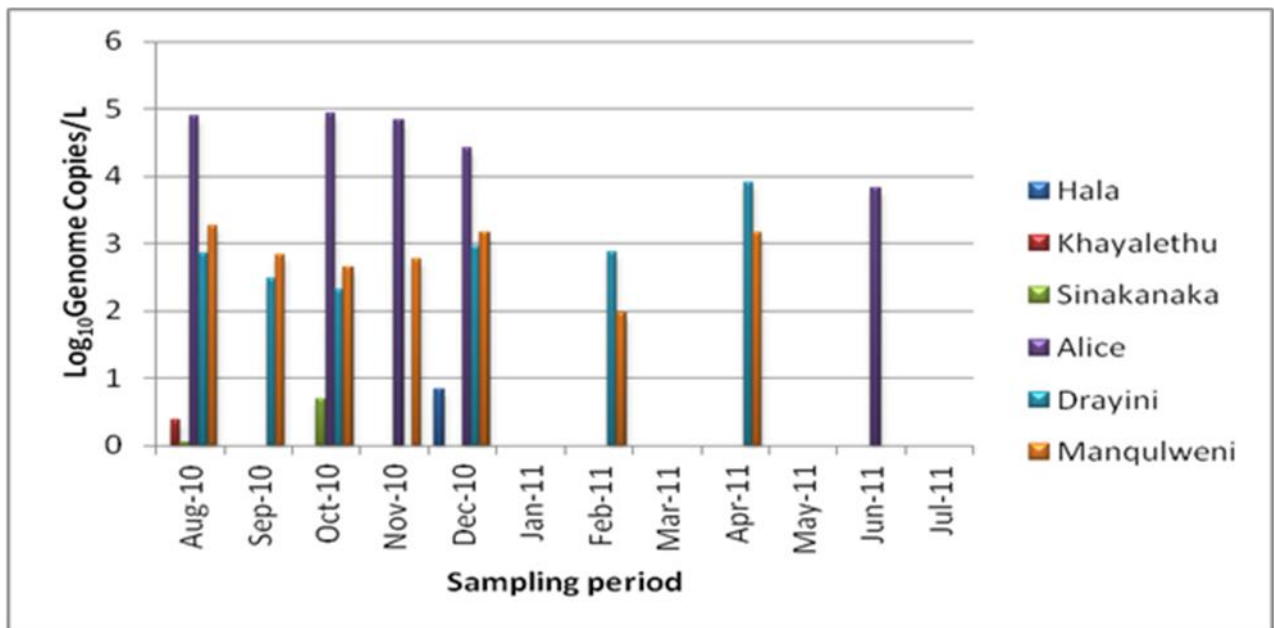
Figure 6.2: **$\log_{10}$ genome copies/ℓ of adenovirus at selected sites along Tyume River**

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 site between August 2010 and June 2011. Of the 22 samples which were positive for adenovirus by real-time PCR, 17 were positive for Species C adenovirus and of this, 6 were positive for both adenovirus serotypes 6 and 7, 1 sample was positive for each of serotypes 6 and 7, 5 were positive for adenovirus serotype 2 (Figure 6.3), while 4 were positive for adenovirus serotype 1 (Figure 6.4). None of the samples were positive for adenovirus species B or A while 4 of the samples were positive for adenovirus species F serotype 41 (Figure 6.5).
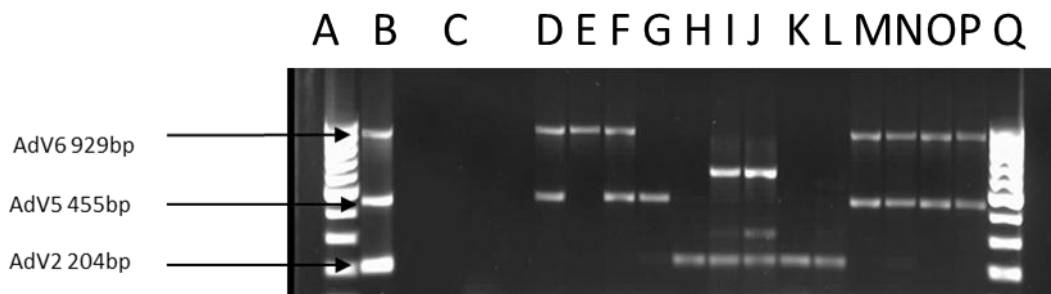
Figure 6.3: **EtBr stained agarose gel picture showing HAdV Species C serotypes 2, 5 and 6**

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).
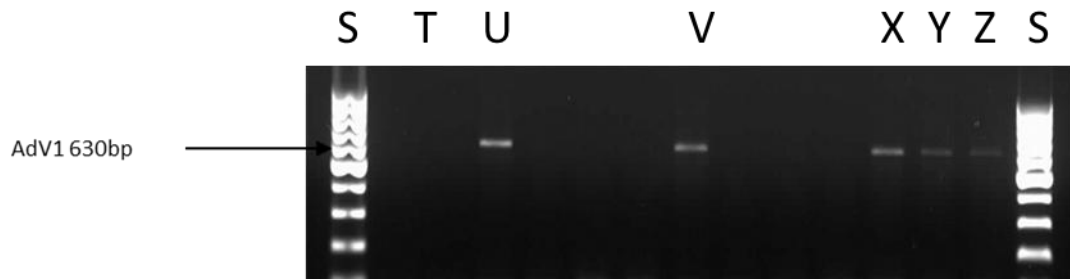
Figure 6.4: **EtBr stained agarose gel picture showing HAdV Species C serotype 1**

Lanes S = DNA ladder; Lane T = negative control; Lane U = positive control; Lane V = Manqulweni (Oct 2010); Lane X = Drayini (Dec 2010); Lane Y = Drayini (Sept 2010); Lane Z = Drayini (Feb 2011).
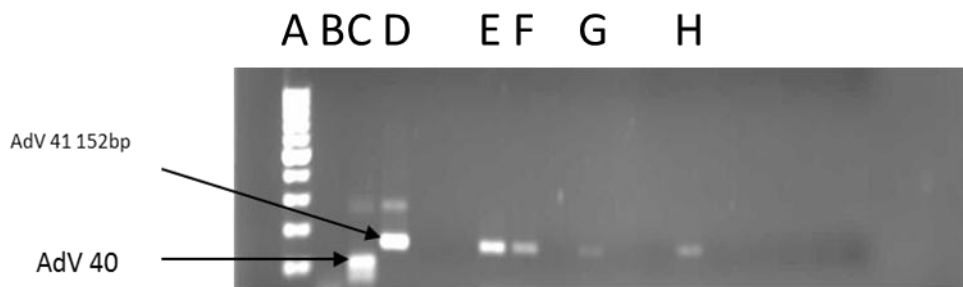
Figure 6.5: **EtBr stained agarose gel picture showing HAdV Species F serotypes 40 and 41**

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).

6.4 Discussion

The downstream stretch of Tyume River flows through areas of high population density characterised by the presence of the small town of Alice, the University of Fort Hare and Victoria Hospital whose combined population is approximately 48 000 (UFH, 2012). Compared with the downstream Tyume, the extreme upper reaches of Tyume River are sparsely inhabited with a rural settlement setting. 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. The most probable of the anthropogenic activities contributing to contamination of natural water sources with enteric pathogens could be the discharge of partially/untreated wastewater effluents from domestic and municipal sewage into the river. A study done by Rosa *et al.* (2010) on wastewater effluents found not only eleveted concentrations of enteric viruses, among them adenovirus, but also found infective virions in the final effluents. The primers used for the TaqMan real-time PCR assays in this study were specific for human adenoviruses and had previously been used to detect human adenoviruses in environmental samples (Jothikumar *et al*., 2005). Results obtained in this study seem to consolidate this fact since the detection rate was heavily skewed to the downstream of the river where human influence is greater. This is despite the fact that the upstream Tyume is exposed to other potential sources of pollution such as livestock watering which happens directly in the river channel.

The highest concentrations of adenovirus were found at the Alice sampling site which lies immediately downstream from sewage outfall points from Victoria Hospital and the northern surburbs of Alice Town which include Ntselamantsi and, Lower and Upper Gqumashe. The discharge of hospital wastewater effluents into the river could be a major source of enteric pathogens owing to the presence at the hospital, of patients suffering from a wide range of ailments, viral gastroenteritis a possibility among them. 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).

Among the downstream sampling points, adenovirus 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 human adenoviruses (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). Also, the increased detection especially at Drayini compared to Alice could be attributed to effluent discharge from the University of Fort Hare wastewater treatment plant which discharges just upstream of the Drayini sampling site. The university comprise a large number of people in a relatively small piece of land, many of them coming from different geographical regions of the african continent. There is therefore a possibility of a range of enteric pathogens (including adenoviruses) being prevalent in the sewage of such an institution.

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). Its sporadic detection may also probably have been encouraged by the temperature of the river which was generally within the range of optimal virus survival ($<23^{o}C$) throughout the year (data not shown), in agreement with previous findings Lipp *et al*. (2001). Also, there was no direct relationship between adenovirus detection in this study and rainfall events

suggesting that the pollution of the river from human sources may be sporadic throughout the year; which is independent of rain events, as has been reported elsewhere (Choi and Jiang, 2005).

6.4.1 Adenovirus characterisation

Real-time PCR positive samples for HAdV were further subjected to multiplex conventional PCR for detection of clinically important adenovirus species B, C and F and their serotypes. The HAdV serotypes 40 and 41 have long been recognised as the main etiological agents of 1-20% of acute viral gastroenteritis in children (Jothikumar *et al*., 2005 Cruz *et al*., 1990; Uhnoo *et al*, 1986; Shimizu *et al*., 2007). While Shimizu *et al*. (2007) state that 50% of all adenoviruses found in stool specimens are types 40 and 41 (species F), there were more samples positive for AdV C than F in this study. This result was unexpected but could as well served to indicate the most common AdV species in circulation in the human population in Tyume River catchment at the time. Of the AdV F positive samples, 100% were AdV 41 (Figure 6.5: lane E-H). This corroborates the findings of other researchers (Samarbaf-Zadeh *et al*., 2010, Fukuda *et al*., 2006) who also found that AdV 41 is more prevalent than AdV 40, a result other researchers attributed to a decline in AdV 40 infection and elevation of infection with AdV 41 (Grimwood *et al*., 1995; De Jong *et al*., 1993). AdV types 40 and 41 can cause mortalities as much as 50% in immunocompromised individuals (De Jong *et al*., 1993). Considering that these viruses are shed for extended periods in faeces, urine, and respiratory secretions of infected persons (Elsheik *et al*., 2012; Li *et al*., 2010), their low prevalence in this study suggests a low incidence of species F infections in the host population.

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). Type 2 adenoviruses are generally associated with pneumonia and childhood respiratory diseases (Fong et al., 2010). AdV serotypes 1, 2 and 5 are implicated in 5-10% of childhood respiratory diseases, which are however mostly self-limiting (Jiang, 2006). These viruses also cause conjunctivitis in healthy adults (Jiang, 2006). The unusual prevalence of AdV C serotypes I this study could be explained by the fact that after acute infection, AdV types 1, 2 and 5 may be shed in stool for months to years (Jiang, 2006), which probably causes the endemic spread to other susceptible groups largely through ingestion of contaminated water. 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 adenovirus detections. Since enteric viruses, of which HAdV is one, are present in the faeces of infected patients in high concentrations (Fong and Lipp, 2005; Haramoto et al., 2008), 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).

Monitoring of river water for enteric viruses could be one of the suitable approaches to understand the actual prevalence of viruses in the river catchment area, because most urban rivers receive effluents from multiple wastewater treatment plants that contain viruses shed

from all patients in the catchment area (Kitajima *et al*., 2010). In this study, adenovirus was detected at all the six sampling sites along the Tyume River howbeit in varying frequencies and titres. The results obtained seem to correlate adenovirus occurrence in river water to increased population densities in river catchments. Potential risk of infection from waterborne adenovirus infections may therefore be higher in downstream as compared to upstream stretches of the river, especially where untreated river water is used for drinking and recreational purposes. Previous studies done elsewhere also demonstrated increased prevalence of enteric viruses in downstream stretches of rivers compared to the upstream stretches (Kitajima *et al*., 2010). Areas with high population densities also seem to shed more species of adenovirus into the environment as evidenced by the detection of AdV 1, AdV 2, AdV 5, AdV 6 and AdV 41 at Alice and Drayini sampling sites which are located in areas commanding higher population densities than at any other sites along Tyume River.

6.5 Conclusions

It should be noted that this is the first report of HAdV detection in Eastern Cape environmental waters using qPCR methods to the best of our knowledge. This study and numerous other studies of its nature have demonstrated that real-time PCR is a powerful tool for rapid determination of enteric viruses in environmental samples and represents a considerable advancement in pathogen quantification in aquatic environments. The high prevalence of HAdV in Tyume River waters obtained in this study indicates an elevated public health risk in consuming or coming into contact with these waters given that a significant proportion of the rural populace in this province still rely on untreated surface water sources for their domestic water needs.

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CHAPTER SEVEN

GENERAL DISCUSSION

Rapid development in agriculture, mining, urbanisation and industrialisation has brought with it the undesirable consequences of pollution of the world's natural resources. As such, surface water contamination with hazardous waste and wastewater has become a common global phenomenon (Kumar *et al*., 2010). Such unabated pollution of surface water resources has led to a dramatic decline in both the quality of life of the world's human population and the health of aquatic ecosystems. Surveillance of water quality to ensure microbiological and chemical safety is a vital public health function the world-over (Haruna *et al*., 2005); especially since every human use of water demands that such water be of "acceptable" quality. In a study by Omoruyi *et al*. (2011) in which they assessed the environmental and demographic risk factors associated with the incidence of *Cryptosporidium* infection in the rural settlements of Alice in the Eastern Cape Province of South Africa, they found that high infection rates per their target specific groups was associated with exposure to a contaminated water supply. Their results further indicated that *Cryptosporidium* infection was highly prevalent in adult faecal specimens from the Nkonkobe Municipality, an observation that they attributed to active infection and on the basis of their finding, postulated that *Cryptosporidium* was likely to emerge as a major human pathogen in that locality owing to socioeconomic changes that favoured its transmission. Coincidentally, it is in this same Nkonkobe Municipality that Tyume River, the study site of the present study, is located.

The current methodologies for determining the quality of surface water involve assessing its physicochemical and microbiological parameters. Besides the microbiological parameters, physicochemical parameters of water can also be used to indicate faecal

pollution. For instance, Sharma *et al*. (2010) making an inference from their findings in which they assessed the bacterial indicators and physiochemical qualities of some North Indian lakes, suggested that an increase in the population of coliforms in a lake environment is directly proportional to the degree of sewage and human waste pollution, which is reflected by high biochemical oxygen demand (BOD) and chemical oxygen demand (COD) levels. These inferences are in agreement with the findings of Howard *et al*. (2004) who also suggested that total suspended solids (TSS), BOD, total nitrogen, total phosphorus, fats, and total dissolved solids (TDS) all provide information about the possible organic or faecal contamination of a water sample while indirect chemical indicators of faecal contamination include conductivity, ammonia, phosphate, COD and nitrite. In this study, as in other studies also (Kumar *et al*., 2010; Sharma *et al*., 2010), some water quality parameters like temperature, pH, total dissolved solids (TDS), turbidity and bacteriological counts showed seasonality. Major fluctuations in physicochemical parameters are a function of external factors like meteorological events and pollution. Physicochemical parameters are major drivers of biochemical reactions that occur within the water. Sudden changes in the levels of these parameters may be indicative of changing conditions in the water (Hacioglu and Dulger, 2009). For instance, pH determines the suitability of water for various purposes, including toxicity to animals and plants and, in natural waters; pH is governed by the carbonate-bicarbonate-carbon dioxide equilibrium. Slightly alkaline pH may be preferable because of its capacity to remove heavy metals as carbonate or bicarbonate precipitates (Hacioglu and Dulger, 2009). Surface water temperature is also an important property that determines the amount of dissolved oxygen (DO) water can contain and consequently its suitability for human use and aquatic ecosystem functioning. Water temperature is controlled primarily by the climate and is generally warmer in the summer and colder in the winter. Aquatic organisms depend on a narrow temperature range for optimal growth and survival.

Temperatures outside the optimal range for prolonged periods of time cause stress or even the death of aquatic organisms. Thermal pollution occurs in surface water when the temperature of the water is made unusually warmer or colder for the appropriate season. Possible causes of thermal pollution of surface water include removal of streamside vegetation that provides shading, river impoundments, hot water discharges from industrial cooling operations.

Faecal indicator bacteria are also used as water quality indicators specifying faecal contamination and thus can be used to determine the source of such contamination, its intensity and the possible mitigation measures (Hacioglu and Dulger, 2009). While the detection of faecal indicator bacteria has always been taken to indicate the possible presence of enteropathogens in water (Ahmed *et al*., 2006), in practical terms, as was also observed in this study, the correlation between FIBs and enteric viruses is usually very poor (Ahmed *et al*., 2009). This lack of correlation has been witnessed even in studies dealing with raw wastewater (Harwood *et al*., 2005). A few studies have however, demonstrated that there can be a correlation between FIBs and enteric viruses. One such example is the work done by Lipp *et al*. (2001) which found the presence of enteroviruses to be significantly related to concentrations of faecal coliform bacteria (80.0% concordance, $p = 0.045$) and enterococci (78.5% concordance, $p = 0.028$). However, whether or not such correlations exist, FIBs have not lost their value as indicators of faecal pollution and they are still used the world-over to determine the microbiological quality and safety of surface water resources.

Drinking water treatment plants (DWTPs) can become weighed down when water resources are heavily polluted. This may cause pathogenic viruses, bacteria, protozoa and helminths, which survive in the environment for long periods (Mane *et al*., 2005); to breach the drinking water treatment processes and contaminates the finished product. Diarrhea still remains one of the leading causes of morbidity and mortality throughout the world (Kosek *et al*., 2003). It is the second largest cause of premature mortality and disability (Murray and

Lopez, 1997). An estimated 4.6 million people, including 2.5 million children, die from diarrhea every year (Thapar and Sanderson, 2004). Eighty to 90% of these diarrheal cases are related to environmental conditions, in particular, contaminated water and inadequate sanitation (Agenbag and Gouws, 2004). Food and water contamination by bacterial enteropathogens are among the main causes of infectious diarrhea (WHO, 1998). Incidents of diarrhoea pose an economic burden for developing countries owing to its expensive methods of treatment and loss of working hours (Dongdem *et al*., 2009).

In South Africa, diarrheal diseases have been identified as the primary cause of death in infants that are younger than 5 years, leading to about 160-200 deaths per day (Nemarude *et al*., 2008). The situation is worsened by the fact that many people, especially in rural areas, still depend on surface and/or groundwater sources for their daily water needs (CSIR, 2012). Water from these sources is used without treatment by communities and in many cases the water sources are faecally contaminated (Momba and Notshe, 2003). Many of these water bodies are often impacted by inadequately treated effluents from municipal wastewater treatment plants as receiving water bodies (Fatoki *et al*., 2003). In some cases, even where treated water is available, inadequate treatment processes have led to insufficient removal of viruses from source waters leading to waterborne outbreaks of gastroenteritis (Lodder and de Roda Husman, 2005).

Dilapidated infrastructure and poor maintenance of wastewater treatment plants reduces their efficiency in removing viruses. Depending on the applied processes, treated sewage discharged onto surface waters may significantly enhance the virus concentrations in the environment (Lodder and de Roda Husman, 2005). Even the first documented outbreak of waterborne hepatitis A in New Delhi (India) between December 1955 and January 1956 was attributed to contamination by sewage, of Jumna River, the source of water for the drinking water treatment plant (Bosch, 1998). Surface waters may also become contaminated with

viruses during heavy rainfall which cause the overflow of raw sewage and wash off of animal manure. The deposition of animal manure into rivers is of public health concern since it may expose swimmers and bathers to zoonotic pathogens such as hepatitis E virus. As in this study, where enteric viruses were detected in surface water samples, Lodder and de Roda Husman (2005) also found noroviruses, rotaviruses and enteroviruses in each of the samples taken from two large rivers in The Netherlands. Sewage contaminated waters may contain over 100 virus species which cause a wide variety of illnesses in man (Bosch, 1998). While other viruses are found in the aqueous phase of the wastewater effluents, others are adsorbed to solids and when discharged into aquatic environments, they accumulate in the sediments where they persist longer than in the water column (Bosch, 1998). Such sediments act as reservoirs from which viruses are resuspended into the water column by natural or artificial phenomena. Apart from particle-adsorption, persistence of enteric viruses in the aquatic environment is also affected by temperature, with which an inverse relationship occurs; pH, microbial antagonism, sunlight penetration (turbidity status) and the type of virus, with DNA viruses generally persisting longer than RNA viruses (Bosch *et al*., 1991). This provides some measure of correlation between virus detection and certain physicochemical and microbiological variables. Due to their stability and persistence, enteric viruses subsequently become pollutants in environmental waters resulting in human exposure through pollution of drinking water sources and recreational waters (Okoh *et al*., 2010; Pusch *et al*., 2005) as well as foods (Pinto *et al*., 2009). As such, although inanimate surfaces can also serve as vehicles for virus infection, ingestion of sewage contaminated water or food remains the major route of infection with human enteric viruses. The broader water environment therefore poses a risk in the transmission of enteric viruses chiefly because routine monitoring for the presence of these viruses in the environment is not done and also due to the fact that enteric viruses are resistant to commonly employed disinfection methods. Though enteric viruses cannot

multiply in the environment, they can survive longer in water than most enteric bacteria and are more infectious than most other enteropathogens (Health Canada, 2010).

While PCR detection (quantitative or qualitative) of enteric viruses in surface water environments is adequate to indicate the presence of viral genomes in the water, it does not distinguish between infectious and non-infectious viral agents in these environments. Till now, only cell culture assays can be used to detect and quantitate infectious enteric viruses in water environments. However, some studies (Pinto *et al*., 2009), including this current study, have used ratios previously obtained when both real-time PCR and cell culture were concurrently used to quantitate viruses in environmental water samples to calculate the proportion of infectious viruses from real-time PCR results. De Medici *et al*. (2001) applied integrated cell-culture RT-PCR to samples that were positive by RT-PCR alone and found that of the 34.5% samples which tested positive by RT-PCR alone, as much as 12.7% were positive for the presence of infectious virus. Also, while detection of viral genomes by an integrated cell-culture RT-PCR method in environmental samples is limited to the period when the viruses are still infectious, molecular detection methods overlap the infective and non-infective but detectable periods (Arnal *et al*., 1998). Because enteric viruses have very low infectious doses in the order of tens to hundreds of virions (Okoh *et al*., 2010), PCR positive results should be treated with as much caution as cell-culture RT-PCR positive results could have been if public health is to be protected. In addition to hepatitis, more than half of the non-bacterial outbreaks of gastroenteritis, respiratory infections, conjunctivitis, meningitis, encephalitis and paralysis have been attributed to enteric virus infections (Okoh *et al*., 2010).

Rotavirus and norovirus genomes have been found to be present in concentrations of between 1×10^4 and 2×10^7 genome copies per litre of raw wastewater while previous studies have also found a correlation between the presence of enteric viruses in wastewater and

outbreak(s) of disease(s) in the community (Bucardo *et al*., 2011). Concentrations of enteric viruses have been observed to peak in sewage samples during the autumn or winter owing either to higher incidence of illness during this time of the year or better survival of enteric viruses in cold temperatures (Health Canada, 2010). Unlike other viruses whose presence and concentrations were statistically associated with effluent-dominated waters in this study, the situation was different for enterovirus, confirming the findings of Aslan *et al*. (2011) who also found that enterovirus concentrations were not linked to effluent impacted waters. In a study, La Rosa *et al*. (2010) found that the proportion of samples positive for enterovirus was the highest in sewage influents and effluents though their concentrations were the lowest compared to adenovirus and norovirus. Explaining their observations, the authors suggested that enteroviruses are excreted more consistently, even in cases of asymptomatic infection, while other enteric viruses are mostly associated with outbreaks and are therefore excreted less consistently, but at greater concentrations.

7.1 Conclusions

This study shows that anthropogenic activities are largely to blame for the pollution of surface water resources as evidenced by the poor physicochemical and microbiological qualities of water in sections of the river bordered by human settlements. High counts of faecal indicator bacteria attest to faecal contamination whose risk to public health was high lightened by the detection of enteric viruses. Results of this study, though not presenting a universal picture of the quality of surface water resources in the Eastern Cape Province, are enough to conclude that users and/or consumers of raw surface water, especially from rivers running through human settlements, risk getting infected and contracting waterborne illnesses. However, while people cannot be completely dissuaded from using raw surface

water due to the unavailability of potable water sources in some places, rural areas in particular, they can be educated on some cheap but effective water purification methods applicable in the home set-up, like sand filtration and/or boiling just to protect public health.

7.2 Recommendations

- Developing countries need to upgrade their existing sanitary facilities to cater for their growing populations, which may also go a long way in reducing microbiological contamination of surface water resources.

- Educational campaigns to improve health literacy need to be conducted to reduce the risks of contracting waterborne illnesses. Actions to protect public health may include permanently discouraging recreational activity in contaminated water, for example by fencing or signposting.

- It is also recommended that future projects of this kind incorporate identification and enumeration of protozoan parasites.

- Since faecal contamination of surface waters has been affirmed by the detection of FIBs and enteric viruses; there is need for future research to assess these surface water resources 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.

- Over and above, governments in developing countries should make every concerted effort to ensure adequate provision of clean water to their citizens in order to improve their quality of life.

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APPENDIX

		TYUME RIVER TURBIDITY (NTU): SEPTEMBER 2010- JULY 2011						
		Hala	Khayalethu	Sinakanaka	Alice	Drayini	Manqulweni	
Sep-10	a	9.47	6.43	25.5	18.7	20	36	
	b	9.13	7.49	27.9	19	9.46	35.5	
	c	9.45	7.88	26	19.1	11	36.8	
Oct-10	a	14.3	5.61	18.3	29.9	11.7	29.9	
	b	14.3	5.73	18	22	11.8	29.8	
	c	14.3	5.74	18	24.6	11.8	29.8	
Feb-11	a	13.4	6.32	19.5	22.4	11	61.4	
	b	13.7	6.19	17.7	19.9	10.9	61.4	
	c	14.2	6.34	18.1	19.6	11.4	59.8	
Mar-11	a	18.3	10.5	22.9	25.3	20.4	31.9	
	b	17.5	10.7	22.4	24.3	20.3	31.2	
	c	16.8	10.2	21.8	25.2	19.1	30.8	
Apr-11	a	44.7	45.1	146	250	453	276	
	b	44.4	44.4	142	253	444	274	
	c	43.7	43.7	140	259	434	278	
May-11	a	31.7	28.2	148	157	174	768	
	b	29.7	26.6	151	154	165	757	
	c	30.3	28.8	153	154	167	760	
Jun-11	a	15.8	12.6	33.6	36.2	43.2	35	
	b	14.2	12.6	32.8	36.7	42.9	33.1	
	c	14.3	12.7	32.4	36	42.3	33.4	
Jul-11	a	13.4	11.8	45.9	40.4	39.9	46.9	
	b	14.2	11.6	44.7	39.7	40.3	48.5	
	c	13.9	11.5	44.9	40	39.3	47.7	
		TYUME RIVER DISSOLVED OXYGEN (mg/ℓ): SEPTEMBER 2010-JULY 2011						
		Hala	Khayalethu	Sinakanaka	Alice	Drayini	Manqulweni	
Sep-10	a	8.74	8.46	7.56	7.92	7.38	7.52	
	b	8.74	8.38	7.61	7.91	7.37	7.56	
	c	8.74	8.38	7.61	7.91	7.37	7.56	
Oct-10	a	8.55	8.65	8.03	8.54	7.59	7.46	
	b	8.53	8.63	8.02	8.61	7.55	7.4	
	c	8.53	8.63	8.02	8.61	7.55	7.4	
Nov-10	a	8.64	8.41	8.21	8.59	7.94	7.56	
	b	8.6	8.43	8.23	8.64	7.94	7.54	
	c	8.6	8.43	8.23	8.59	7.96	7.54	
Dec-10	a	8.53	8.47	7.55	7.89	7.38	7.52	
	b	8.53	8.48	7.56	7.88	7.38	7.56	
	c	8.54	8.48	7.56	7.88	7.38	7.56	
Jan-11	a	8.53	8.46	7.55	7.89	7.38	7.52	

	b	8.53	8.46	7.56	7.89	7.38	7.52
	c	8.54	8.46	8.56	7.89	7.38	7.52
Feb-11	a	8.94	8.65	8.05	8.59	8.11	7.9
	b	8.97	8.77	8.01	8.64	8.11	7.9
	c	8.97	8.77	8.01	8.64	8.11	7.9
Mar-11	a	9.51	9.54	9.04	8.73	8.67	8.68
	b	9.51	9.42	9.04	8.73	8.65	8.8
	c	9.52	9.42	9.14	8.72	8.65	8.8
Apr-11	a	10.28	10.43	10.28	10.63	9.56	9.99
	b	10.97	10.44	10.44	10.66	9.56	10.02
	c	10.28	10.44	10.44	10.66	9.56	10.02
May-11	a	9.65	9.72	9.6	9.2	8.9	9.06
	b	9.72	9.48	9.63	9.18	8.9	9.12
	c	9.72	9.48	9.63	9.18	8.9	9.12
Jun-11	a	10.47	10.47	10.58	10.41	9.71	10.02
	b	10.51	10.52	10.56	10.43	9.73	10.02
	c	10.51	10.52	10.62	10.44	9.73	10.01
Jul-11	a	11.05	10.68	10.52	11.19	10.39	10.88
	b	11.08	10.68	10.52	11.19	10.37	10.88
	c	11.08	10.72	10.51	11.2	10.37	10.88
		TYUME RIVER ELECTRICAL CONDUCTIVITY (µS/cm): SEPTEMBER 2010-JULY 2011					
		Hala	Khayalethu	Sinakanaka	Alice	Drayini	Manqulweni
Sep-10	a	57	65	125	216	343	392
	b	60	57	128	213	345	390
	c	60	63	131	220	338	403
Oct-10	a	76	72	131	229	363	426
	b	76	59	127	224	355	418
	c	76	74	129	228	359	420
Nov-10	a	59	50	239	168	330	371
	b	49	47	225	223	340	376
	c	49	51	225	424	611	727
Dec-10	a	63	54	154	234	384	419
	b	78	54	148	233	383	407
	c	79	56	139	236	381	407
Jan-11	a	57	43	156	203	288	228
	b	62	52	146	188	297	223
	c	64	54	156	208	298	212
Feb-11	a	69	45	160	283	459	436
	b	50	64	125	295	460	421
	c	45	38	159	370	465	426
Mar-11	a	69	50	136	253	399	502
	b	69	41	138	253	388	508

	c	71	48	139	304	397	522	
Apr-11	a	50	42	148	208	330	313	
	b	51	55	148	200	334	313	
	c	39	45	147	200	334	312	
May-11	a	52	53	153	270	407	184	
	b	54	43	154	274	412	184	
	c	55	41	168	342	409	182	
Jun-11	a	51	52	122	226	294	256	
	b	51	46	122	226	293	256	
	c	47	53	115	525	292	246	
Jul-11	a	47	53	162	241	324	277	
	b	45	53	150	242	334	277	
	c	53	57	153	236	339	268	
		TYUME RIVER PHOSPHATE (mg/ℓ): SEPTEMBER 2010- JULY 2011						
		Hala	Khayalethu	Sinakanaka	Alice	Drayini	Manqulweni	
Sep-10	a	1.21	1.71	2.67	1	2.07	1.19	
	b	1.21	1.71	2.67	1	2.07	1.18	
	c	1.21	1.71	2.65	1	2.04	1.17	
Oct-10	a	1.81	1.24	2.77	1.14	2.18	2	
	b	1.81	1.23	2.54	1.01	2.15	2	
	c	1.81	1.22	2.43	1.11	2.16	2	
Nov-10	a	0.18	0.242	0.334	0.194	0.466	0.918	
	b	0.18	0.243	0.323	0.193	0.486	0.917	
	c	0.16	0.243	0.322	0.192	0.476	0.916	
Dec-10	a	1.164	1.132	1.174	1.173	1.134	1.219	
	b	1.164	1.133	1.173	1.174	1.132	1.217	
	c	1.163	1.134	1.172	1.176	1.131	1.216	
Jan-11	a	0.954	0.938	1.264	1.011	1.5	2.16	
	b	0.954	0.934	1.263	1.102	1.15	2.16	
	c	0.599	0.876	1.233	1.032	1.15	2.11	
Feb-11	a	0.02	0.03	0.07	0.03	0.07	0.3	
	b	0.03	0.05	0.016	0.06	0.07	0.31	
	c	0.03	0.05	0.016	0.03	0.07	0.3	
Mar-11	a	0.03	0.03	0.01	0.1	0.53	0.34	
	b	0.03	0.03	0.01	0.1	0.53	0.17	
	c	0.04	0.03	0.01	0.1	0.52	0.17	
Apr-11	a	0.1	0.16	0.25	0.35	1.29	0.83	
	b	0.11	0.16	0.25	0.34	1.33	0.81	
	c	0.13	0.08	0.24	0.32	1.33	0.75	
May-11	a	0.203	0.194	0.212	0.095	0.097	0.125	
	b	0.203	0.191	0.208	0.095	0.096	0.126	
	c	0.213	0.195	0.203	0.096	0.094	0.124	

		Hala	Khayalethu	Sinakanaka	Alice	Drayini	Manqulweni	
Jun-11	a	0.07	0.17	0.28	0.36	0.6	0.41	
	b	0.03	0.2	0.28	0.42	0.64	0.47	
	c	0.03	0.23	0.59	0.21	0.56	0.44	
Jul-11	a	0.22	0.09	0.27	0.1	1.13	0.17	
	b	0.19	0.06	0.28	0.08	1.13	0.19	
	c	0.19	0.05	0.57	0.11	1.12	0.19	
TYUME RIVER ENTEROCOCCI COUNTS (cfu/100 mℓ): SEPTEMBER 2010- JULY 2011								
		Hala	Khayalethu	Sinakanaka	Alice	Drayini	Manqulweni	
Sep-10	a	40	56	60	200	190	70	
	b	40	64	60	240	150	130	
	c	40	60	66	230	170	130	
Oct-10	a	40	35	40	190	90	80	
	b	40	45	40	210	130	88	
	c	30	50	60	200	120	90	
Nov-10	a	670	134	93	5900	3000	1500	
	b	680	122	97	6700	3000	1500	
	c	660	120	90	6700	3000	1500	
Dec-10	a	400	118	100	3500	1400	500	
	b	420	122	100	3900	1400	480	
	c	420	120	110	3900	1300	490	
Jan-11	a	470	134	87	5200	4800	1500	
	b	510	138	93	5200	4400	1700	
	c	500	140	93	5100	4400	1600	
Feb-11	a	33	43	30	1500	210	520	
	b	33	43	30	1580	310	490	
	c	30	43	30	1600	300	360	
Mar-11	a	32	40	90	4000	390	150	
	b	36	48	60	4400	400	210	
	c	40	50	110	4200	330	160	
Apr-11	a	30	40	500	4400	3300	2300	
	b	30	40	460	4800	4100	1400	
	c	30	40	460	4800	4000	1900	
May-11	a	130	260	40	3700	5200	500	
	b	138	240	46	3300	5200	500	
	c	146	240	43	3200	5300	630	
Jun-11	a	100	50	210	2200	2800	200	
	b	50	60	150	1600	3200	200	
	c	80	60	130	1400	2000	200	
Jul-11	a	30	30	40	300	30	90	
	b	30	40	40	400	80	100	
	c	30	30	40	300	80	80	

TYUME RIVER pH: SEPTEMBER 2010- JULY 2011		Hala	Khayalethu	Sinakanaka	Alice	Drayini	Manqulweni	
Sep-10	a	7.43	7.25	7.05	7.85	8.09	8.05	
	b	7.42	7.21	7.04	7.69	7.09	7.93	
	c	7.41	7.6	7.02	7.41	7.55	7.66	
Oct-10	a	8.59	7.72	7.34	7.51	8.75	8.32	
	b	8.32	7.63	7.35	7.49	8.31	8.32	
	c	8.18	7.55	7.37	7.47	8.31	8.32	
Nov-10	a	7.87	7.6	8.05	8.34	8.57	8.83	
	b	7.55	7.49	7.77	8.53	8.68	9.07	
	c	7.56	7.48	7.66	8.47	8.59	9.04	
Dec-10	a	7.19	7.76	7.66	7.82	8.04	7.92	
	b	7.25	7.69	7.64	7.82	8.04	8.03	
	c	7.25	7.69	7.64	7.82	8.04	8.03	
Jan-11	a	6.72	6.48	6.63	6.67	9.24	9.02	
	b	6.72	6.58	6.34	6.67	9.19	9.94	
	c	6.8	6.58	6.34	6.89	9.19	9.94	
Feb-11	a	9.82	9.87	8.7	8.01	9.97	9.97	
	b	10.02	9.92	8.84	7.99	9.91	9.96	
	c	10.06	9.92	8.78	7.61	9.85	9.91	
Mar-11	a	10.3	9.88	10.14	9.84	9.73	9.82	
	b	10.28	9.85	10.16	9.84	9.74	9.8	
	c	10.38	9.88	10.24	9.73	9.72	9.84	
Apr-11	a	9.86	10.7	9.5	9.7	9.81	9.87	
	b	9.73	10.71	9.58	9.71	9.8	9.85	
	c	9.68	10.57	9.67	9.62	9.79	9.83	
May-11	a	9.77	9.7	10	10.08	9.34	9.29	
	b	9.77	9.72	10.04	10.39	9.36	9.36	
	c	9.77	9.77	10.07	10.37	9.37	9.4	
Jun-11	a	10.9	10.66	11.46	11.12	9.77	10.03	
	b	10.94	10.7	11.46	11.11	9.78	10.1	
	c	11.02	10.74	11.51	11.26	9.76	10.15	
Jul-11	a	8.27	7.93	9.64	10.48	7.28	7.37	
	b	8.34	7.94	9.67	10.46	7.29	7.41	
	c	8.41	7.96	9.72	10.19	7.25	7.45	
TYUME RIVER TOTAL DISSOLVED SOLIDS (mg/ℓ): SEPTEMBER 2010-JULY 2011								
		Hala	Khayalethu	Sinakanaka	Alice	Drayini	Manqulweni	
Sep-10	a	35	34	64	106	173	197	
	b	37	32	69	110	169	201	
	c	36	29	63	106	172	195	
Oct-10	a	38	33	63	114	179	210	
	b	38	37	64	112	179	209	

	c	53	30	66	112	179	209
Nov-10	a	24	26	112	212	338	362
	b	25	23	190	111	170	188
	c	29	25	217	184	165	186
Dec-10	a	39	29	77	114	193	203
	b	39	27	74	118	192	203
	c	40	27	74	114	190	204
Jan-11	a	31	22	78	104	149	114
	b	29	26	73	94	149	111
	c	29	26	73	94	149	114
Feb-11	a	34	22	80	141	230	218
	b	35	32	65	147	230	211
	c	22	19	79	185	233	213
Mar-11	a	35	25	68	127	200	251
	b	35	20	69	127	199	254
	c	36	24	69	152	199	261
Apr-11	a	25	21	74	104	165	157
	b	19	28	74	107	167	157
	c	25	22	73	107	167	156
May-11	a	26	27	77	137	204	95
	b	27	22	77	137	206	92
	c	27	21	84	171	205	91
Jun-11	a	26	26	61	113	147	128
	b	25	23	61	113	146	128
	c	23	26	57	263	146	123
Jul-11	a	24	26	81	120	162	138
	b	23	26	72	121	167	139
	c	27	29	77	118	170	134

TYUME RIVER NITRATE (mg/ℓ): SEPTEMBER 2010- JULY 2011

		Hala	Khayalethu	Sinakanaka	Alice	Drayini	Manqulweni
Sep-10	a	3.34	3.56	3.45	3.1	3.77	4.21
	b	3.34	3.56	3.45	3.1	3.77	4.21
	c	3.34	3.56	3.45	3.1	3.77	4.21
Oct-10	a	3.11	3.71	3.88	3.93	3.14	3.61
	b	3.11	3.71	3.88	3.93	3.14	3.61
	c	3.11	3.71	3.88	3.93	3.14	3.61
Nov-10	a	1.056	1.553	1.09	1.484	1.478	1.615
	b	1.056	1.553	1.09	1.484	1.478	1.616
	c	1.056	1.545	1.1	1.485	1.479	1.616
Dec-10	a	2.77	1.909	2.587	2.081	2.235	2.817
	b	2.77	1.909	2.59	2.034	2.236	2.817
	c	2.78	1.91	2.4	2.036	2.24	2.645

Jan-11	a	2.992	2.593	3.305	3.33	3.151	3.506	
	b	2.992	2.345	3.3	3.321	3.151	3.503	
	c	2.845	2.123	3.278	3.22	3.134	3.234	
Feb-11	a	0.39	0.7	0.34	1.2	0.71	0.24	
	b	0.36	0.7	0.32	1.2	0.63	0.29	
	c	0.37	0.6	0.34	1.7	0.69	0.31	
Mar-11	a	0.25	0.93	0.44	1.24	2.5	1.8	
	b	0.14	0.92	0.49	1.24	2.6	0.65	
	c	0.15	0.92	0.5	1.26	2.5	0.6	
Apr-11	a	0.24	0.86	0.52	1.36	1.47	0.49	
	b	0.19	0.72	0.46	1.29	1.3	0.49	
	c	0.16	0.91	0.46	1.34	1.45	0.37	
May-11	a	0.7	1.2	1.1	2.1	3.7	2	
	b	0.78	1.1	1.9	2.1	3.6	2.1	
	c	0.68	1.1	1.9	2.1	3.5	2	
Jun-11	a	0.6	0.41	0.9	0.65	1.53	0.61	
	b	0.8	0.41	0.8	0.7	1.55	0.78	
	c	0.6	0.35	0.8	0.59	1.47	0.7	
Jul-11	a	1.4	1.3	1.4	1.6	2.4	3.2	
	b	1.4	1.4	0.52	1.7	2.4	3.1	
	c	1.4	1.3	1.7	1.8	2.5	3.1	

TYUME RIVER TOTAL COLIFORM COUNTS (cfu/100 mℓ): SEPTEMBER 2010- JULY 2011

		Hala	Khayalethu	Sinakanaka	Alice	Drayini	Manqulweni	
Sep-10	a	8400	12600	9800	32000	21600	12900	
	b	12200	10800	10600	32000	20000	11700	
	c	12200	10900	10700	31000	20000	12000	
Oct-10	a	8000	11900	15700	35000	23100	12600	
	b	9400	14000	17100	33000	21300	14000	
	c	9600	13000	16600	33000	22000	13000	
Nov-10	a	9600	13000	7900	29000	23600	18000	
	b	8300	11200	7700	29000	24200	18000	
	c	8500	13600	7700	28000	24500	18000	
Dec-10	a	2300	2900	4000	10500	8900	10000	
	b	1900	2700	4200	9700	8500	6400	
	c	2000	2700	4000	10200	8700	10000	
Jan-11	a	1400	9700	7000	45000	31000	26000	
	b	1380	10000	6600	43000	25800	28200	
	c	1300	9900	6900	44000	28000	27000	
Feb-11	a	990	2300	5400	30000	8800	14200	
	b	1000	2300	5600	36000	8700	14000	
	c	1220	2300	5200	33000	8600	14000	
Mar-11	a	3000	3000	5100	69000	14000	10100	

	b	2600	2900	5500	59000	13000	10500
	c	2800	3100	5400	64000	15000	10000
Apr-11	a	2200	3800	11000	63000	24000	18000
	b	2400	3900	11400	59000	16000	18400
	c	2400	4000	11300	60000	18000	18000
May-11	a	330	530	4200	26400	20000	1600
	b	280	510	3400	29200	26000	1600
	c	210	520	4700	28000	25000	1600
Jun-11	a	60	230	2100	8000	52000	3000
	b	90	200	1600	11000	51000	2700
	c	90	200	2100	13000	53000	3300
Jul-11	a	280	310	700	20700	27000	3100
	b	280	310	1000	20700	27000	3100
	c	270	290	700	20000	26000	3100

TYUME RIVER TEMPERATURE (°C): SEPTEMBER 2010-JULY 2011							
		Hala	Khayalethu	Sinakanaka	Alice	Drayini	Manqulweni
Sep-10	a	9.6	12.41	15.18	15.07	14.99	16.49
	b	9.54	12.46	15.26	15.06	14.37	16.53
	c	9.41	11.86	15.04	15.08	14.23	16.54
Oct-10	a	16.41	20.02	22.09	21.13	20.59	22.09
	b	16.38	19.95	22.11	20.87	20.01	22.32
	c	16.71	19.94	22.11	20.87	20.01	22.32
Nov-10	a	16.03	18.64	22.34	21.51	22.11	23.09
	b	16.26	18.98	22.29	21.03	22.19	23.2
	c	15.27	18.41	22.66	22.9	21.88	22.89
Dec-10	a	13.57	15.11	22.01	19.16	19.12	22.5
	b	13.87	15.11	22.02	19.16	19.12	23.2
	c	13.87	15.14	22.02	19.16	19.12	23.2
Jan-11	a	17.5	19.02	24.94	22.66	24.57	24.33
	b	17.04	19.7	24.97	22.81	24.09	24.92
	c	17.04	19.7	24.97	22.81	24.09	24.92
Feb-11	a	19.69	23.29	27.91	25.08	26.65	28.87
	b	20.12	23.16	28.12	25.21	27.32	29
	c	19.93	23.07	28.43	25.31	26.98	29.07
Mar-11	a	16.18	13.7	25.57	20.98	22.01	24.11
	b	16.52	13.97	22.5	20.98	23.07	24.19
	c	17.1	14.17	22.52	21.1	22.17	24.22
Apr-11	a	12.92	18.23	17.79	16.62	17.68	18.16
	b	13.38	18.67	17.78	16.63	17.99	18.31
	c	13.9	18.54	17.54	16.74	18.04	18.61
May-11	a	12.7	13.49	15.16	15.04	15.78	16.01

		Hala	Khayalethu	Sinakanaka	Alice	Drayini	Manqulweni	
	b	12.86	13.84	15.21	15.03	15.98	16.17	
	c	13.33	13.72	15.27	15.54	16.53	16.29	
Jun-11	a	7.57	8.5	11.61	11.25	11.85	11.83	
	b	7.7	8.59	11.54	11.36	12.16	11.96	
	c	8.29	8.59	11.56	12.1	12.66	12.56	
Jul-11	a	6.33	7.67	10.12	9.57	11.61	10.41	
	b	6.36	7.97	10.1	9.46	10.43	10.64	
	c	7.26	8.28	10.26	9.97	10.78	11.46	
TYUME RIVER BIOCHEMICAL OXYGEN DEMAND (mg/ℓ): SEPTEMBER 2010-JULY 2011								
		Hala	Khayalethu	Sinakanaka	Alice	Drayini	Manqulweni	
Sep-10	a	0.46	0.74	0.74	0.93	1.4	1.17	
	b	0.46	0.74	0.74	0.93	1.4	1.17	
	c	0.46	0.74	0.74	0.93	1.4	1.17	
Oct-10	a	1	2.7	1.9	2	2.5	1.7	
	b	1.2	2.7	1.9	2	2.5	1.7	
	c	1.2	2.7	1.9	2	2.5	1.7	
Nov-10	a	2.1	6.2	0.8	1.43	2.23	3.56	
	b	2.5	6.8	1.2	1.71	2.57	3.84	
	c	2.1	6.8	1.2	1.71	2.57	3.84	
Dec-10	a	1.18	1.13	0.96	1.64	1.66	1.82	
	b	1.18	1.13	0.96	1.64	1.66	1.82	
	c	1.18	1.13	0.96	1.64	1.66	1.82	
Jan-11	a	0.27	0.55	0.98	0.97	1.44	1.46	
	b	0.27	0.55	0.98	0.97	1.44	1.46	
	c	0.27	0.55	0.98	0.97	1.44	1.46	
Feb-11	a	0.54	0.48	0.95	1.07	1.53	1.62	
	b	0.54	0.98	0.98	1.41	1.42	2.56	
	c	0.89	0.68	0.98	1.4	1.43	2.54	
Mar-11	a	0.92	1.01	1.14	1.69	1.15	2.44	
	b	1.16	1.02	1.13	1.48	3.14	2.07	
	c	1.16	1.02	1.13	1.48	3.14	2.2	
Apr-11	a	2.1	2.13	2.11	2.68	3.41	3.4	
	b	2.6	2.05	2.31	4.52	3.96	3.48	
	c	2.6	2.05	2.13	4.5	3.9	4.39	
May-11	a	1.39	0.44	1.97	1.44	0.72	1.81	
	b	1.48	1.2	1.49	1.34	2.52	1.67	
	c	1.48	1.2	1.4	1.34	2.52	1.67	
Jun-11	a	0.86	0.92	1.12	0.99	0.13	0.89	
	b	0.92	1.02	0.98	0.96	1.19	0.83	
	c	0.92	1.02	0.98	0.97	1.1	0.83	
Jul-11	a	1.31	1.34	1.65	1.65	1.5	1.35	
	b	1.14	1.26	1.56	1.57	1.48	3.74	

		1.14	1.26	1.56	1.57	1.48	3.74	
	c							

TYUME RIVER NITRITE (mg/ℓ): SEPTEMBER 2010- JULY 2011

		Hala	Khayalethu	Sinakanaka	Alice	Drayini	Manqulweni	
Sep-10	a	2.28	2.35	2.19	2.46	2.46	2.32	
	b	2.28	2.35	2.19	2.46	2.46	2.32	
	c	2.26	2.35	2.19	2.46	2.46	2.32	
Oct-10	a	2.39	0.9	2.19	1.6	2.24	1.76	
	b	2.39	0.95	2.19	1.45	2.22	1.74	
	c	2.39	0.95	2.16	1.34	2.12	1.64	
Nov-10	a	0.528	0.127	0.449	0.217	0.466	1.042	
	b	0.528	0.127	0.443	0.211	0.432	1.042	
	c	0.532	0.122	0.443	0.213	0.454	1.034	
Dec-10	a	1.23	1.265	1.11	1.172	1.143	1.32	
	b	1.23	1.243	1.11	1.17	1.133	1.33	
	c	1.22	1.233	1.09	1.164	1.132	1.31	
Jan-11	a	0.172	0.367	3.05	3.2	2.054	0.121	
	b	0.172	0.367	3.02	3.01	2.043	0.122	
	c	0.163	0.354	3.03	2.43	2.044	0.122	
Feb-11	a	0.03	0.01	0.017	0.04	0.03	0.1	
	b	0.02	0.01	0.04	0.04	0.02	0.09	
	c	0.02	0.02	0.05	0.04	0.04	0.1	
Mar-11	a	0.031	0.01	0.04	0.09	0.09	0.05	
	b	0.03	0.03	0.03	0.09	0.1	0.06	
	c	0.03	0.01	0.04	0.09	0.09	0.06	
Apr-11	a	0.037	0.046	0.084	0.25	0.38	0.117	
	b	0.036	0.046	0.083	0.25	0.37	0.118	
	c	0.037	0.046	0.076	0.23	0.36	0.116	
May-11	a	1.19	1.21	1.25	1.26	1.22	1.41	
	b	1.19	1.22	1.24	1.28	1.22	1.42	
	c	1.19	1.21	1.21	1.16	1.23	1.4	
Jun-11	a	0.069	0.09	0.043	0.049	0.07	0.043	
	b	0.064	0.12	0.13	0.05	0.068	0.05	
	c	0.13	0.04	0.043	0.053	0.16	0.045	
Jul-11	a	0.18	0.25	0.15	0.1	0.14	0.13	
	b	0.12	0.21	0.17	0.16	0.13	0.13	
	c	0.1	0.21	0.15	0.13	0.13	0.13	

TYUME RIVER FAECAL COLIFORM COUNTS (cfu/100 mℓ): SEPTEMBER 2010- JULY 2011

		Hala	Khayalethu	Sinakanaka	Alice	Drayini	Manqulweni	
Sep-10	a	190	320	650	10300	1470	1780	
	b	210	360	590	7500	1530	1420	

	c	200	360	600	8500	1530	1400
Oct-10	a	160	280	470	4900	1300	12600
	b	200	340	550	6900	1500	14000
	c	180	320	530	6000	1500	14000
Nov-10	a	1000	910	1450	15900	7200	3000
	b	970	870	1050	17700	6900	3100
	c	970	890	1400	17000	7500	3200
Dec-10	a	220	680	460	9300	8900	10000
	b	260	640	620	7900	8500	6400
	c	260	640	600	8200	8500	7300
Jan-11	a	920	650	880	11000	7300	2000
	b	960	690	920	11200	7300	2400
	c	960	980	920	11000	7400	2000
Feb-11	a	50	300	90	2000	370	80
	b	70	200	50	2900	580	20
	c	80	400	50	3100	380	40
Mar-11	a	50	120	50	7400	490	70
	b	50	110	60	11900	360	80
	c	50	130	50	6400	420	80
Apr-11	a	140	210	440	6400	2600	380
	b	134	210	440	6400	3200	380
	c	130	210	450	6300	3000	390
May-11	a	170	140	1370	3400	16000	430
	b	140	140	1350	3600	16600	480
	c	140	180	1360	3200	16600	500
Jun-11	a	40	80	120	4200	13000	460
	b	40	80	100	4000	18000	480
	c	40	90	110	4400	13000	470
Jul-11	a	130	150	170	2000	16400	470
	b	110	140	140	2000	16600	470
	c	110	154	150	2000	16800	470

TYUME RIVER ROTAVIRUS RESULTS (genome copies/ℓ): AUGUST 2010-JULY 2011												
	Aug-10	Sep-10	Oct-10	Nov-10	Dec-10	Jan-11	Feb-11	Mar-11	Apr-11	May-11	Jun-11	Jul-11
Hala	0	0	0	0	0	0	0	0	0	0	11.46476	9.223051
Khayalethu	0	0	0	0	0	0	0	0	0	0	0	0
Sinakanaka	0	5641.521	0	0	0	0	0	0	0	0	0	0
Alice	0	0	0	0	0	0	0	0	0	0	0	0
Drayini	0	0	0	0	0	0	0	0	0	0	0	0

213

Manqu lweni	0	0	0	0	0	0	0	0	0	0	0	0

TYUME RIVER HEPATITIS A VIRUS RESULTS (genome copies/ℓ): AUGUST 2010- JULY 2011

	Jan-11	Feb-11	Mar-11	Apr-11	May-11	Jun-11	Jul-11	Aug-10	Sep-10	Oct-10	Nov-10	Dec-10
Hala	0	0	0	0	0	10321.63	0	0	0	0	14348.77	1670.119
Khayal ethu	9334.168	0	0	0	0	16389.78	2654.121	0	0	4205.566	0	0
Sinaka naka	7647.986	0	0	0	0	5890.797	0	0	0	0	0	0
Alice	0	0	0	0	0	0	0	0	0	0	0	0
Drayini	0	0	0	0	0	0	0	0	0	0	0	0
Manqu lweni	0	0	0	0	0	0	0	0	0	0	0	0

TYUME RIVER ADENOVIRUS RESULTS (genome copies/ℓ): AUGUST 2010-JULY 2011

	Aug-10	Sep-10	Oct-10	Nov-10	Dec-10	Jan-11	Feb-11	Mar-11	Apr-11	May-11	Jun-11	Jul-11
Hala	0	0	0	0	5.696879	0	0	0	0	0	0	0
Khayal ethu	3.439884	0	0	0	0	0	0	0	0	0	0	0
Sinaka naka	0	0	4.191578	0	0	0	0	0	0	0	0	0
Alice	65904.82	0	72731.74	57582.26	21974.86	0	0	0	0	0	5608.889	0
Drayini	599.7449	253.1886	173.8216	0	776.1967	0	628.2046	0	6841.264	0	0	0
Manqu lweni	1554.823	582.4478	369.6176	499.8803	1247.655	0	80.44839	0	1253.326	0	0	0