# Investigating the presence of microbial pathogens in the Umhlangane River, Durban, South Africa

By

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Submitted in fulfilment of the academic requirements for the degree of Master of Science in Microbiology

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## PREFACE

The experimental work described in this dissertation was carried out in the Discipline of Microbiology of the School of Life Sciences, College of Agriculture, Engineering and Science, University of KwaZulu-Natal (Westville campus), from March 2013 to May 2015 under the supervision of Professor Johnson Lin.

The experimental findings represent original work by the author and have not otherwise been submitted in any form for any degree or diploma to any University. Where use has been made of the work of others, it is duly acknowledged in the text.

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Prof Johnson Lin (Supervisor)

## DECLARATION – PLAGIARISM

I, **Miss Veronna Marie** declare that:

1. The research reported in this dissertation, except where otherwise indicated, is my original research.

2. This dissertation has not been submitted for any degree or examination at any other university.

3. This dissertation does not contain other scientists‟ data, pictures, graphs or other information, unless specifically acknowledged as being sourced from other scientists.

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5. This dissertation does not contain text, graphics or tables taken from the internet, unless specifically acknowledged, and the source being detailed in the dissertation and in the reference section.

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*Declaration Plagiarism 22/05/08 FHDR Approved*

## PRESENTATIONS

Part of the findings observed in this study was presented at many conferences/symposiums as follows:

1. *Poster presentation* at the College of Agriculture, Engineering and Science Post Graduate Research Day, held at the University of KwaZulu-Natal (Howard campus) on 1st November 2013.

2. *Paper (oral) presentation* at the eThekwini-University Research Symposium (EURS), held at the Southern Sun-Elangeni Hotel, Durban from the 21st – 22nd of November 2013.

3. *Poster presentation* at the National South African Society for Microbiology (SASM) Conference, held at Forever Resorts Warmbaths, Bela-Bela from the 24th – 27th of November 2013.

4. *Paper (oral) presentation* at the International Water Association (IWA) 7th International Young Water Professionals (YWP) Conference, held at Howard Civil Service International House, Taipei (Taiwan) from the 7th – 11th of December 2014.

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## ABSTRACT

The use of rivers for recreational and domestic practices makes it imperative to scrutinize the water quality circulating within the surrounding communities. The presence of potential pathogens in the Umhlangane River was monitored at five points (Phoenix industrial: P1; upstream KwaMashu wastewater/residential: P2; natural wetlands: P3; Riverhorse Valley industrial/business estate: P4; and Springfield industrial: P5) on a monthly basis from October 2013 to September 2014. Commonly measured physico-chemical parameters were determined according to standard protocols. Bacterial indicators were enumerated using the membrane filtration technique. A tangential flow filtration process was set up to remove the bacteria and to concentrate the virus populations from 25 ℓ of river water samples. Somatic and $F^+$RNA coliphages were enumerated using plaque assays. The virus-like particles (VLPs) were estimated using epifluorescence microscopy and viral morphology was observed using transmission electron microscopy (TEM). The potential infectious nature of the concentrated viruses was assessed using cytopathic effect (CPE) of tissue culture. The specific detection of some virus populations was determined by a two round nested-PCR reaction using virus-specific primer sets and confirmed by sequencing. Chemical and biological oxygen demand (COD; BOD) fluctuated at all sampling points and months with BOD ranging from 0.48 mg/ℓ (Riverhorse Valley; April 2014) to 12.4 mg/ℓ (Phoenix industrial; June 2014), respectively. The highest COD content was recorded at the Phoenix industrial site in May with 269 mg/ℓ. The total dissolved solid (TDS) content and electrical conductivity (E.C.) fluctuated throughout all sampling months and points with all measurements exceeding the Department of Water Affairs recommended limits of 0 – 100 mg/ℓ and 0 – 15 mS/m, respectively. High counts of *E. coli* (EC), total and faecal coliform (TC; FC) and *Shigella* (SHIG) were recorded at the industrial sites in Phoenix and Springfiled and upstream of the KwaMashu WWTP in Phoenix while the total heterotrophic bacteria (THB) depicted the highest and lowest counts at the Phoenix industrial natural wetland sites ranging from $14.9 \times 10^6$ cfu/100mℓ to $1.3 \times 10^6$ cfu/100mℓ, respectively. Somatic and $F^+$RNA coliphages produced its highest counts at the industrial site in Phoenix ranging from 765 pfu/mℓ and 585 pfu/mℓ in January 2014, respectively. Direct VLP counts were substantially lower (105 vlp/mℓ; April 2014) than the plaques produced by the somatic and $F^+$RNA coliphages. Morphological changes of HEK293, Vero and Hep-G2 cell lines were indicative of a positive CPE for viral concentrates. Apart from visualization of bacteriophages belonging to the *Siphoviridae*, *Myoviridae* and *Podoviridae* families, presumptive *Picornaviridae*, *Adenoviridae*,

*Herpesviridae*, *Coronaviridae*, *Reoviridae*, *Polyomaviridae* and *Orthomyxoviridae* VLPs were revealed based on size and comparisons to electron micrographs of known viruses. Adenovirus, polyomavirus, and hepatitis A and C virus-specific nested primers revealed the detection of these waterborne pathogens in the Umhlangane River. Moreover, sequence data confirmed the presence of these virus populations by comparisons made in GenBank. An increase in the amount of chemical pollutants entering the water would allow for the high COD, BOD and changing E.C. and TDS levels. Elevated THB populations at all sampling points and months indicate poor water quality. High EC, TC, FC and SHIG are indicative of possible faecal pollution, which could be attributed to faecal contamination entering the catchment. The presence of these indicators as well as the somatic and $F^{+}$RNA coliphages could be due to anthropogenic activities, changing climatic conditions and the excreta of infected and non-infected individuals entering the river. Viruses or phages in the river water samples are morphologically diverse. Phage diversity further indicates diversity in their bacterial counterparts. The presence of various VLPs revealed by TEM together with substantial CPE on human tissue cell lines and the confirmation of adenoviruses, polyomaviruses as well as hepatitis A and C viruses by molecular detection and sequencing data raise the health concerns of the river system. The present study highlights the importance of routine environmental surveillance of human enteric viruses for a better understanding of the actual burden of these viral infections on those who might be using the water directly without treatment.

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# Chapter *one*

# Literature review

## 1.1 Introduction

Due to the rise in population numbers and an expanding economy, great concern is being put on the world‟s natural resources, particularly, easily accessible and clean drinking water (Savichtcheva and Okabe, 2006). Anthropogenic activities such as increased land practices and decreased rainfall due to changing climatic conditions have also contributed to the demand of water sources that can be exploited by humans (Olaniran *et al*., 2012). Much focus is put on water quality where the major concern is contamination caused by nefarious sources that ultimately leads to the emergence of waterborne pathogens (Savichtcheva and Okabe, 2006).

Monitoring water is imperative to protect the public‟s health (Puig *et al*., 2000), social and economic status (Spinner and di Giovanni, 2001; Hamza *et al*., 2011; Wong *et al*., 2012). Pathogens present in human and animal faeces are not only confined to bacterial origin instead a variety of protozoa and viruses co-exist (Aw and Rose, 2011). Detection of these pathogens by public water systems depends on bacterial indicators such as *Escherichia coli* and total coliforms. However, it has been found that these indicators are effortlessly eradicated by water treatment processes and are poorly correlated with the presence of protozoa and viruses (Straub and Chandler, 2003; Rodríguez *et al*., 2008). Monitoring of viruses in water is of most importance since they are highly stable and are not easily eliminated by conventional water treatment processes (Lee and Jeong, 2004; Gibson and Schwab, 2011; Tong and Lu, 2011).

In spite of the importance of viruses in water, detection methods are much more difficult than that of bacterial indicators. Often the cost of equipment and specialized apparatus are the limiting factors in the direct evaluation of viral indicators in routine water quality testing (Karim *et al*., 2004; Fong and Lipp, 2005). Methods required for reproducible, rapid and cost-effective viral detection needs to be evaluated. Many key issues need to be addressed in order to understand the large viral communities present in common water environments and possibly prevent waterborne-viral diseases (Craun *et al*., 2006; Hamza *et al*., 2011). Since water scarcity and quality can be imperatively linked to socio-economic and political expansions (Fatoki *et al*., 2001), water quality management is just as important, if not more in water-related issues (Yilmaz and Harmancioglu, 2010).

## 1.2 Water availability, management and quality in South Africa

In 2012 the World Health Organization concluded that 97 out of every 100 people do not have access to portable water and 14% rely on ponds, rivers and lakes (WHO, 2012).

Furthermore, without these facilities women and children have the responsibility to carry water from their designated water sources (WHO, 2012). However, even in the presence of the necessary water supply infrastructure it has been found that sustainability of these facilities becomes a problem (Gibson *et al*., 2011). South Africa is a water-scarce, semi-arid country that relies on dams and rivers for drinking water, recreational and agricultural practices (Chigor and Okoh, 2012). Water scarcity is mainly attributed to low precipitation rates where the estimated annual rainfall (500 mm) falls below the global acceptable limit of 860 mm (Water Rhapsody, 2009). In addition, low rainfall is coupled with uneven and seasonal distribution, allowing for the South-Easterly parts of the country to receive more rain than the Western and Northern parts (Olaniran *et al*., 2012). Much focus is put on freshwater resources in an attempt to conserve and manage the already limiting sources of water to provide enough water for the countries‟ expanding population. According to Water Rhapsody (2009), 12-14 million South African citizens do not have access to safe drinking water and increasing consumption rates could lead to the lack of any available drinking water before 2040. Rural areas, particularly in developing countries such as South Africa rely on water from nearby surface and groundwater due to the lack of water supply facilities (Sibanda and Okoh, 2013).

The Integrated Water Resource Management (IWRM) programme has been used to manage water sources in South Africa. Water supplies are controlled according to social, economic and environmental factors that distribute water quantities based on detailed objectives (Karar, 2008; Yilmaz and Harmancioglu, 2010). In addition to water conservation management, water quality management is also imperative since unsafe drinking and recreational water cause an estimated 2.6% deaths in South Africa annually (Lewin *et al*., 2007).

Water quality is affected by various physical, chemical and biological factors (Taljaard and Botes, 1995). The natural regulation of these factors can make water sources appear healthy enough to be a negligible risk for humans, animals and plants alike or contaminated enough to cause waterborne diseases to any biota in close proximity (Sadeghi *et al*., 2007). Microbial and heavy metal quantities are of particular interest since the accumulation of metals can cause many neurological and muscular impairments while microorganisms can account for cholera, hepatitis and typhoid fever outbreaks. The comparison of water quality parameters to a national standard is done by the Department of Water Affairs (DWA). Acceptable standards for each water quality parameter is noted and compared upon monitoring (Neysmith and Dent, 2010). These standards are merely a guideline and do not

completely evoke the risk of possible water-related sickness. It should be noted that any risk that is currently tolerated is considered as an acceptable risk.

### 1.3 Anthropogenic sources of pollution on freshwater environments

Freshwater resources are frequently exposed to quality alterations (Zhou *et al*., 2012). Rivers in particular, undergo flow variation due to changing landscape and tidal interferences (Gregory, 2006; Mendiguchia *et al*., 2007). Anthropogenic activities such as urban, agricultural and industrial practices are amongst the major drivers of river pollution and water quality altercations (Mendiguchia *et al*., 2007; Zhou *et al*., 2012). These practices often carry large amounts of human and animal faecal matter. Of most concern is the level of pathogens excreted within the faeces into aquatic ecosystems (Spinner and di Giovanni, 2001; Jurzik *et al*., 2010; Gibson and Schwab, 2011). These pathogens can enter aquatic ecosystems through urban and agricultural runoffs, broken sewers, indecorous septic tanks and inadequately treated wastewater that cause waterborne illnesses (Aw and Rose, 2011; Gibson and Schwab, 2011).

### 1.4 Physico-chemical and heavy metal constituents affecting river water quality

Rivers are highly complex in terms of their heterogeneity at both temporal and spatial scales and this complexity can often be monitored using the physico-chemical dynamics of the river (Singh *et al*., 2010). These chemical and physical characteristics often affect water quality and readily fluctuate throughout time and seasonal changes. Drastic alterations in these parameters can cause a major decline in water quality as they have a profound impact on microbial growth or deterioration (EPA, 2006; Walton and Hunter, 2009).

Monitoring physico-chemical parameters over time allows changes in the aquatic environment to be detected. Moreover, the compilation of physico-chemical and biological data can generate information for organizations protecting and managing natural resources. This allows them to determine if the water quality is worsening or improving with human use and time. In addition to aquatic fitness, monitoring of physico-chemical constituents are important for (i) human health, (ii) some agricultural or industrial purposes and (ii) are part water quality standards and criteria (Farrell-Poe, 2000).

Commonly measured physico-chemical properties include temperature, pH, turbidity, total dissolved solids (TDS), dissolved oxygen (DO), electrical conductivity (E.C), biological oxygen demand (BOD), chemical oxygen demand (COD) and nutrients such as nitrates,

nitrites, ammonia, phosphorous and chloride content (DWAF, 1996a; Zamxaka *et al*., 2004; Bellos and Sawidis, 2005; EPA, 2006; Prego *et al*., 2008; Zhou *et al*., 2012).

Since rivers carries matter from inlands to drain into the oceans, it is imperative to monitor TDS in water quality testing. The amount of settled or particulate matter in water is referred to as total dissolved solids. Concentrations of these solids fluctuate within natural water sources and their occurrences are due to soil and rock mineral dissolution as well as organic plant decomposition. This suggests a direct relationship between TDS and varying geological conditions (DWAF, 1996a). Turbidity measures the clearness of water in corroboration with the amount of suspended material present in the water source (DWAF, 1996a; EPA, 2006). Various ecological conditions such as water or climatic changes are related to turbidity. Thus, a change in turbidity infers a change in the environment (DWAF, 1996a). Electrical conductivity is the ability to conduct a current, of which the same concept is applied to conductance in water bodies (DWAF, 1996a; EPA, 2006). A linear relationship between E.C. and TDS has been observed. Since TDS contain various organic and inorganic matter composed of salts and ions, water currents a charge through these ions (DWAF, 1996a). Since conductivity is dependent on ion concentration, this parameter can be applied for the determination of metal toxicity or pollution in anthropogenically affected waters, specifically industrial areas (Chapman, 1992; Jeena *et al*., 2012). Temperature has many effects on chemical and biological surroundings (Sang, 2012). Thus, climatic changes are important to note when monitoring the quality of water (Farnsworth and Milliman, 2003). Temperature can repress or elevate microbial growth. Lower temperatures are indicative of decreasing microbial activity (improved water quality) whereas higher temperatures elevate microbial growth by providing optimal growth conditions (Kolawole *et al*., 2011). The measurement of DO in water is important in regulating and controlling various factors in the environment (Caraco *et al*., 2000). BOD is the determination of the amount of organic matter broken down by microbial populations in water. Chemical oxygen demand (COD) is used to determine the level or organic substances in water by assessing the amount of oxygen molecules used during oxidation (Vyrides and Stuckey, 2009; Yang *et al*., 2011). These parameters can predict the levels of pollution in water resources. pH is the measurement of hydrogen ions found within a solution where readings can either be acidic, basic or neutral (Chapman, 1992; Davies, 2009). pH values greater than 7 are alkaline while values lower than 7 are acidic (DWAF, 1996a). These values are important in water quality testing because it is affected by toxic substances that could be released from industrial plants (Davies, 2009). However, it does not pose a detrimental effect on human or animal health except when it is

highly acidic or basic (DWAF, 1996a). Nutrients are found in low quantities in water. However, high amounts of nutrients (nitrogen, chloride, etc.) can be released as industrial and agricultural effluents into water. These parameters can be measured using various chemical analyses (DWAF, 1996a).

These properties are affected by societal activities that can also alter the level of pollution in water environments (Zhou *et al*., 2012). Physical and chemical properties of rivers are also affected by climate change (Li *et al*., 2008). Undistributed rainfall can alter the quantity of water flowing through a river (Chen *et al*., 2007). This occurrence coupled with the flow of water through changing landscapes alters the natural properties of water, thus affecting water quality and ultimately microbial proliferation (Chen *et al*., 2007; Zhou *et al*., 2012). According to an extensive study on river water quality via physical and chemical properties, Singh *et al*. (2010) concluded that river water quality deteriorates with excessive anthropogenic influences.

Apart from physico-chemical properties affecting water quality, the accumulation of heavy metals in aquatic environments can also affect the quality of water (Singh *et al*., 2010; Wu *et al*., 2013). Trace elements including lead, cadmium, aluminium, zinc, iron, cobalt, manganese, copper and nickel are found everywhere in nature (Mohamed and Osman, 1998; Jarup and Akesson, 2009; Ma *et al*., 2009). Metal ions are usually transported in urban and industrial wastes at very high levels (Vesk and Allaway, 1997; Korai *et al*., 2008). They can accumulate within the environment and in the organs of aquatic organisms. The accumulation of these metals in organisms such as fish can directly cause harm to people who consume them. Additionally, individuals who ingest the water can become very sick from the toxic poisoning (Korai *et al*., 2008).

## 1.5 Waterborne pathogens that have plagued mankind

Waterborne sickness has affected people even before the turn of the century. Infectious agents that could not be viewed to the naked eye was severely feared and mostly classified as “animalcules” capable of infecting hundreds of individuals at a time. Drinking water treatments were dated to 4000 BC according to scriptures obtained from Sankrit and Greek archaeological digs (Barry and Hughes, 2008). Hippocrates often regarded as the father of medicine, was among one of the first scientists to acknowledge water purification and subsequently invented a bag filter unit (Barry and Hughes, 2008).

Despite these minor beliefs and developments regarding the purity of water, no actual acknowledged threat came to light until sometime during the 18$^{th}$ century when physician

John Snow uncovered a cholera pandemic in London (Ashbolt, 2004). He did not trust that the cholera outbreak was due to the miasma-theory of transmission instead was spread through faecally-contaminated water. His scepticism which led him to interview local residents eventually revealed a pattern between the infected individuals and residential well that locals used to collect water for everyday purposes. This was one of the main events linking pathogens to waterborne infections (Smith, 2002).

Since then numerous waterborne illnesses mostly associated with protozoan and particularly bacterial organisms have been noted due to their ubiquitous nature (Das, 2009). To date, exemplary amounts of sickness (mostly diarrhoeal) are caused by bacteria whereas protozoan infections are somewhat rare but still present nevertheless. As depicted in Table 1.1, they cause a variety of sudden onset symptoms as well as chronic effects.

**Table 1.1** Microbial-induced diseases and their respective symptoms identified over the years (Das, 2009).

| Name of Disease | Causative agent | Symptoms/ chronic effects |
|---|---|---|
| **Cholera** (Bacterium) | *Vibrio cholerae* | Diarrhoea, occasional cramps and vomiting |
| **Typhoid Fever** (Bacterium) | *Salmonella typhimurium* | Fever, nausea, diarrhoea, headache, constipation, delirium and nose bleeds |
| **Campylobacteriosis** (Bacterium) | *Campylobacter jejuni* | Diarrhoea, abdominal discomfort and fever |
| **Salmonellosis** (Bacterium) | *Salmonella* spp. | Diarrhoea and fever |
| **Shigellosis** (Bacterium) | *Shigella* spp. | Fever, seizures, diarrhoea, renal failure and coma |
| **Hemolytic Uremic Syndrome** (Bacterium) | *Escherichia coli* O157:H7 | Diarrhoea, infarction, kidney failure and stomach discomfort |
| **Legionnaire's Disease** (Bacterium) | *Legionella* spp. (*pneumophila*) | Fever, CNS dysfunction, pulmonary failure and pneumonia |
| **Cryptosporidiosis** (Protozoan) | *Cryptosporidium parvum* | Abdominal pain and diarrhoea |
| **Giardiasis** (Protozoan) | *Giardia lamblia* | Leak flux, diarrhoea and pain in the abdomen |

## 1.6 Microbial indicators of faecal pollution

A number of methods can be applied to detect the presence of faecal pollution in water. These may include the detection of organic compounds (coprostanol), anaerobic bacteria (*Bacteroides* spp. and *Clostridium* spp.) and bacteriophages (F-specific and somatic

coliphages) (Savichtcheva and Okabe, 2006). Currently, the preferred method for the detection of faecal pollution in water involves microbial assessment using indicator microorganisms. The detection of indicator bacteria was initially designed for drinking water assessment and relied on the absence or presence of these bacteria within definite groups of water (Griffin *et al*., 2001). Indicator microorganisms are normal microflora found in human and animal gastrointestinal tracts. They are excreted in faeces and are easy to count, have survival rates similar to pathogens of concern and are considered non-pathogenic however; their presence could indicate the presence of pathogenic microorganisms (Griffin *et al*., 2001; Scott *et al*., 2002). At present, the most widely used indicators of water quality include *Escherichia coli* (*E. coli*), total and faecal coliforms, as well as intestinal *Enterococci* (Scott *et al*., 2002; Savichtcheva and Okabe, 2006; Jurzik *et al*., 2010). The convenience of using microbial indicators is to circumvent the detection of every pathogen that could be present within the water samples (Scott *et al*., 2002).

### 1.6.1 *Escherichia coli*

In the 1890s *E. coli* was suggested as the universal indicator for drinking water testing after being studied by Theobald Smith. At the time proper assays to detect this pathogen had not been developed, requiring continuous sub-culturing techniques to identify the bacterium. With time a number of tests were developed and it was found that *E. coli* was by far the most superior candidate for water quality testing since it exhibited higher thermotolerant growth patterns than other lactose-fermenting, Gram-negative, enteric bacteria (Edberg *et al*., 2000).

The large intestine is the natural habitat of *E. coli* and studies have shown that, with some exceptions that the persistence of this bacterium outside of the gut is not sound. The existence of *E. coli* in water, food and inanimate objects generally indicates faecal pollution that may be the cause of poor hygiene or sanitation during food practices and storage conditions. Its presence in a given sample specifies a heightened threat of other pathogenic microorganisms such as *Salmonella* spp. and viruses making it an ideal indicator (Odonkor and Ampofo, 2013). *E. coli* is the most widespread (Scott *et al*., 2002) and specific faecal indicator bacteria (Odonkor and Ampofo, 2013) used in water quality monitoring today. It displays ideal characteristics including their usually non-pathogenic effect on humans and its presence at high concentrations (Scott *et al*., 2002).

In spite of all the advantages this bacterium offers, recent establishments in water quality monitoring indicate that it can reproduce in the soil of subtropical and tropical environments (Scott *et al*., 2002; Figueras and Borrego, 2010). Furthermore, *E. coli* has

depicted a short lifespan in aquatic, temperate environments (Figueras and Borrego, 2010). Studies have shown that high concentrations and genera of *E. coli* were found in numerous animals as depicted in Table 1.2 (Edberg *et al*., 2000). Since *E. coli* is found in warm-blooded animals, their presence does not only indicate human faecal contamination and can therefore present a drawback for its use in detecting specifically human faecal pollution.

**Table 1.2** Percentage of coliform genera found in animal and human faecal matter (adapted from Edberg *et al*., 2000).

| | Sample size (*n*) | *E. coli* (%) | *Klebsiella* spp. (%) | *Enterobacter*/ *Citrobacter* (%) |
|---|---|---|---|---|
| **Pig** | 15 | 83.5 | 6.8 | 9.7 |
| **Chicken** | 11 | 90 | 1 | 9 |
| **Sheep** | 10 | 97 | - | 3 |
| **Horse** | 3 | 100 | - | - |
| **Cow** | 15 | 99.9 | - | 0.1 |
| **Dog** | 7 | 91 | - | - |
| **Goat** | 8 | 92 | 8 | - |
| **Cat** | 7 | 100 | - | - |
| **Human** | 26 | 96.8 | 1.5 | 1.7 |
| **Average** | | 94.5 | 1.9 | 1.7 |

**-** Not detected

### 1.6.2 Coliforms (total and faecal) and faecal streptococci

Gram-negative, (lactose production with gas) non-spore forming bacilli (production of red-metallic colonies on m-Endo agar) that are aerobic or facultative anaerobic are referred to as coliforms (Rompré *et al.*, 2002). In the early 1900s methods for coliform detection was modified to reduce the experimental temperature in an attempt to enumerate all members of the *Enterobacteriaceae* family with the ability to produce acid and gas upon lactose fermentation. This became known as the total coliform (TC) group which included *E. coli* and other non-intestinal bacteria (Edberg *et al*., 2000). Members of this family include *Klebsiella*, *Escherichia* and *Enterobacter* and genera of *Leclercia*, *Citrobacter* and *Kluyvera* (Figueras and Borrego, 2010). However, total coliforms have been found to replicate in natural and public water systems making them unreliable indicators (Tallon *et al*., 2005). Additionally, correlations between the number of pathogenic microbes and coliforms are not stable (Figueras and Borrego, 2010).

Faecal coliforms (FC) are distinguished from total coliforms by their ability to grow higher temperatures (44.5°C) (Griffin *et al*., 2001; Figueras and Borrego, 2010). Survival capabilities of FCs were found to be similar to those of pathogenic microorganisms however their correlation to viruses and protozoa are inadequate (Figueras and Borrego, 2010). Total and faecal coliforms have been used as water quality indicators for decades however, studies have revealed their resistance, ecology and pervasiveness to stress factors differ from pathogens they are a substitution for. The realization of these paramount differences limits their use as sole indicators of faecal pollution in water sources (Scott *et al*., 2002). Research has also shown that coliforms can be transported to other water sources via groundwater sources (Griffin *et al*., 2001).

Faecal streptococci (FS) are Gram-positive cocci with a Lancefield group D antigen and are catalase-negative (Tallon *et al*., 2005). These bacteria are beneficial indicators of faecal pollution because their presence in the intestinal tract of warm-blooded animals is constant, they show a strong relationship to pathogenic microbes, they have a much higher survival expectancy than coliforms and they cannot reproduce in water (Figueras and Borrego, 2010).

One of the most beneficial outcomes of enumerating both faecal coliforms and faecal streptococci is the calculation of the faecal coliform/faecal streptococcus ratio (FC/FS ratio). This ratio can be used to differentiate between human and animal faecal matter to aid in microbial source tracking. A ratio of >4 is indicative of human faecal pollution while a ratio of ≤0.7 is animal contamination. The method does not require extensive training of personnel and yields rapid results. However, it can be unreliable due to changing detection methods, variation in faecal streptococci survival rates and sensitivity to water treatment processes (Scott *et al*., 2002).

### 1.6.3 *Salmonella* and *Shigella* species

The continuous pollution of faecal waste into water environments is contributing to the emergence of pathogenic microorganisms such as *Salmonella* (SAL) species (Morinigo *et al*., 1990). *Salmonella* spp. is constantly prevalent in environmental water as they are defecated by animals and humans alike. Various runoffs from sewage plants, storm water from urban areas and agricultural wastes are sources of these pathogens (Arvanitidou *et al*., 2005). Research suggests that its capability to persist in the natural environment may be dependent on the type of strain and the level of pollution.

*Shigella* (SHIG) species are non-motile, Gram-negative, non-lactose fermenting, facultative anaerobes. These bacteria are pathogenic to humans and are transmitted through body contact with infected individuals and via contaminated water and food (Theron *et al*., 2000; Kinge and Mbewe, 2010). There are 4 species belonging to the genus which include *S. boydii, S. dysentriae, S. sonnei and S. flexneri* (Theron *et al*., 2000). *Shigella* spp. cause shigellosis or bacillary dysentery in immuno-compromised individuals, elderly and children. In 2009, this disease caused illness in South Africa in over 1812 children under the age of 5 that were both non-invasive and invasive shigellosis (Kinge and Mbewe, 2010). The sudden outbreak showed that these pathogens are becoming much more prevalent in the surrounding environments. *Shigella* spp. is largely seen in the faeces of infected persons and should therefore not be present in high numbers in natural water environments (Ozmert *et al*., 2011).

### 1.6.4 *Vibrio* species

*Vibrio* (VIB) species are facultative anaerobic, Gram-negative, curved or straight shaped bacteria (Cabral, 2010). The genus contains over 60 species most of which originate in marine environments (Igbinosa and Okoh, 2009). Sodium concentration and temperature affects the level of species distribution of *Vibrio* species. Species that have a low sodium requirement are generally found in freshwater environments such as rivers (Cabral, 2010). Apart from the well-known *Vibrio cholerae*, other species such as *V. mimicus, V. parahaemolyticus* and *V. vulnificus* are capable of causing disease in humans (Igbinosa and Okoh, 2009). Since a large proportion of the *Vibrio cholerae* population occur in the viable but non-cultivable state in water environments only approximately 1% of these bacteria are detectable in water (du Preez *et al*., 2010). *Vibrio* species can indicate faecal contamination in water from many domestic and farm animals (Keshav *et al*., 2010).

### 1.6.5 Total heterotrophic bacteria

Total heterotrophic bacteria (THB) are usually detected to indicate the general quality of a particular water source (Edberg and Smith, 1989; DWAF, 1996a). Total heterotrophs are enumerated after water treatment processes to evaluate the efficiency of the process. They cannot be used as indictors of faecal pollution or as a representation of the total bacterial community present in water sample (DWAF, 1996a). Representation of THB allow for the evaluation of a spectrum of omnipresent bacteria that are aerobic or facultative anaerobic. Heterotrophic counts are dependent on the type of media used to enumerate the organisms

(Edberg and Smith, 1989). Water pollution allows fluctuating nutrient conditions which can allow for the increased growth or sharp decline in microorganisms (DWAF, 1996a).

### 1.6.6 Bacteriophages as indicators of faecal contamination

Bacteriophages are gaining acceptance as faecal pollution indicators in water environments over the years due to the fact that bacterial indicators can reproduce within the natural environments (Donaldson *et al*., 2002; Hot *et al*., 2003) and are not easily detectable when faecal contamination is low (Stewart *et al*., 2008). Numerous studies have shown that bacterial indicators are effortlessly eradicated by water treatment processes and are poorly correlated with the presence of protozoa and viruses (Straub *et al*., 2003; Rodríguez *et al*., 2008). Many of the general requirements of a water quality indicator are met by bacteriophages. Bacterial viruses share similar characteristics to human viruses such as shape, surface chemical properties, size, isoelectric points, morphology and internal chemistry. It is also improbable for phages to replicate within the environment due to the lack of a suitable host and other surrounding factors. Furthermore, bacteriophages are safer in terms of human, animal and plant health and are a cost-effective and an easier way to detect the presence of viruses than the viruses itself (Leclerc *et al*., 2000).

#### 1.6.6.1 Somatic and F-specific RNA bacteriophages

Coliphages are bacteriophages infecting *E. coli* and other coliform bacteria therefore, their presence is indicative of their bacterial counterparts. Like many coliform bacteria, coliphages are excreted within the faeces of humans and warm-blooded animals. They can therefore be applied as indicators of faecal pollution and enteric viruses. Coliphages are divided into the somatic and male F-specific coliphages (DWAF, 1996a).

Somatic coliphages are largely used as indicators due to the inexpensive, less labour intensive and quick methodology and results. A relationship between the existence of somatic coliphage and faecal pollution has been identified in many aquatic environments (Burbano-Rosero *et al*., 2011). Somatic bacteriophages attach to numerous sites in the cell walls of *E. coli* (Donnison and Ross, 1994). These coliphages belong to 4 families: (i) *Siphoviridae* – non-contractile, long tails; (ii) *Myoviridae* – contractile, long tails; (iii) *Podoviridae* – contractile, short tails and (iv) *Microviridae* – no tail (Grabow, 2001). *Siphoviridae*, *Myoviridae* and *Podoviridae* have double-stranded DNA genomes while phages belonging to *Microviridae* have single-stranded DNA (Burbano-Rosero *et al*., 2011). Literature has reported a relationship between the level of infectious enteroviruses or their genomes and

somatic coliphages in treated wastewater samples (Hot *et al*., 2003). Plaque counts of this bacteriophage from human and animal faeces fluctuate from less than 10 plaque-forming units per gram (pfu/g) to $10^8$ pfu/g. However, counts in human faecal matter and freshwater sources do not generally exceed $10^3$ pfu/g. These phages can be detected in high numbers often due to the collection of sewage. Suspended solids, temperature and sunlight are some of the factors that can inactivate somatic coliphages in water (DWAF, 1996a). Somatic coliphages have also been shown to exceed $F^+$RNA phages in freshwater and wastewater sources by a factor of 5 and cytopathogenic human viruses by approximately a factor of 500. Thus, these phages can be applied as useful indicators of faecal pollution and enteric viruses in water as well as valuable surrogates of enteric viruses (Grabow, 2001).

F-specific RNA coliphages belonging to the *Leviviridae* family within the genera *Allolevivirus* and *Levivirus* are single-stranded RNA bacteriophages (Friedman *et al*., 2009) which are recognized as model systems for the observation of viral behaviour such as the general adsorption and replication process of viruses within the host cells (Grabow, 2001). These bacteriophages are used as indicators of water sanitation in various water sources, assessing fresh produce in terms of microbial safety, monitoring shellfish habitats and evaluating wastewater treatment plants. Male-specific F-RNA coliphages can also be used to evaluate the emergence of waterborne pathogens and as a viral indicator of faecal waste (Friedman *et al*., 2011). Unlike somatic coliphages, F-RNA phages attach via the fimbriae (sex pili) coded by the F-plasmid (Grabow, 2001; Dryden *et al*., 2006; Rodríguez *et al*., 2012). Bacteriophages that infect through pili are not necessarily constrained to specific hosts or related species since the plasmid can be conferred to a wide range of Gram-negative bacteria (Lucena *et al*., 2004). Successful transfer and expression of these plasmids have been observed in *Shigella* species, *Proteus* and *Salmonella typhimurium* (Cole *et al*., 2003; Sinton *et al*., 1996). Due to explicit growth conditions required to produce these pili such as temperatures above 30°C, F-RNA coliphages are unlikely to replicate within the environment (Grabow, 2001).

$F^+$RNA phages have been found to be quite useful in microbial source tracking. Microbial source tracking is defined as the discrimination between human and animal faecal matter from point and non-point source contamination (Schaper *et al*., 2002; Scott *et al*., 2002; Sundram *et al*., 2002). $F^+$ bacteriophages ($F^+$ total) consist of $F^+$DNA and $F^+$RNA phages. F-DNA phages contain circular, single-stranded DNA and are rod-shaped, filamentous, non-enveloped bacteriophages belonging to the *Inoviridae* family (Vinje *et al*., 2004; Lucena *et al*., 2004). These phages show greater resistance to sunlight inactivation and

exist in much higher numbers than $F^+$RNA phages. However, since the amount of information regarding their role in the natural environment and use as microbial source trackers is lacking their application is limited (Sinton *et al*., 1996; Vinje *et al*., 2004).

$F^+$RNA phages are much more useful as faecal source trackers since these bacteriophages are classified into serogroups that can be used to distinguish between animal and human faecal contamination. Serogroups II and III are predominately excreted within human faeces while groups I and IV and predominately found in animal faecal matter (Vinje *et al*., 2004; Dryden *et al*., 2006; Savichtcheva and Okabe, 2006; Sundram *et al*., 2006; Ogorzaly *et al*., 2009).

Research indicated that male-specific $F^+$RNA bacteriophages greatly outnumber cytopathogenic viruses by a factor of 100 in natural and wastewater sources (Burge *et al*., 1981; Grabow, 2001). In a study conducted by Yanko *et al*. (1999) on the evaluation of secondary and tertiary treatment efficacy at a wastewater treatment plant, $F^+$RNA phages showed greater resistance to chlorination than enteric viruses. Resistance to chlorine treatment by these phages have been documented (Havelaar and Nieuwstad, 1985; Chesler and Jacangelo, 1993). Thus, $F^+$RNA phages as an indicator of process efficacy are questionable.

#### 1.6.6.2 Bacteriophages active against *Bacteroides fragilis*

*Bacteroides fragilis* is a Gram-negative obligate anaerobe and the most prevalent bacterial member of the human microflora (Ferreira *et al*., 2008). Other members of the *Bacteroides* genus are also found within the human gastrointestinal tract (Grabow, 2001). *Bacteroides* species have many disadvantages as faecal indicators because they do not produce spores and are rapidly inactivated by exposure to exogenous oxygen sources (Grabow, 2001). Recent developments in molecular technology have allowed for the rapid detection of *Bacteroides* species. Since these bacteria are host-specific, faecal contamination can be discriminated by identifying the bacterial species (Savichtcheva and Okabe, 2006).

*Bacteroides fragilis* strain HSP40 was initially present in 10% of human faecal samples but was not detected in animal stool samples by Tartera and Jofre (1987). This led to the use of bacteriophages infecting *B. fragilis* HSP40 as a faecal pollution indicator. Additional strains were found over the years that phenotypically resembled HSP40 and other *Bacteroides* strains (Scott *et al*., 2002). Since these bacteria cannot reproduce in the environment, are found in large numbers in faeces and have a stringent host-specificity the detection of *B. fragilis* phages were gaining interest as faecal indicators. Bacteriophage

ATCC 700786-B1 infecting *B. fragilis* RYC2056 is now considered an International Standardization Organization (ISO) standard reference phage. However, this strain is still susceptible to animal faecal matter (Hawkins *et al*., 2008). Furthermore, *B. fragilis* phage detection by their hosts possesses great geographical variation and some phages cannot distinguish between human and animal faecal contamination (Payan *et al*., 2005). Thus, phage detection using strain HSP40 produces high numbers in South Africa and the Mediterranean regions but not in Northern Europe. In contrast, RYC2056 detects comparable phage numbers in many geographical locations but cannot discriminate between faecal sources (Payan *et al*., 2005). Additionally, the ability to recover these bacteriophages is difficult for water bodies that have low faecal contamination requiring the need for methodological improvement (Savichtcheva and Okabe, 2006).

### 1.7 Current methodology used to detect bacterial and phage water quality indicators

The introduction of indicator microorganisms as faecal indicators has led to the development and optimization of numerous methods to evaluate their presence in aquatic environments. Methods to evaluate the presence of bacterial indicators in water quality analysis have been standardized to develop established laboratory testing (Green *et al*., 1999; Edberg *et al*., 2000; Rompré *et al*., 2001; Tallon *et al*., 2005). Three methods have been most commonly used in water quality monitoring: (i) standard plate counts; (ii) membrane filtration technique and (iii) multiple tube fermentation technique.

The standard plate counts are a simple way of monitoring a broad range of microorganisms in a water sample. This method therefore provides a way to evaluate the efficiency of disinfection and treatment processes. Once the samples have been inoculated onto the plates it is incubated at 35°C for 2 days to allow the possible growth of faecal bacteria. Samples are usually represented as the number of enumerated colonies per millilitre (DWAF, 1996a).

The use of membranes as a way to trap bacteria was evaluated in 1943 by Mueller in Germany in combination with Endo broth for the examination of coliforms in portable water systems. In the 1950s the membrane filtration technique was being applied as a water quality monitoring technique as opposed to methods that required the production of acid and gas formation (Ashbolt *et al*., 2001). Membrane filtration relies on trapping the bacteria on a membrane filter with a 0.45 µm pore size, incubating the filter on selective media and enumerating the colonies present on the filter (Rompré *et al*., 2002). This method has been used for many years and is the standard method for evaluating coliforms in water samples

(APHA *et al*., 1998). Various types of selective media were developed for different types of indicator microorganisms such as m-Endo (total coliforms), m-FC (faecal coliforms) and recently developed chromocult (*E. coli*) for filtration analyses (Rompré *et al*., 2002). Despite the easy and fast yielding results produced by this method, membrane filtration cannot detect stressed or injured bacteria, assays are difficult when the sample is highly turbid, non-coliform bacteria can grow on many of the selective media (APHA *et al*., 1998) and the procedure can become tedious when large samples have to be processed (Rompré *et al*., 2002).

Multiple tube fermentation relies on the formation of acid and gas in tubes incubated for 48 hours at 35°C that was previously inoculated with dilutions of the water sample. This positive result is a presumptive test which then further requires a confirmation by the production of gas in brilliant green lactose bile broth within 48 hours at 35°C (Rompré *et al*., 2002). Once the confirmed test has been done the positive tubes are then used to isolate the organisms onto various types of selective media using the streak plate technique (DWAF, 1996a). Detection of coliform bacteria using multiple tube fermentation is easy to conduct and is an advantageous method of use when the samples are turbid. However, accurate evaluation of coliforms is inhibited by the growth of non-coliform bacteria during the presumptive test and inhibition of the desired microorganisms because of components within the media used. Additionally, this method is time consuming and lacks accuracy in quantitative and qualitative terms (Rompré *et al*., 2002).

The colilert test is based on the ability of coliform bacteria to produce the enzyme β-galactosidase. This enzyme hydrolyses o-nitrophenyl-β-D- galactopyranoside (ONPG) to yellow nitrophenol. The colilert test is performed by adding the sample to a bottle or MPN tube containing only specific substrates or powder comprising salts to serve as the carbon source. After a 24 hour incubation period, the samples positive for coliforms will turn yellow (Rompré *et al*., 2002). More recently, molecular methods (polymerase chain reaction; PCR) and immunological methods (enzyme-linked immunosorbent assay; ELISA or immunofluorescence assay; IFA) are some of the methods used to detect coliforms in water environments (Rompré *et al*., 2002).

Bacteriophages can be detected by many techniques most of which are continuously undergoing modification and optimization for precise results. The main reason for this is discrepancies in the data which is mostly due to the host bacteria required to enumerate the phages. Nonetheless, international standards have been developed for the detection of phages in water samples such as the International Organisation for Standardisation (Ashbolt *et al*.,

2001). Currently the method of choice for all bacteriophage enumeration is the double-overlay plaque assays (Green *et al*., 1999). Diluted water samples are mixed with molten agar and host bacteria before being poured over a bottom layer of agar. The plates are then allowed to solidify and incubated (Grabow, 2001). Bacteriophage assays carried out in this manner is fast and easy to perform however certain disadvantages occur with the types of phage being enumerated. Male F-specific bacteriophage detection has been found to show difficulty in terms of the host strain. *Salmonella typhimurium* WG49 contains the plasmid for F-pili production that was initially transferred from an *E. coli* K-12 strain. Studies have shown that *S. typhimurium* can suddenly lose the plasmid leading to a loss in the pili production and hence phage detection. Therefore reliable hosts for phage enumeration can be a problem (Mooijman *et al*., 2002). Some phages like those of *B. fragilis* are time consuming, require complex media and are expensive to evaluate. Additionally, many types of antibiotics are needed to prevent the growth of unwanted microbes during the test. Furthermore, concentration methods are generally required to accurately evaluate the presence of the bacteriophages from water which could be expensive and time consuming (Grabow, 2001).

### 1.8 Viral presence in aquatic (marine and freshwater) ecosystems

In nature all organisms are susceptible to predation by larger organisms. This can be seen in a number of environments. An example would be that larger fish are known to devour smaller fish in aquatic ecosystems. Similarly this can be seen on a microbial scale microzooplankton prey on unicellular eukaryotes and bacteria (Strom, 2002). Some organisms can take advantage of their prey from the inside such as viruses (DeBruyn *et al*., 2004). Virological studies in the environmental and medical fields are invaluable as their roles in the environment help to maintain diversity on a microscopic level. Furthermore, understanding of the natural order in how microbial communities regulate chemical and nutrient cycles to maintain a balance (release of nutrinets, controlling the level of bacterial abundance and regulating species diversity) is imperative reiterating that the importance of viral studies, particularly in water environments cannot be overstated (Hutchinson, 1961; DeBruyn *et al*., 2004).

Viruses are the most abundant biological entities in aquatic environments particularly, the oceans where viral abundance has been estimated to exceed about $10^{30}$ viruses (Danovaro *et al*., 2008). Pelagic marine environmental virology has led to expansive research in viral studies in other habitats such as freshwater (lakes, rivers and groundwater), soils and marine and freshwater sediments (Middelboe *et al*., 2008). Due to their abundance it has been

hypothesized that viruses are major players in microbial food webs. Viruses contribute to the control of organic and nutrient cycling, moulding microbial assortment and shaping the dynamic structure of microbial food webs (Ram *et al*., 2011). However, biological and genetic diversity of aquatic viruses remains largely unknown (Suttle, 2007) due to practical difficulties and the lack of an adequate environmental gene bank (Colombet *et al*., 2007).

By far, the most apparent role of viruses in microbial regulation would be its role in host population mortality. This allows viruses to control microbial diversity in a hypothesis known as "killing the winner". The Ktw hypothesis is based on negative frequency-dependant selection as bacterial fitness is indirectly related to its frequency in the community (Koskella and Meaden, 2013). The general logic of "killing the winner" relies on the fact that infective viruses introduced by migration or mutation provide density dependent regulation of dominant competitive bacteria while allowing less dominant bacterial populations to prevail (Thingstad and Lignell, 1997; Brockhurst *et al*., 2006). Preferentially attacking the dominant species provides a self-regulating negative feedback so that the dominant species are down-regulated by the predators while the populations of the rests can recover by granting them a predatory refuge (Vallina *et al*., 2014). This hypothesis is based on the Lotka-Volterra-type equations and has been applied in many laboratory experiments (Shapiro and Kushmaro, 2011). Usually viral-induced mortality causes a separate response by their hosts called antagonistic coevolution. During this process the viral hosts constantly evolve immunity to viral susceptibility while the virus continues to evolve to counteract this immunity. The investigation of phage-host coevolution has been conducted on wastewater treatment plant microbes (Shapiro and Kushmaro, 2011).

While many bacterial viruses exist in freshwater and marine environments, a number of eukaryotic viruses such as those that infect humans, plants or aquatic animals are present in variable amounts. Viral outbreaks have been linked to drinking water, groundwater (Maunula *et al*., 2005) and river water (Bosch, 1998). A study conducted by Scarcella *et al*. (2009) revealed that a viral gastrointestinal outbreak was linked to municipal drinking water in Italy where out of 399 probable cases, 30 were confirmed to be caused by astrovirus, enterovirus, norovirus and rotavirus over a one month period. This further reiterates the fact that viruses can resist drinking water purification procedures.

## 1.9 Human enteric viruses in water

The world"s increasing population and societal expansions have led to the accumulation of enteric pathogens into surface waters (Mocé-Llivina *et al*., 2005). Enteric viruses of human

origin have been found in drinking and recreational water (Lambertini *et al*., 2010). Studies based on drinking water quality have shown that at least 37 different human viruses have been isolated from drinking water sources around the globe (Tsai *et al*., 1994). Viral concentrations in surface waters are usually low due to the absence of an available host and constant dilution and degradation effects, however, they are still able to cause disease with the ingestion of low numbers (Haramoto *et al*., 2005; Verheyen *et al*., 2009). Enteric viruses are highly resistant to pH changes, heat and disinfectants which allows them to persist and retain their infectious nature for longer periods of time in water (Steyer *et al*., 2011). Therefore, eradication of enteric viruses through conventional water treatment processes (e.g. chlorination) have proved insufficient as their presence has been detected in treated water (Lee and Jeong, 2004; Gibson *et al*., 2011).

Human and animal faecal matter contains over 150 types of enteric viruses (Fong and Lipp, 2005). These viruses are excreted at elevated concentrations from the stools of infected individuals usually ranging from $10^5 – 10^{13}$ particles per gram of stool (Vieira *et al*., 2012). These viruses can be transported through estuaries, rivers, sea water, groundwater, drinking water, aerosols emitted from sewage treatment plants or untreated wastewater flowing into a water source (Lee *et al*., 2004). Enteric viruses are transmitted through the faecal–oral route, consumption of contaminated food or water or by direct person-to-person contact (Haramoto *et al*., 2012).

## 1.10 Waterborne enteric viruses of a public health concern

The diversity of viruses colonizing the human gastrointestinal tract is profound. However from a health perspective the most important include hepatitis A and E viruses (HAV; HEV), noroviruses (NoVs), adenoviruses (AdVs), astroviruses (AstVs), enteroviruses (EVs) and rotaviruses (RVs) (Bosch, 1998; Okoh *et al*., 2010; Steyer *et al*., 2011). Additionally, it has been found that other novel viruses are beginning to emerge from the faeces of humans such as the human parechovirus, SARS coronavirus and zoonotic *orthomyxoviridae* (Wyn-Jones *et al*., 2011). These viruses have been associated with waterborne gastroenteritis, conjunctivitis, respiratory tract infections, hepatitis, paralysis, meningitis and encephalitis (Okoh *et al*., 2010) as well as chronic diseases such as insulin-dependent diabetes (Hot *et al*., 2003; Fong and Lipp, 2005). These infections are common in immunocompromised individuals in undeveloped and developed parts of the world (Okoh *et al*., 2010).

### 1.10.1 Enteroviruses

Enteroviruses belong to the family *Picornaviridae* which consist of single-stranded RNA, non-enveloped viruses with an icosahedral capsid (La Rosa *et al*., 2010). Four structural proteins (VP1 – VP4) and 7 non-structural proteins involved in mutation and replication are encoded by these viruses (Okoh *et al*., 2010). They include echoviruses, coxsackieviruses (A and B) and polioviruses (Abbaszadegan *et al*., 1999; La Rosa *et al*., 2010). According to numerous molecular techniques, 80 serotypes have been identified within the genera and are classified into 4 species (A – D). Phylogenetic studies revealed that 23 coxsackie A viruses, 6 coxsackie B viruses, 31 echoviruses and 3 poliovirus serotypes were found within these groups (Okoh *et al*., 2010).

Most enteroviral infections are asymptomatic and cause mild respiratory tract infections (Okoh *et al*., 2010). However, enteroviruses can also cause many illnesses such as myocarditis, poliomyelitis, neonatal enteroviral disease, gastroenteritis and aseptic meningitis (Mocé-Llivina *et al*., 2005). Enteroviral transmission is through the faecal-oral route thus, water used for recreational or other societal purposes can be a source of enteroviruses (Wyn-Jones and Sellwood, 2001). The prevalence of these viruses in water is much more persistent during early winter and summer which could be due to the increased exposure of people to recreational activities (Wyn-Jones and Sellwood, 2001; Fong and Lipp, 2005).

Enteroviruses have been found in rivers, marine water, groundwater, drinking water and treated sewage under fluctuating environmental conditions (Fong and Lipp, 2005). These viruses are stable at pH 3-10 and are sensitive to UV radiation and chlorine treatment (Wyn-Jones and Sellwood, 2001). Studies have indicated that EVs are easy to detect with inexpensive cultivable methods (Mocé-Llivina *et al*., 2005). They display a cytopathic effect (CPE) by resulting in the obliteration of tissue cells during cell culture experiments (Okoh *et al*., 2010). However, tissue culture experiments is time consuming, lacks sensitivity if low concentrations of the virus is present and does not allow the detection of all serotypes. Advanced molecular detection methods such as reverse transcription PCR can bypass culture based methods. Primers for the 5′ non-coding region (5′ NCR) or the VP1/VP2 capsid protein-coding regions are used to detect these viruses in water and from infected individuals. Moreover, antigen capture-PCR, restriction enzyme analysis and sequencing can be used to differentiate between EV genotypes (Ehlers *et al*., 2005).

### 1.10.2 Noroviruses

Noroviruses (previously known as small round or Norwalk-like viruses) are single-stranded, positive sense polyadenylated RNA viruses and are members of the *Caliciviridae* family (Lodder and de Roda Husman, 2005; La Rosa *et al*., 2010; Kishida *et al*., 2012). These viruses show large genetic variation due to unprompted mutations during its replication cycles which is the main reason for the spurt of different genotypes. Noroviruses are subdivided into five genogroups including GGI, GGII, GGIII, GGIV and GGV each consisting of various genotypes represented by specific prototype viruses. Genogroups GI, GII and GIV infect humans with GII responsible for epidemic acute gastroenteritis in all age groups (La Rosa *et al*., 2010; Kishida *et al*., 2012).

Since noroviruses are shed in the faeces of infected persons in fluctuating numbers and may be present within the environment at low concentrations (Karim *et al*., 2004) waterborne NoV outbreaks are well known (Lodder and de Roda Husman, 2005; Hewitt *et al*., 2007; Kishida *et al*., 2012; Mans *et al*., 2013). These viruses have a strong stability rate in the environment and a substantially low infectious dose (Mans *et al*., 2013). Human voluntary experiments revealed that 6-10 polymerase chain reaction-detectable units were the minimum dose required to cause infection (Karim *et al*., 2004). The spread of NoVs through surface water and sewage systems are common however, norovirus illness caused by exposure to recreational water is less heard off and may be founded on circumstantial and epidemiological information (Wyn-Jones and Sellwood, 2001).

### 1.10.3 Adenoviruses

Adenoviruses were first isolated in the 1950s from military recruits who were suffering from epidemic respiratory disease (Fong and Lipp, 2005). Adenoviruses are non-enveloped, double-stranded DNA viruses belonging to the *Mastadenovirus* genera and the *Adenoviridae* family. They comprise 51 serotypes which are grouped into 6 (A – F) species (La Rosa *et al*., 2010; Tong and Lu, 2011; Kishida *et al*., 2012). These viruses have an icosahedral capsid with fibre-like protrusions from the 12 vertices and are approximately 90 nm in size (Kishida *et al*., 2012).

Adenoviral populations are able to replicate within the host respiratory and intestinal epithelial cells (Okoh *et al*., 2010) and are shed in faeces (Kishida *et al*., 2012). On a global scale, AdVs are most prevalent than other enteric viruses in sewage and therefore, aquatic environments (Haramoto *et al*., 2010). In addition, human AdVs cause a variety of clinical implications in virtually every visceral system in humans and are associated with outbreaks

caused by exposure to recreational water (Vieira *et al*., 2012). Adenovirus serogroups 40 and 41 have been found to have a central role in adenoviral gastroenteritis in children and is the second leading cause of this illness next to rotaviruses (Chapron *et al*., 2000). However, their role in waterborne disease is questionable since these organisms cause asymptomatic infections amongst adults due to the development of immunity to this virus and endemic illness cases (Jiang, 2006; Kishida *et al*., 2012).

Human adenoviruses are remarkably resistant to disinfection and purification processes such as chlorination or UV treatment. Human AdV serotype 40 was only inactivated after 1 – 3 hours and 57 minutes using 1 mg $\ell^{-1}$ free chlorine. Damage caused to the viral DNA by UV treatment may be repaired by the host cell‟s DNA-repair mechanisms using both the AdV DNA strands as templates for replication. These characteristics have placed adenoviruses on the United States Environmental Protection Agency (USEPA) candidate contaminant list that dictates for priority on rapid methodological development for AdV detection in water quality testing (van Heerden *et al*., 2005).

Adenoviruses can be propagated on various cell lines for infectivity tests. However viral replication varies according to the serotype being propagated. Serotypes 40 and 41 grow extremely slow and may not produce a visible cytopathic effect on most cell lines. Therefore, detection limits using tissue culture may be restricted (Jiang *et al*., 2009). To overcome this, molecular methods such as PCR and nested-PCR have been applied (van Heerden *et al*., 2005).

### 1.10.4 Rotaviruses

Rotaviruses are double-stranded RNA viruses of 70 nm in dimension with a non-enveloped icosahedral capsid (Okoh *et al*., 2010; Wong *et al*., 2012). They belong to the family *Reoviridae* and are comprised of 7 species (A – G) with groups A to C infecting humans. Rotaviruses encode 6 viral proteins to make the capsid (VP1 – VP4, VP6 and VP7) and 5 non-structural proteins (NSP1 – NSP5) (Okoh *et al*., 2010). The outer capsid is mainly composed of VP4 (protease-sensitive protein, P) and VP7 (glycoprotein, G) which classifies rotaviruses into G and P serotypes (van Zyl *et al*., 2006; Okoh *et al*., 2010).

These viruses are primarily responsible for viral gastroenteritis in children and infants (Kittigul *et al*., 2005) in both developed and developing countries (Chizhikov *et al*., 2002). Globally, rotavirus infections account for approximately 2.4 million hospitalisations (Siqueira *et al*., 2013) and around 611 000 deaths annually with most of the estimated deaths found to occur in Africa (Chigor and Okoh, 2012). Group A rotaviruses are responsible for majority of

human gastrointestinal infections in developing countries. In spite of the fatal diarrhoeal cases reported in children, studies indicate that RV infections in older persons may induce much milder enteric symptoms or be asymptomatic which may be due to cumulative cross-protective immunity as a consequence of recurring infections (Sibanda and Okoh, 2013).

Studies indicate two recurrent patterns observed by rotaviruses during fluctuating climatic conditions in South Africa. The first indicated that rotavirus infectious occurred throughout the year and secondly their numbers increased during the dry and cold conditions. This concludes that the presence of persistent rotaviruses in environmental water and individuals are affected by seasonal changes (Steel and Glass, 2011). However, patterns between rainfall, humidity and rainfall have produced inconsistent data. A number of developing countries are located in tropical regions where rotavirus transmission has shown to lack seasonality and remain active throughout the year (Siqueira *et al*., 2013).

### 1.10.5 Astroviruses

Astroviruses are naked positive-sense, single-stranded RNA viruses with a dimension between 28 and 30 nm (Chapron *et al*., 2000). The origin of their namesake was due to the distinctive stellate design on the virus surface (Pinto *et al*., 1996). Eight serotypes (AstV-1 – AstV-8) have been recognized. Astroviral infections occur primarily in children around the age of 10 and in the elderly however, their infection rates are mild compared to infections caused by rotaviruses (Chapron *et al*., 2000; Nadan *et al*., 2003). Despite the water-associated outbreaks of astrovirus infections, these organisms can be transmitted through the consumption of shellfish compromised with faecal pollution (Pinto *et al*., 1996; Chapron *et al*., 2000). A study conducted by Marx *et al*. (1998) in South Africa detected the presence of astroviral populations in 70% of all environmental samples tested. Similarities between feline and porcine astroviruses suggest possible zoonoses involving cats, pigs and humans however; no interspecies transmission has been observed (Nadan *et al*., 2003).

### 1.10.6 Hepatitis A and E viruses

In primordial Roman, Greek and Chinese eras “jaundice” was most likely viral hepatitis. Infectious hepatitis was coined in 1912 as an etiological agent of jaundice. Hepatitis A is caused by the hepatitis A virus (HAV) – one of 5 viruses, each of which come from a different family and whose main site of replication is the liver. Early epidemiological research grouped hepatitis into serum and infectious forms based on the manner of transmission (Nainan *et al*., 2006). Studies concluded that Hepatitis A is mainly transmitted

through the faecal-oral route (Sánchez *et al*., 2002; Nainan *et al*., 2006; Redwan and Abdullah, 2012; Sibanda and Okoh, 2013).

Hepatitis A viruses (HAVs) have a diameter of 27-32 nm, possess single-stranded RNA and belong to the genus *Hepatovirus* in the *Picornaviridae* family (Pina *et al*., 2001; Chigor and Okoh, 2012). This virus consists of a coat comprising approximately 60 copies of VP1, VP2 and VP3 proteins (Feigelstock *et al*., 2005) and also depicts genetic and antigenic conservation however; many sub-genotypes and genotypes have been identified (Nainan *et al*., 2006). Six genotypes of HAV have been identified, 3 of which are of animal origin (IV – VI) and 3 from human (I – III) (Redwan and Abdullah, 2012).

Hepatitis A viruses are excreted in the faeces of infected of individuals causing symptomatic and asymptomatic infections and under favourable conditions HAV may persist in the environment for months (Sibanda and Okoh, 2013). This virus is responsible for hyperendemic hepatitis A infection in South Africa (Taylor *et al*., 2001; Chigor and Okoh, 2012). Consumption of contaminated water and food, contact with infected individuals and exposure to faecally polluted recreational water may be the possible routes of infection (Nainan *et al*., 2006; Sibanda and Okoh, 2013).

Hepatitis A viruses have 1 antigenic serotype with a single infection conferring permanent immunity. This limited variability confines the use of serological methods to discriminate between different virus isolates (Pina *et al*., 2001; Sánchez *et al*., 2002). Furthermore, the mode of transmission may be difficult to track due to the long incubation period of HAV as the relationship between the consumption of contaminated water and illness may be obscured (de Serres *et al*., 1999). Wild-type HAVs are challenging to grow *in vitro* (Williams-Woods *et al*., 2011) but accumulates mutations during tissue culture adaptation. However, studies have shown that these viruses can be propagated on primate cell lines (Feigelstock *et al*., 2005).

Hepatitis E viruses (HEVs) are non-enveloped, positive-sense, single-stranded RNA viruses and are the etiological agents of hepatitis E with hepatocytes being its primary target site of infection (Vasickova *et al*., 2007). They are classified within the *Hepeviridae* family and the *Hepevirus* genus (Martin-Latil *et al*., 2012) and are 27-30 nm in size (Vasickova *et al*., 2007). HEVs comprise 4 genotypes (GI – GIV), all infecting humans with the exception of GIII and GIV which may also infect animals. In spite of the faecally contaminated water-related transmission of hepatitis E viral infections, it has known to spread through direct contact with infected animals, meat and shellfish (Martin-Latil *et al*., 2012). Inadequate drinking water treatment processes and unsanitary conditions have been associated with

major outbreaks of HEVs in developing nations (Vasickova *et al*., 2007). Infection caused by both hepatitis A and E viruses exhibit analogous symptoms such as nausea, fatigue, fever, vomiting, discoloured urine, jaundice, elevated levels of liver enzymes and abdominal pain (Martin-Latil *et al*., 2012).

### 1.11 Can human enteric viruses be applied as indicators of faecal pollution?

Bacterial indicators provide several rewards as in their use as faecal indicators. They are consistently present in the faeces of warm-blooded animals and are economical to detect. However, due to their presence in the environment and variable association with other pathogens, predominantly enteric viruses, their use is questionable at best. The application of human enteric viruses as indicators of faecal pollution may be much more advantageous for water quality risk assessments for virus communities and in microbial source tracking (Hewitt *et al*., 2013).

Adenoviruses have been proposed as indicators for viruses due to their ability to withstand environmental extremes such as fluctuations in temperature, pH, chlorine concentrations, UV irradiation and sewage treatment processes (Calgua *et al*., 2008), does not show seasonal variation (Faccin-Galhardi *et al*., 2013) and is found in all geographical locations (Bofill-Mas *et al*., 2013). In addition, they have no quantitative relationship with coliforms and are easily detected (Faccin-Galhardi *et al*., 2013). These viruses are present in high concentration in sewage as their numbers are consistent with *E. coli* and may outnumber intestinal *Enterococci* (Calgua *et al*., 2008). Moreover, adenoviruses have been used as indicators of viruses in shellfish (Myrmel *et al*., 2004) and have shown a relationship with hepatitis A viruses isolated from muscles and seawater (Serracca *et al*., 2010).

Myrmel *et al*. (2004) proposed the use of human circoviruses (huCV) as alternative indicators. These viruses contain DNA genomes, are contained within faeces and are present in most of the world‟s population. Analyses of TT virus (TTV) and TTV-like miniviruses (TLMV) were evaluated using PCR-specific primers from 113 muscle samples. The human circoviruses were detected in 8% (TLMV in 5 samples and TTV in 6 samples) and did not show any seasonal distinctions. Since circoviruses are non-envelope they may be considered highly stable however, their low detection rate present in the study indicate they may not be consistently stable in sewage, it was shed in low titres or it was poorly recovered from the shellfish (Myrmel *et al*., 2004).

Another less utilized virus that can be applied as an indicator is the pepper mild motile virus (PMMoV). This virus belongs to the *Tobamovirus* genus that contains single-stranded

RNA genomes and infects bell, hot and ornamental peppers (Rosario *et al*., 2009). The presence of PMMoV in faecally-polluted water, human faeces and raw sewage is high (Wong *et al*., 2012) ranging from $10^6$ to $10^7$ viruses per gram. The non-enveloped virus is very stable and can retain their infectious nature for plants after excretion from the intestinal tract of humans. This virus may be much more abundant in healthy persons than viruses that cause disease since it is dependent on dietary lifestyles (Rosario *et al*., 2009). Detection of PPMoV RNA can be done using RT-PCR, ELISA and electron microscopy (Lin and Ganesh, 2013).

Polymaviruses are icosahedral viruses that contain circular double-stranded DNA infecting many vertebrates (Bofill-Mas *et al*., 2013). Human polyomaviruses (huPyV) belong to the *Polyomaviridae* family (Hewitt *et al*., 2013) and are characterized into KIV, MCV, BKV, WUV and JCV members (Lin and Ganesh, 2013). The BK and JC members have been found in both faeces and urine while the other members were found exclusively in faeces (Hewitt *et al*., 2013). Human polyomaviruses are infrequently pathogenic and have been applied as microbial source trackers since they are stringent to humans (Harwood *et al*., 2013).

Other viruses that have been considered as indicators include Torque teno viruses, picobirnaviruses (Rosario *et al*., 2009) and noroviruses due to their high prevalence in sewage (Hewitt *et al*., 2013). Picobirnaviruses (PBV) have bi-segmented double-stranded RNA that belongs to the *Picobirnaviridae* family. The virus can be detected by standard molecular techniques like RT-PCR or by polyacrylamide gel electrophoresis (Lin and Ganesh, 2013). Torque teno viruses belong to the *Circoviridae* family and *Anellovirus* genera. They possess single-stranded DNA genetic material and are transmitted by the faecal-oral route. Torque teno viruses and picobirnaviruses are consistently present in raw sewage in certain geographical regions. The major problem with using viruses that infect humans or animals is that their presence is dependent on the shedding of faeces by infected individuals (Rosario *et al*., 2009).

## 1.12 Concentration methods for environmental water viruses

Monitoring viral populations from variant ecological niches usually involves the concentration of virus particles and direct enumeration. These techniques are the core methodological basis of all viral studies and are constantly modified to better the efficiency, yield and reproducibility of field and laboratory experiments (Wommack *et al*., 2010).

Virus concentration is of particular importance since viruses present in natural water sources generally exist in low numbers thus imposing the need to concentrate the water

samples prior to analysis (Karim *et al*., 2004; Verheyen *et al*., 2009). Methods involve concentrating large volumes (20 – 50 litres up to 100 – 1500 litres) of water to be assessed. During selection methods for virus concentration, the density of the virus, type of water and the possible presence of interfering components should be evaluated to optimize viral recovery and increase the probability of detecting multiple viral types within the water (Karim *et al*., 2004). Moreover, these methods should allow for efficient and fast viral recovery rates that are compatible to analytical detection methods such as cell culture and PCR (Pei *et al*., 2012; Prata *et al*., 2012), be universal to all enteric viruses, easy to use and applicable to routine water quality analyses (Skraber *et al*., 2009). A number of methods have been applied for viral recovery from water samples including flocculation, ultracentrifugation, ultrafiltration and adsorption-elution methods (Prata *et al*., 2012).

### 1.12.1 Adsorption-elution methods

Adsorption-elution methods are based on the principle that viruses have electrostatic charges that are predominantly near neutral or negative (Grabow, 2001). These methods have been used extensively to concentrate viruses (Haramoto *et al*., 2012) that involve either electronegative or electropositive filters (Skraber *et al*., 2009). Water samples are filtered through membrane filters that capture the virus particles by the electrostatic charge. After adsorption the viruses are eluted using a buffer with an equal to or greater than pH 9.5. The efficiency of adsorption-elution methods are affected by the physical and chemical properties of the filters and the viral particles which in turn fluctuate with pH. Additionally, the amount and type of salts and ionic strength of the medium also has an effect on the efficiency of recovery (Grabow, 2001; Skraber *et al*., 2009). The most challenging aspect of viral recovery is the ability to retain as many viruses as possible without viral loss due to the concentration method. Research has shown that enteroviruses can retain their infectivity at pH 3.5 and pH 9 however; this may not be the case for other viruses such as rotaviruses where infectivity is compromised below pH 5 (Wyn-Jones and Sellwood, 2001).

Adsorption-elution methods can incorporate different types of membranes or matrices such as electropositive membranes, electronegative membranes, glass powder, glass wool, electronegative cartridges and electropositive cartridges (Hamza *et al*., 2011). Positively charged membranes are considered to be one of the most beneficial methods of choice as it has been successfully applied to groundwater, lake water, tap water, river water, marine water and secondary treated water (Katayama *et al*., 2002). However, adsorption-elution methods are 2 step processes that can often become cumbersome and sometimes hinder the

simultaneous processing of different types of water samples requiring concentration (Bofill-Mas *et al*., 2013). Additionally, adjusting the pH of the samples can be time consuming and monotonous that warrants further field evaluation (Wong *et al*., 2012).

### 1.12.2 Organic and chemical flocculation

Flocculation is one of the most recurrently used methods to concentrate viruses from aquatic environments (Prata *et al*., 2012). This procedure involves the adsorption of viruses to flocculants such as aluminium hydroxide followed by disintegration of the flocks by shaking and recovery by centrifugation (Grabow, 2001). One of the most desirable qualities of flocculation is its simple one-step process requirements. Calgua *et al*. (2008) reported a 50% recovery of human adenoviruses from 10 ℓ of seawater by real-time PCR using skim milk flocculants. This procedure has proved to be a cost-effective and easily manipulated protocol for routine water quality testing of large samples showing adequate recovery rates from other laboratories that have evaluated seawater (Calgua *et al*., 2013). Chemical flocculants such as aluminium, iron and polyelectrolytes have been used to concentrate viruses from wastewater with a greater than 99% removal. Ferric chloride as the chemical flocculent was shown to be inexpensive, efficient and non-toxic in flocculation, filtration and re-suspension (FFR) procedures (John *et al*., 2011). However, sample manipulation may be required to tweak fluctuating factors that may affect the rate of recovery including pH and viral isoelectric points (Prata *et al*., 2012).

### 1.12.3 Centrifugation

Centrifugation can be used to purify, analyse and concentrate biological samples based on the size of cells or macromolecules (Lawrence and Steward, 2010). Ultracentrifugation is described as a catch-all method since it can concentrate all viruses in a sample (Wyn-Jones and Sellwood, 2001; Wong *et al*., 2012). Ultracentrifugation provides appropriate gravitational forces that can be used to efficiently sediment virus particles from the samples being tested (Lawrence and Steward, 2010). This method allows for several advantages including minimal manipulation, no elution step, samples can be processed at neutral pH and the pelleted virus particles can be used for a variety of molecular techniques (Prata *et al*., 2012). Furthermore, differential ultracentrifugation can be used to collect specific types of viruses allowing the purification of different viruses for investigative purposes (Wyn-Jones and Sellwood, 2001). Ultracentrifugation can be used as a direct method for the concentration of viruses (Prata *et al*., 2012) or in secondary concentration procedures that require a

prominent viral pellet for visualizing viral structures under electron microscopy (Colombet *et al*., 2007).

### 1.12.4 Ultrafiltration: Tangential flow filtration

A common method used to concentrate viruses from large water samples is ultrafiltration techniques (Wickramasinghe *et al*., 2005). This is a size-dependent process that applies membranes with a specific molecular weight cut-off (Grabow, 2001; Wickramasinghe *et al*., 2005; Pei *et al*., 2012). Most frequently the final product is the concentrated feed suspension or retentate. The advantage of using this method is that a variety of viruses, protozoa and bacteria can be recovered simultaneously with no need for a pre-conditioning step (Wickramasinghe *et al*., 2005). In spite of these advantages, the use of ultrafiltration has resulted in filter clogging, ultimately reducing the rates of recovery. For this reason a tangential (TFF) - or cross-flow filtration (CFF) process has been applied (Hensgen *et al*., 2010; Wommack *et al*., 2010; Rhodes *et al*., 2011). As depicted in Figure 1.1 TFF employs the principle that the water sample flows parallel to the filter surface. Hydrodynamic pressure forces particles smaller that the membrane pore size to filter through while the larger particles are retained to flow into the retentate chamber. Water flowing parallel to the membrane is the retentate while water collected at the bottom of the filter is the permeate (Wommack *et al*., 2010). The retentate is re-circulated through the system reducing the concentrate until a desired amount is attained (Thurber *et al*., 2009).

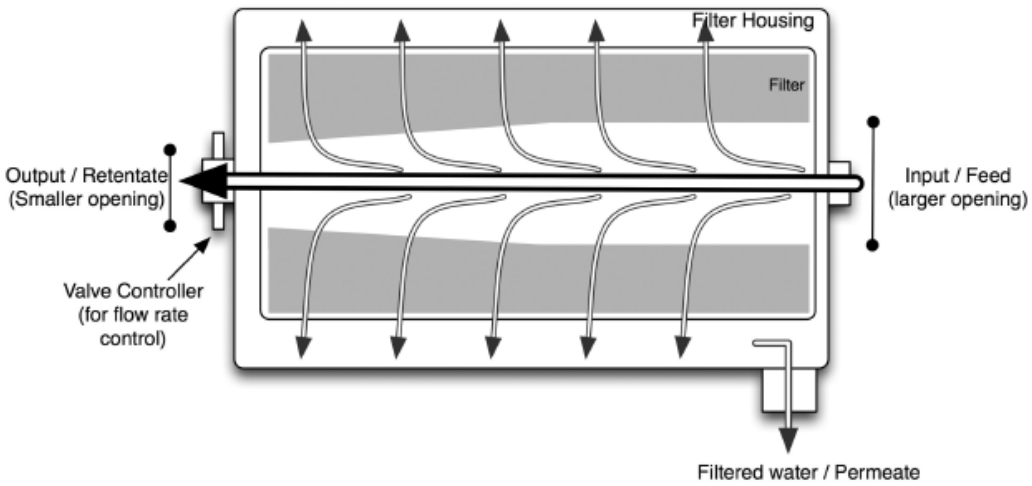


**Figure 1.1** Schematic diagram representing the direction flow of the water samples within a tangential flow filtration cartridge (TFF) (Wommack *et al*., 2010).

Flow rates and speed of the pressure applied can be controlled and a process optimization is usually required for adequate viral recovery (Wommack *et al*., 2010). The experimental pressure should not exceed 10 p.s.i as greater pressures can negatively affect the viruses within the sample. A variety of membrane surface areas and pore sizes are available (Thurber *et al*., 2009) which are usually manufactured from polysulphonate or related materials (Grabow, 2001). Experiments performed indicate that high flow rates can also cause filter clogging thus maintenance of the system is regularly required, particularly to preserve the integrity of the filters (Wommack *et al*., 2010).

The benefits of using tangential-flow filtration over other concentration methods include speedy separation time, it does not require toxic reagents (Wommack *et al*., 2010), viruses are not exposed to varying pH that can affect viral infectivity (Grabow, 2001) and large sample volumes can be processed at a time due to increased surface area (Thurber *et al*., 2009). Although TFF produces high recovery rates and is the most sought out technique for viral concentration, it has many disadvantages. The procedure requires costly equipment and may produce variable recovery efficiencies depending on the type of filter used, sample composition, operator‟s knowledge in sample processing and the amount of backpressure applied to create an accurate transmembrane pressure (John *et al*., 2011). Additionally back-flushing used to maintain the TFF filters can cause shorter life-spans of the filters by damaging the integrity of the filters (John *et al*., 2011). Moreover, the accidental exposure of abrasive chemicals such as chloroform (Thurber *et al*., 2009) and poor storage conditions can foul the filter thus reducing the efficiency of viral recovery (Wommack *et al*., 2010).

## 1.13 Methods to detect viruses in water

Since viruses are constantly causing infections on a global scale it is important to detect enteric viruses using reliably easy methods (Hamza *et al*., 2011). Currently, a number of biological, physical, molecular and serological methods exist (Lee *et al*., 2004; Kreuze *et al*., 2009). Tissue culture is the most common method of choice (Hamza *et al*., 2011), however, polymerase chain reaction assays (Okoh *et al*., 2010) and electron microscopy have also been used (Ogura, 2012).

### 1.13.1 Tissue culture

The standard method for the evaluation of enteric viruses involves the production of a cytopathic effect (CPE) using animal cell culture lines. Common CPEs produced by viruses include the formation of syncytia, the cells become circular, cytoplasmic/nuclear inclusion

bodies start to form and the cells detach from the wall of the flask. These changes can be viewed under a light microscope circumventing the need for staining and fixing (Albrecht *et al*., 1996). The total culturable virus assay (TCVA) has been optimized and applied in the United States to detect and quantify the presence of waterborne viruses (Lee and Jeong, 2004). Examples include buffalo green monkey kidney (BGM) and Madin-Darby bovine kidney (MDBK) cells that viruses attach to and infect (Li *et al*., 2002a). One of the main advantages of cell culture is its high sensitivity rates where a single virus particle can be propagated from a particular water sample (Li *et al*., 2002a).

The disadvantage of using cell culture is that the procedures are labour intensive and costly (Gilgen *et al*., 1997; Hamza *et al*., 2011) with viruses not being able to produce a CPE (Fong and Lipp, 2005). For example, cell culture assays for hepatitis A viruses may take 6-8 weeks and may not necessarily produce pathological cell change (Feigelstock *et al*., 2005). Furthermore, long incubation times (greater than 3 days) are required before any visible CPE is produced by most viruses that are propagated from environmental water samples (Li *et al*., 2002a).

Apart from the quantal assay for detecting infectious viruses the MTT assay has been used to determine the viability of cells in comparison to cell cytopathology (Heldt *et al*., 2006). It involves the reduction of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide, MTT by NADH and NADPH. The cells produce insoluble purple formazan crystals which are solubilized to produce a purple colour. The intensity of the colour of the final solution corresponds to the number of viable cells which can be read spectrophotometrically. This method has been used for influenza viruses, coxsackievirus B6 and picornaviruses (Heldt *et al*., 2006).

### 1.13.2 Electron microscopy

Electron microscopy has been widely applied to classify and identify new viruses in environmental samples. The first electron microscope was designed by Ernst Ruska and Max Knoll in 1931. Eight years after development, Ruska and colleagues were the first to visualize a virus (tobacco mosaic virus) with the electron microscope (Goldsmith and Miller, 2009; Vale *et al*., 2010). Since its development, electron microscopy has become one of the most common techniques applied to identify potentially infectious agents (Hazelton and Gelderblom, 2003).

The most common electron microscopy techniques used to visualize viruses include transmission electron microscopy (TEM) and scanning electron microscopy (SEM). Most

focus is on TEM because it allows for the morphologies of viruses to be observed under an appropriate magnification (Ackermann, 2011). Sample examination usually requires staining procedures to enhance the contrast of the image being viewed. Sample preparation now includes negative staining, glutataldehyde fixation and heavy metal coating (Ogura, 2012). Negative staining is the most common sample preparation technique used to visualize viral particles because it is fast, simple and provides increased resolution of specimens (Fong, 1989). Generally, negative staining requires concentration of the virus and the removal or cell debris and bacteria to allow increased chances and proper visualization of the viruses (Fong, 1989; Goldsmith and Miller, 2009). The specimen grids used for TEM contain a support film to hold the particulate viruses such as Butvar, Formvar, Pioloform and Collodion. The grids are then coated with carbon to prevent them from melting under the electron beam during imaging. The support film is hydrophilic to allow an even spread and stain across the grid. Negative stains include uranyl acetate, ammonium molybdate and phosphotungstic acid. The stain of preference depends on the individual laboratories and a number of advantages and disadvantages are associated with each stain of choice:

i. Uranyl acetate (0.05 to 2%) – Behaves as a stain and an adhesive. Virus samples can be viewed after long periods of time and still remain intact. It also allows for the viewing of some finer details since the images appear granular. However, uranyl acetate can precipitate under phosphate salts and is considered radioactive.
ii. Ammonium molybdate (0.05 to 5%) – Allows detail in the images because of a granular appearance.
iii. Phosphotungstic acid (1 to 2%) – Does not precipitate under phosphate salts and can sometimes outline spikes or protrusions on enveloped viruses. However, PTA can degrade some viruses upon long intervals of visualization (Goldsmith and Miller, 2009).

A number of viruses were first seen from clinical specimens with TEM including chicken pox (varicella-zoster virus of the Herpes family), smallpox (variola virus of the Poxvirus family), norovirus and various other viral outbreaks such as the henipavirus outbreaks in Asia and Australia were first discovered by electron microscopy. Apart from diagnostic purposes, electron microscopy has also been used to evaluate virus attachment and replication which is advantageous is the development of antiviral drugs (Goldsmith and Miller, 2009).

The diversity of aquatic viruses is quite astonishing and their presence has now been recognized as key players in microbial evolution, influencing geochemical cycles and probably climate change (Pollard, 2012). Epifluorescence microscopy (EFM) has been adopted for the direct enumeration of virus-like particles (VLPs) in environmental samples (Diemer *et al*., 2012). The general procedure involves collecting the viruses onto a filter and subsequently stained with a fluorescent dye. A specific wavelength is then applied to elicit glowing particles that can then be counted digitally (Ortmann and Suttle, 2009). A number of dyes have been employed for enumerating viruses such as 4',6-diamidino-2-phenylindole (DAPI), Yo-Pro-1, SYBR Green I and SYBR Gold (Wen *et al*., 2004; Patel *et al*., 2007).

### 1.13.3 Polymerase chain reaction (PCR)

Molecular techniques like PCR have been extensively used to study non-cultivable or isolated viruses. In spite of its success, two major drawbacks are associated with these techniques: (i) separate tests must be carried out to properly identify each pathogen making the identification of unknown pathogens difficult or impossible and (ii) the lack of sequence based information of known viruses makes it difficult to characterize emerging viral pathogens (Bibby, 2013).

The detection of human enteric viruses has greatly increased since the development of PCR. Molecular detection by PCR or reverse-transcription PCR (RT-PCR) has especially worked well for viruses that cannot be cultured on conventional cell lines (Rodríguez *et al*., 2009; Bosch *et al*., 2011). The viral capsid protein or nucleic acid genome (DNA or RNA) is targeted for virus detection (Mattison and Bidawid, 2009) using specific primers (Rodríguez *et al*., 2009). Viruses with a RNA genome would first need to be transcribed to cDNA prior to RT-PCR (Rodríguez *et al*., 2009).

The detection of specific and potentially infectious pathogens that may cause similar symptoms is important. Many illnesses are very infrequently caused by a single infectious agent and would therefore require the simultaneous detection of multiple organisms. To overcome this drawback many studies have now also incorporated multiplex RT-PCR for virus detection (Piao *et al*., 2012). Multiplex PCR encompasses multiple primers within a single PCR reaction. However, optimization of this type of PCR can be problematic due to the varying annealing temperature requirements of the primers. In some instances, oligonucleotide hybridization is needed for the confirmation of the multiplex PCR products (Rodríguez *et al*., 2009). Moreover, the assay is limited by the amount of pathogens that can be detected in a single multiplex assay (Piao *et al*., 2012). Researchers have also used nested

PCR to increase the sensitivity of the initial PCR assay. Nested PCR can be applied to all types of PCR methods such as conventional PCR and real-time PCR assays (van Heerden *et al*., 2005). Puig *et al*. (1994) applied nested PCR to all RT-PCR assays to decrease false positives, increase sensitivity, increase the simplification signal and to ensure the specificity of detection for enterovirus and adenoviruses from river water and sewage effluent. During nested PCR additional primers and the product from the first PCR reaction (template) are used for a second round to increase the throughput of the initial reaction (Gajardo *et al*., 1995; Mayer and Palmer, 1996).

Molecular detection by direct PCR/RT-PCR has many advantages and disadvantages associated with its use. Some of the advantages include its high specificity and sensitivity (Mattison and Bidawid, 2009) as well as the attainment of large PCR products since larger volumes can be processed (Bosch *et al*., 2011). In addition, typing and characterization of the viruses for epidemiological studies and faecal tracking can be done (Mattison and Bidawid, 2009). The assay is also inexpensive compared to other detection methods that may be applied (Bosch *et al*., 2011).

The disadvantages of using PCR include the production of false positive amplification due to the presence of inhibitory compounds (Rodríguez *et al*., 2009) and its inability to differentiate between non-infectious and infectious viruses (Bosch *et al*., 2011). To overcome the latter, integrated cell culture PCR (ICC-PCR) was developed to reduce long incubation periods for cytopathic effects (CPE). This method has been used for the investigation of many enteric viruses that have shown an infectious nature (Bosch *et al*., 2011). The principal behind ICC-PCR is that after infection of the cell line, only the infectious viruses will propagate. The cell are then harvested and tested for viruses by PCR. Although ICC-PCR has been adopted to test for infectious viruses, this method is also useful for viruses that cannot produce CPE but can still infect and grow (Fong and Lipp, 2005).

### 1.13.4 Real-time PCR

Most studies previously focused on qualitative rather than quantitative evaluation of viral populations (Choi and Jiang, 2005). Recently, real-time PCR has been commonly used in the detection of viruses from sewage (treated and untreated), seawater and river water (Haramoto *et al*., 2010). The use of real-time PCR for the quantification of viruses has greatly influenced the knowledge of viral infectious agents (Sibanda and Okoh, 2013). The target sequences are detected by the production of a fluorescence signal by fluorophore-labelled primers, intercalating dyes or sequence specific probes. Sequence specific probes are much

more precise in comparison to agarose gel-based PCR assays. The number of amplification cycles or copy numbers of the PCR product (fluorescence) at the start of the reaction to cross a threshold fluorescence line (threshold cycle (Ct)) is used to quantify the targeted sequence (Hoffmann *et al*., 2009a).

Real-time PCR provides increased sensitivity with rapid results in comparison to convectional PCR (Jothikumar *et al*., 2006). Furthermore, the reduced risks of contamination have made real-time PCR a preferable alternative to agarose-based PCR, immunoassay-based methods and tissue culture (Hoffmann *et al*., 2009a).

The drawbacks of using real-time PCR is (i) the incompatibility of some instruments with certain fluorogenic dyes, (ii) the inability to determine the product size without opening the system, (iii) starting real-time PCR in certain laboratories may be expensive and (iv) the notable restrictions in multiplex reactions can be problematic (Mackay *et al*., 2002).

### 1.13.5 Metegenomic analyses of viral communities in aquatic environments

Viral sequence information from environmental research is a desired topic of interest as it provides information on the amount of extracellular genetic assortment that planktonic organisms are constantly in contact with and the variations of viruses that exist in particular habitats (Steward and Preston, 2011). Metagenomics is defined as sequence based studies that investigate many organisms that may not be able to be grown in culture-based systems. Unlike bacteria, viruses do not contain universal conserved regions within their genomes and viral metagenomic studies employ shotgun or random sequence analyses (Roossinck, 2012). After the high throughput sequencing data sets are obtained the sequences are compared to known sequences complied in public databases using computational algorithmic programmes such as BLAST (Hall *et al*., 2013). Since metagenomics analyse all nucleic acids present within a sample, researchers have to confirm that there are no contaminating bacterial or eukaryotic cells that could compromise the investigation of viral genomes. Samples can be treated with RNase or DNase I to eliminate free nucleic acids. However, DNase I may not completely rid the sample of all contaminants and RNase may result in the loss of viral particles containing RNA in their nucleocapsids. Thus, to fully confirm that only viruses are present 18S or 16S primers can be used in PCR assays (Thurber *et al*., 2009).

The general routine of metagenomic analysis of viruses is depicted in Figure 1.2. Viruses are isolated and the RNA or DNA is extracted, converted to cDNA (RNA), fragmented and then sequenced. Subsequent to sequencing, short sequences may be assembled into longer sequences to assist in annotation. The assembled or raw sequence data

is then annotated through global or local alignments with reference genes or genomes. Further analyses are done on annotated sequences of human pathogens (Bibby, 2013).

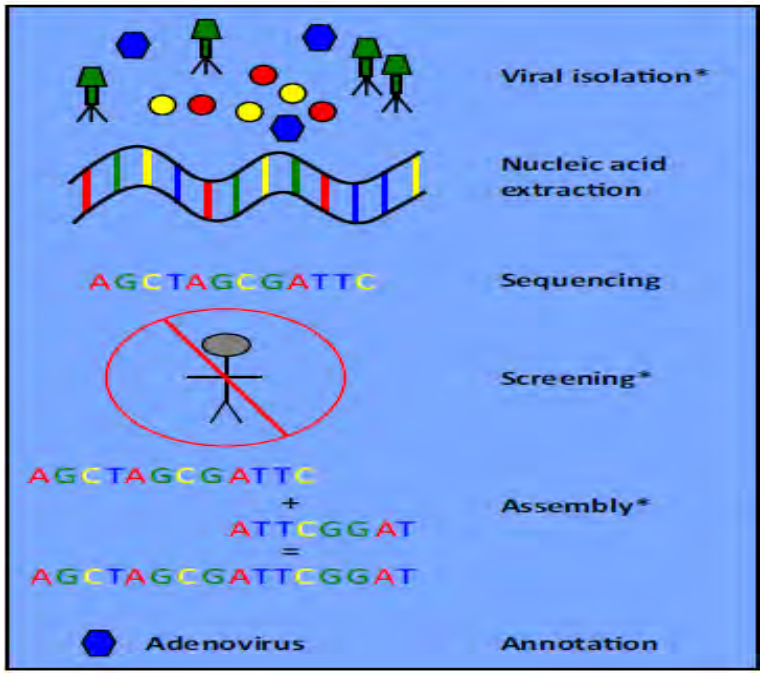


**Figure 1.2** Generalized metagenomic shotgun approach to viral genome construction. To begin, the viruses are isolated based on density and/or size. The nucleic acids are extracted using standard extraction protocols. The nucleic acids are prepared and sequenced which are then screened to eliminate non-viral sequences (bacterial or human-derived hosts). The sequences are then assembled into longer sequences and annotated. *Denotes application-dependent or optional steps (Bibby, 2013).

## 1.14 Significance of study

Viral contamination of recreational water such as rivers has been reported over the years. Combined sewer overflows, treated and possibly untreated wastewater as well as storm water runoffs carry a diversity of viral communities into surface water (Aslan *et al*., 2011). Despite the advent of advanced technology in wastewater and drinking water treatments, viral-induced disease outbreaks still persevere (Hamza *et al*., 2011). South Africa is a water-scarce, semi-arid country that relies on dams and rivers for drinking water, recreational and agricultural practices (Chigor and Okoh, 2012). Viral disease outbreaks have also occurred in South Africa (Mardon, 2003). South Africa experienced 2 norovirus outbreaks that caused severe cases of gastroenteritis in individuals who consumed contaminated foods such as salads and oysters and drinking water. The severity of these outbreaks earned them the "Grootbrak" and "de Christmas" names, respectively. Researchers believe these outbreaks

may have been as a consequence of improper sewage disposal, the use of wastewater for irrigation and fishing from contaminated rivers which may have also allowed for the (in)direct consumption of contaminated water (Dongdem *et al*., 2009).

In spite of all the disease occurrences caused by viruses, their role in these infections and the health impact created by them is still well underestimated and largely not understood (Kiulia *et al*., 2010). While viruses are the most abundant organisms inhabiting Earth, their diversity, history and functional aptitude is poorly understood (Diemer and Stedman, 2012). The persistence of viruses in the environment and their morphology is becoming a relevant field of study considering the fact that viral populations have a major impact on other organisms co-existing in the same habitat as well as the human population (Bettarel *et al*., 2000; Cantalupo *et al*., 2011). Furthermore, studies involving viral populations in South Africa are inexplicably limited and confined to a few locations and provinces (Chigor and Okoh, 2012).

Thus, this study demonstrated the advantage of environmental monitoring of a freshwater resource, namely the Umhlangane River in an attempt to evaluate the presence and diversity of viruses (bacteriophages and enteric viruses), their interaction within the immediate environment (physico-chemical constituents) and other microbial pathogens (bacterial indicators) as well as appraise the possible use of enteric viruses as indicators of faecal pollution in water samples. The Umhlangane River was chosen due to the rapid development of industrial and domestic infrastructure surrounding the catchment. This river flows through a substantial part of Durban including Inanda, KwaMashu and Ntuzuma which comprises inadequate infrastructure and wide unused open spaces (Water Rhapsody, 2009). According to an eThekwini Municipality case report, 2% of the population surrounding the river are rural, 32% are informal and 64% are formal inhabitants. Furthermore, large industrial (709 ha) areas, market gardening (68 ha) regions and approximately 170 households directly utilize water from the river (Mgeni case report, 2011). In spite of restoration efforts of these areas by government officials, backlog accumulation remains high as new urban migrants still continue to these areas absconding rural poverty (Water Rhapsody, 2009). The Umhlangane River is constantly exposed to a variety of changing conditions while simultaneously serving as a conduit for wastewater effluent emanating from the KwaMashu and Northern WWTPs. The combination of this wastewater, industrial (Phoenix, Riverhorse Valley and Springfield) and residential effluents, informal settlement wastes as well as storm water runoffs from all the surrounding areas are conveyed to the Umgeni estuary which is a key component in KwaZulu-Natal‟s drinking water supply

(Hadlow, 2011). The mounting declination in river water quality is primarily due to the subsequent accumulation of anthropogenic pollution within rivers (Mendiguchia *et al*., 2007; Wang *et al*., 2007; Zhou *et al*., 2012). This polluted water source may be a potential risk factor for individuals who may directly or indirectly utilize the river water. Moreover, the Umhlangane River has an expansive history of flooding where the river rose up to 2 m in depth in 1996. This uncontrollable rise in the river water can cause increased pollution to collect throughout the catchment causing bigger water quality concerns (Wildlands, 2012).

## 1.15 Hypothesis

It is hypothesized that the Umhlangane River contains a broad range of enteric pathogenic viruses. It is further hypothesized that concentrating and monitoring these viruses and their approximate numbers would allow for much more accurate knowledge for the risk assessment of waterborne viral infections.

## 1.16 Objectives

The following objectives were created:

**1.16.1** To determine the water quality of the Umhlangane River, Durban, South Africa;

**1.16.2** To evaluate the diversity and presence of viruses (bacteriophages and enteric viruses) in the Umhlangane River and;

**1.16.3** To statistically analyse the spatial and temporal variations between all tested water quality parameters.

## 1.17 Aims

The subsequent aims were established:

**1.17.1** To monitor the physico-chemical and bacterial indicators in the river water samples;

**1.17.2** To maximize viral recovery by concentrating the water samples using tangential flow filtration;

**1.17.3** To enumerate somatic and F-specific RNA coliphages from the collected water samples;

**1.17.4** To evaluate viral morphology, diversity and abundance in the water samples with transmission electron and epifluorescence microscopy;

**1.17.5** To monitor four viral communities and infectivity using nested PCR/RT-PCR and cell culture assays, respectively and;

**1.17.6** To investigate the correlations between all physico-chemical, bacterial and viral indicators evaluated in this study.

## 1.18 Experimental outline

This dissertation was structured into four chapters that are presented as follows:

- **Chapter *one*** represents the introduction and literature review. The review covers the water availability and management in South Africa that focuses on anthropogenic, physico-chemical and heavy metal effects on river systems. The employment of bacterial indicators of faecal pollution is also discussed. The presence of enteric viruses in water environments, their possible use as faecal indicators and the subsequent concentration and detection methods are also reviewed.

- **Chapter *two*** depicts the water quality of the Umhlangane River that focused on the detection of physico-chemical and microbiological examination, specifically bacterial and coliphage populations. The relationships between all the tested water quality indicators were evaluated using canonical correspondence analysis (CCA).

- **Chapter *three*** describes the abundance of virus-like particles in the Umhlangane River in relation to the coliphage populations and microbial indicators. The detection of bacteriophages and enteric viruses using a number of techniques are also presented. Finally, multivariate correlations to describe the relationships between the viruses, physico-chemical and bacterial indicators are presented.

- **Chapter *four*** describes a summary of research findings observed in the study with future recommendations also being discussed.

# Chapter *two*

# Physico-chemical parameters and microbiological analyses of the Umhlangane River

## 2.1 Introduction

The National Water Act of 1998 and the National Water Policy of 1997 put forward by the South African Government have a visualization of a society where every individual possesses the opportunity to participate in activities and lead a healthy and dignified life (CSIR, 2010). This audacious statement shows that the importance of water cannot be overstated and is a key player to human health, dignity, and socio-economic endeavours. The pertinent nature of water based on the level of importance in terms of human needs starts with drinking water, sanitation, health and agricultural practices. The importance of water for mining, industrial practices, tourism and power generation follows after (CSIR, 2010). Furthermore, water is essential for all life on Earth because it is a component of food and has a role in many biological metabolic processes (Mienik, 2004). Globally, water scarcity can lead to extreme political pressures or water stress (Molobela and Sinha, 2011). At present a number of countries are faced with the lack of water resources resulting in water scarcity coinciding with expanding population growth (Rajiv *et al*., 2012).

In countries like South Africa where 90% of the country is considered semi-arid or arid, water is particularly precious due to the onset of changing climatic conditions that inadvertently cause drought (Hoffman *et al*., 2009b). Geographical and indigenous traits results in low amounts of freshwater in comparison to the African Sub-Saharan regions and the world. The relative amount of freshwater that flows through the hydrological cycle in South Africa is estimated to be 86% less than the average for developing countries (85% less than the Sub-Saharan average) and approximately 87.2% less than the world average (Langstaff, 2010). Annual rainfall is subject to evapo-transpiration that reduces the total amount of available rain water. This effect is greatly pronounced due to changing temperature and precipitation levels experienced over time (Langstaff, 2010). Rivers are one of the major sources of water for agricultural, industrial and domestic purposes (Paulse *et al*., 2009; Kolawole *et al*., 2011), particularly in South Africa (van der Merwe-Botha, 2009). The inter-basin method for the transfer of drinking water from freshwater resources in South Africa means that all rivers are either directly or indirectly employed for water purposes (Nomquphu *et al*., 2007; van der Merwe-Botha, 2009). Thus, safe water resources are imperative to sustain human health particularly to the elderly, children and immunosuppressant individuals (Paulse *et al*., 2009).

In certain regions and sectors of South Africa water quality is a key concern. Water holds unique chemical, biological and physical properties resulting in a characteristic signature (Taljaard and Botes, 1995). Rivers flowing through areas of human interest such as

industrial sites may contain increased concentrations of heavy metals thus affecting the water quality (Sadeghi *et al*., 2007). Therefore, standard methods have been developed to assess the microbial, physical and chemical composition of water bodies to ensure that these characteristics are present at acceptable levels in order to safeguard human and animal health (Boyacioglu, 2007; Rajiv *et al*., 2012).

Microbial communities are naturally present in the environment. However, some may enter through water, soil or domestic and industrial effluents. Pathogenic microorganisms are of most concern since they originate from excreta and may travel to enter water systems (Saati and Faidah, 2013). These pathogens may cause waterborne diseases that can be acquired through indirect or direct use of the water (Sinclair *et al*., 2009). The measurement of human activities on the quality of water is currently carried out under the supervision of Department of Water Affairs (DWA) and the Foundation for Research Development programmes (Nomquphu *et al*., 2007). These programmes established a standard method for the management and accurate monitoring of water resources in South Africa (Nomquphu *et al*., 2007). Bacterial indicators are currently the method of choice for the detection of faecal pollution in environmental and potable water supplies (Griffin *et al*., 2001; Rippy *et al*., 2013; Vierheilig *et al*., 2013). These indicators are microflora of animal and human gastrointestinal tracts (Luyt *et al*., 2012) and belong to the faecal coliform group (Sengupta and Saha, 2013). The estimation of bacterial indicators in water environments is indispensible as it provides valuable knowledge regarding the salubrious nature of the water source in question (Miernik, 2004).

The realization that bacterial indicators have many disadvantages such as their replication in the natural environment, their faster die-off rates (Scott *et al*., 2002; Savichtcheva and Okabe, 2006) and the lack of correlation to diarrhoeal incidences, has led water technicians to the use of bacteriophages as indicators of water quality (McMinn *et al*., 2014). The somatic and $F^{+}$RNA bacteriophages were proposed as indicators of faecal pollution because they share survival characteristics to enteric viruses, they cannot replicate in the environment and their presence signifies the presence of bacterial populations capable of replicating the phage (Grabow, 2001; Rodríguez *et al*., 2012).

This chapter describes the measurement of both physico-chemical and microbial indicators (bacterial and coliphage populations) in an attempt to assess the water quality of the Umhlangane River with respect to the amount of possible anthropogenic influences impacting the river system at different time points and sampling sites.

## 2.2 Materials and methods

### 2.2.1 Study area and sampling procedure

This study was conducted on the Umhlangane River (sub-catchment of the Umgeni River) because it is surrounded by a plethora of developed and undeveloped societies receiving wastewater, storm and urban water runoffs as well as the possibility of faeces from freely roaming fauna around the area (Hadlow, 2011). The river flows through Phoenix, Riverhorse Valley and Springfield which is densely populated areas experiencing great anthropogenic influences with a number of different land use zones. The river spans approximately 15 km and is a key component in KwaZulu-Natal‟s drinking water supply since it drains directly into the Umgeni River (Hadlow, 2011).

The 5 sampling points (designated P1 to P5) are depicted in Figure 2.1. Each sampling point represents different land use zones that are described as follows: P1 (Phoenix industrial), P2 (upstream KwaMashu Wastewater Treatment Plant; tributary from KwaMashu area), P3 (natural wetlands; downstream KwaMashu Wastewater Treatment Plant), P4 (Riverhorse Valley business estate; upstream Northern Wastewater Treatment Plant) and P5 (Springfield industrial; downstream Northern Wastewater Treatment Plant).

Water samples were collected on a monthly basis (second week of each month) commencing in October 2013 and concluding in September 2014. Samples were collected in 5 ℓ plastic containers (previously disinfected with 70% (v/v) alcohol and rinsed with deionized water) (Olaniran *et al*., 2012). At each sampling point, the drums were rinsed with river water prior to being plunged approximately 0.3-0.5 m below the water surface to circumvent the disinfectant effect of UV light (Zamxaka *et al*, 2004; Jurzik *et al*., 2010). Samples collected at sites that contained bridges employed the grab sampling method using a bucket and rope (Hadlow, 2011). All water samples were transported to the Discipline of Microbiology, University of KwaZulu-Natal (UKZN; Westville campus) and stored at 4°C until further sample processing and analyses (Skraber *et al*., 2004; Espinosa *et al*., 2008). All samples were processed within 48 h of collection (Olaniran *et al*., 2012).

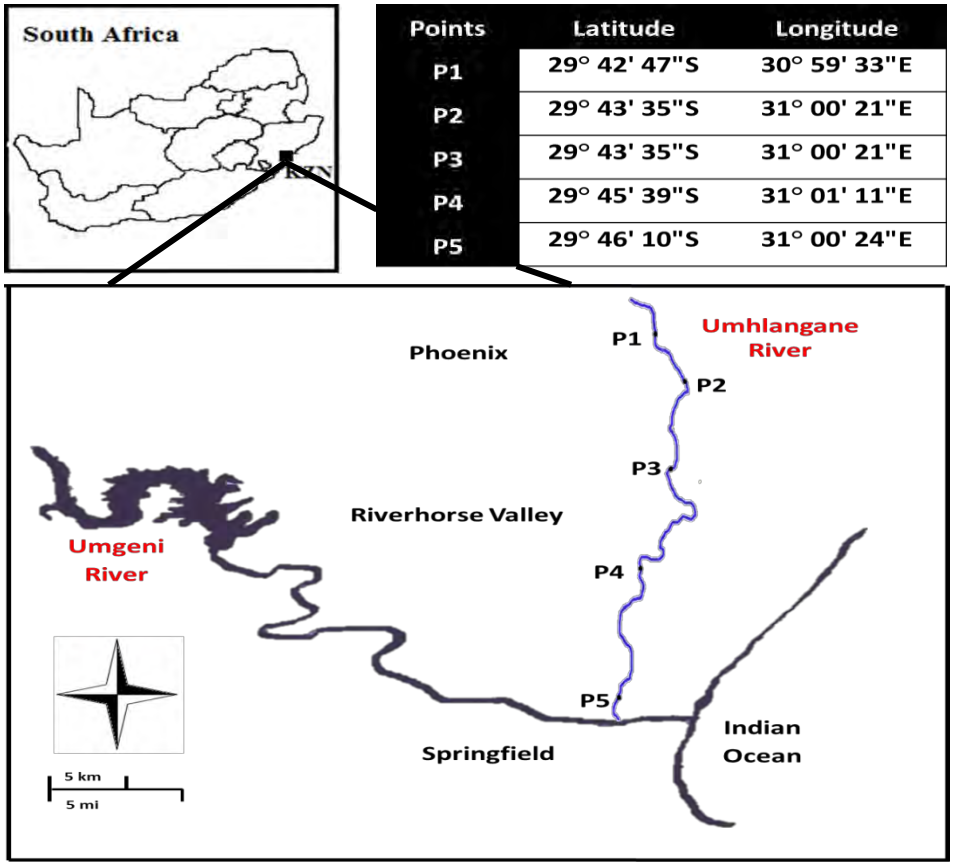


**Figure 2.1** Geographical map of the Umhlangane River with the 5 sampling locations and the GPS coordinates.

### 2.2.2 Physico-chemical assessment of the river water samples

Commonly measured physico-chemical parameters including temperature, pH, dissolved oxygen, turbidity, electrical conductivity (E.C.), total dissolved solids (TDS), salinity, biological oxygen demand (BOD) and chemical oxygen demand (COD) were determined. Temperature was measured *in situ* (°C) using a thermometer while the salinity, electrical conductivity and the total dissolved solid content was measured using the HACH CDC401 probe. Turbidity and pH was determined using a portable 2100P turbidometer and pH meter (Hanna), respectively. Additional precipitation data was also obtained (Accuweather, 2014).

#### 2.2.2.1 Chemical oxygen demand

An estimated range of 10 − 150 mg/ℓ COD vials (Merck-Millipore Corp.) was used to determine the COD levels of the river water samples. Three millilitres of each water sample was added to a previously mixed COD vial and vortexed to homogenize the samples.

Distilled water was added to one vial to serve as a control. The prepared vials were then digested in a thermoreactor (HACH DRB 200) at 148°C for 120 minutes. After 2 hours the vials were allowed to cool at room temperature for 10 minutes in a dry, dark place, vortexed and cooled before readings were taken using a photometer (Spectroquant NOVO 60).

#### 2.2.2.2 Biological oxygen demand

Biological oxygen demand was determined using the 5-day BOD test. The estimated BOD mg/ℓ was taken as 4 mg/ℓ for polluted river water samples. Two-hundred millilitres of each water sample was transferred into the respective BOD bottles. Process inhibitors (HACH) were added to each bottle to circumvent the oxidation of nitrogenous compounds. Dilution water (1 sachet of nutrient buffer pillow dissolved in 3 ℓ of autoclaved distilled water) was added to each bottle. A control with only dilution water and process inhibitors was also prepared. All bottles were inverted several times to mix and the initial dissolved oxygen ($DO_1$) was read using the HACH HQ40d portable meter and LD101 DO probe. After readings, the bottles were topped up with dilution water, stoppered and incubated for 5 days at 20°C. The $DO_5$ measurements were taken on day 5 and the $BOD_5$ was calculated according to the following formula (Delzer and McKinzie, 2003):

$$BOD_5\ (mg/l) = \frac{\mathrm{DO_1} - \mathrm{DO_5}}{\mathrm{P}}$$

$DO_1$ : Initial DO

$DO_5$ : DO after 5 days incubation

P : Fraction of volume of sample used

### 2.2.3 Bacterial indicator enumeration

The detection of eight bacterial indicators was done using the membrane filtration technique according to standard methods (APHA, 1998). Appropriate dilutions of each water sample was made before filtering 50 mℓ through a 0.45 µm membrane filter (PALL corp.) into a previously autoclaved glass filtration unit (GLASCO). The membrane filters was transferred onto 65 mm petri plates of selective media and incubated at specific incubation conditions (Table 2.1). Water samples were processed in duplicates to reduce the scale of error. Growth was then enumerated as colony forming units per 100 mℓ (cfu/100 mℓ).

The FC/FS ratio was used to determine the potential source of faecal pollution present in the Umhlangane River. The FC/FS ratio was calculated after performing the membrane

filtration technique. The FC/FS standard ratios are as follows: (i) >4 – human origin, (ii) 2.0–4.0 – predominantly human wastes in mixed population, (iii) 0.7–2.0 – predominantly animal wastes in mixed population and (iv) <0.7 – animal origin (Scott *et al*., 2002).

**Table 2.1** Bacterial indicators with their appropriate incubation conditions and selective media (Griesel and Jagals, 2002; Rompré *et al*., 2002; Olaniran *et al*., 2009; Patra *et al*., 2009).

| INDICATORS | SELECTIVE MEDIA | INCUBATION CONDITIONS |
|---|---|---|
| Total heterotrophs (TH) | Nutrient agar | 24 hrs at 37°C |
| Presumptive *Escherichia coli* (EC) | Chromocult agar | 24 hrs at 37°C |
| Total coliforms (TC) | m-Endo agar | 24 hrs at 35°C |
| Faecal coliforms (FC) | m-FC agar | 24 hrs at 44.5°C |
| Faecal streptococci (FS) | KF-*streptococcus* agar | 48 hrs at 42°C |
| *Vibrio* spp. (VIB) | Thiosulphate citrate bile salts sucrose agar | 18 – 24 hrs at 37°C |
| Presumptive *Salmonella* spp. (SAL) | *Salmonella-Shigella* agar | 24 hrs at 35°C |
| Presumptive *Shigella* spp. (SHIG) | *Salmonella-Shigella* agar | 24 hrs at 35°C |

### 2.2.4 Somatic and F-specific RNA coliphage determinations

#### 2.2.4.1 Bacteriophage host preparation

The hosts for the somatic and F-specific RNA phages (obtained from the Discipline of Microbiology, UKZN, Westville) was *E. coli* WG5 and *Salmonella typhimurium* (*S. typhimurium*) WG49, respectively (ISO, 1995; ISO, 1998). The somatic host was grown by inoculating 1 vial of *E. coli* WG5 (glycerol stock) into 50 mℓ nutrient broth and 1% NaCl. The bacteriophages were grown overnight at 37°C and 100 revolutions per minute (rpm).

The F-specific RNA coliphage host was grown on the day of use by inoculating 1 glycerol stock vial into 50 mℓ tryptone yeast extract-glucose broth (TYGB) and grown in a shaking incubator of 37°C and 100 rpm (ISO, 1995). Absorbency was taken at 30 minute intervals ($OD_{600}$) until an optical density of 0.75 was achieved, thus indicating the production of F-pili. The culture was immediately placed on ice to prevent the loss of the surface appendages and used within 2 hours (ISO, 1995; Grabow, 2001).

#### 2.2.4.2 Presence-absence spot test

Appropriate dilutions of the concentrated water samples (0.22 µm filtered) were made prior to both the presence-absence spot test and bacteriophage enumeration assays. Single layer agar plates for the somatic and $F^{+}$RNA coliphages were prepared (Appendix i) for the presence-absence spot test. The agar plates were inoculated with 0.1 mℓ of host and spread using a sterile cotton swab. Twenty microlitres of diluted and undiluted water samples was spotted onto the lawn of bacterial host and incubated at 37°C for 24 hours. The presence of plaques or clearings was indicative of a positive phage result (Grabow, 2001).

#### 2.2.4.3 Double-overlay plaque assay for bacteriophage enumeration

The double overlay agar technique was used to enumerate the somatic coliphages according to ISO 10705-2 standard (ISO, 1998) and ISO 10705-1 standard (ISO, 1995) for the F-specific RNA coliphages. Briefly, 1 mℓ of the respective host culture and the previously prepared dilutions containing the concentrated water samples was added to 8 mℓ soft agar (Appendix i). The mixture was vortexed to distribute the particles in suspension evenly and poured over the prepared agar plates. All samples were processed in duplicates. The plates were allowed to solidify before being inverted and incubated for 24 hours at 37°C. Plaques were enumerated as plaque-forming units per millilitre (pfu/mℓ) (Jiang *et al*., 2001).

### 2.2.5 Statistical analysis

Correlation between the sampling months, sampling points, physico-chemical parameters and microbial indicators was determined using the Pearson‟s correlation test (Student‟s *t*-test) using SPSS programme version 22 (SPSS Inc., Illinois). The level of significance was set at $p < 0.01$ and $p < 0.05$ (Olaniran *et al*., 2012).

Canonical correspondence analysis (CCA) was used to evaluate the relationship between all the physico-chemical and microbial indicators during the varying sampling points and months. Ratios were then compared to the set standard. Correlations was generated in the form of an ordination bi-plot where the length of an arrow indicates a rate of change therefore a longer arrow indicates a larger change in the variable being investigated. A Monte Carlo permutation test of 499 random permutations was used to establish the significance of the axes with the species data. Canoco for Windows version 4.5 was used to determine the CCA statistical ordination plots (ter Braak, 1994; ter Braak and Verdonschot, 1995).

## 2.3 Results

### 2.3.1 Physico-chemical analyses

The physico-chemical parameters varied throughout all sampling months and points along the Umhlangane River and are depicted in Table 2.2. Temperature varied significantly throughout the sampling months ($p < 0.01$) and points ($p < 0.05$) ranging from 28.5°C (January 2014; P1) to 18.0°C in July and September 2014 (P3 and P4, respectively). Interestingly, temperature depicted higher values at P1 and P2 compared to the other 3 sampling points during all months with the exception of May, June and September. The pH of the river water samples ranged from 9.04 (June 2014; P1) to 6.00 (December 2013; P3) and varied significantly at all the sampling months and points ($p < 0.01$).

The $BOD_5$ and COD content (Table 2.2) fluctuated throughout all sampling months and points with $BOD_5$ ranging from 0.48 mg/ℓ (April 2014; P4) to 12.4 mg/ℓ (June 2014; P1). Significant difference ($p < 0.01$) between $BOD_5$ and the sampling points was observed while a weak positive correlation ($r = 0.365$; $p < 0.004$) between the pH and $BOD_5$ values was also seen. The highest COD measurement was recorded at P1 in May 2014 with a value of 269 mg/ℓ. A COD value of < 10 mg/ℓ was recorded at sampling points P2 and P3 (February 2014), P3 (April 2014), P2 – P5 (May 2014), P3 and P4 (June 2014) and in P2, P3 and P5 (September 2014). No significant difference ($p > 0.05$) was seen between the COD content and the sampling months but a significant difference was observed ($p < 0.05$) between COD and the sampling points. The DO (Table 2.2) measurements varied and ranged from 9.46 mg/ℓ (June 2014; P1) to 3.28 mg/ℓ (March 2014; P4). A significant difference ($p < 0.01$) between the sampling months and DO values was also noted.

Turbidity (Table 2.2) ranged from 1.16 NTU (April 2014; P5) to 62.4 NTU (September 2014; P1) and showed weak negative correlations ($r = -0.362$; $p < 0.005$) with the sampling points. The TDS and E.C. (Table 2.2) values varied throughout all sampling months and points. The highest and lowest TDS values were observed at P2 in February 2014 (430 mg/ℓ) and P5 in December 2014 (201 mg/ℓ), respectively. The E.C. values ranged from 869 mg/ℓ (July 2014; P1) to 425 mg/ℓ at P3 in December 2013 and June 2014, respectively. Salinity was noted at all points and months along the Umhlangane River ranging from 0.43% (July 2014; P1) to 0.21% (February 2014; P4). No significant differences ($p > 0.05$) were seen between the TDS content, E.C., salinity and sampling points and months.

**Table 2.2** Physico-chemical parameters recorded at all sampling points from October 2013 to September 2014.

| Points | Month | T (°C) | pH | $BOD_5$ (mg/ℓ) | COD (mg/ℓ) | DO (mg/ℓ) | TDS (mg/ℓ) | Turbidity (NTU) | E.C. (mS/m) | Salinity (%) |
|---|---|---|---|---|---|---|---|---|---|---|
| P1 | October 2013 | 24.0 | 7.30 | 11.1 | 35.0 | 8.07 | 361 | 9.85 | 737 | 0.36 |
| P2 | | 23.5 | 7.32 | 4.48 | 19.0 | 8.10 | 263 | 6.04 | 542 | 0.26 |
| P3 | | 22.0 | 6.51 | 4.48 | 23.0 | 8.36 | 306 | 6.01 | 627 | 0.30 |
| P4 | | 23.0 | 6.98 | 3.06 | 22.0 | 7.99 | 331 | 5.08 | 677 | 0.33 |
| P5 | | 23.0 | 6.92 | 2.89 | 15.0 | 8.33 | 330 | 5.42 | 678 | 0.33 |
| P1 | November 2013 | 24.0 | 6.20 | 10.5 | 24.0 | 7.16 | 379 | 12.1 | 763 | 0.38 |
| P2 | | 24.0 | 7.00 | 8.07 | 18.0 | 8.03 | 240 | 11.9 | 630 | 0.25 |
| P3 | | 23.0 | 6.45 | 4.01 | 12.0 | 8.42 | 232 | 4.80 | 601 | 0.33 |
| P4 | | 21.0 | 6.04 | 3.20 | 14.0 | 7.79 | 328 | 5.72 | 648 | 0.31 |
| P5 | | 22.0 | 6.06 | 3.19 | 7.00 | 8.07 | 329 | 6.12 | 699 | 0.31 |
| P1 | December 2013 | 27.5 | 7.11 | 3.20 | 19.0 | 8.44 | 426 | 7.72 | 789 | 0.37 |
| P2 | | 27.0 | 7.59 | 3.99 | 16.0 | 8.14 | 374 | 7.73 | 671 | 0.23 |
| P3 | | 25.0 | 6.00 | 0.99 | 14.0 | 8.53 | 361 | 6.15 | 425 | 0.22 |
| P4 | | 26.0 | 6.04 | 1.42 | 7.00 | 9.02 | 218 | 3.30 | 598 | 0.24 |
| P5 | | 25.0 | 6.19 | 2.12 | 3.00 | 8.80 | 201 | 3.98 | 777 | 0.26 |
| P1 | January 2014 | 28.5 | 8.05 | 9.99 | 58.0 | 8.00 | 386 | 10.1 | 788 | 0.38 |
| P2 | | 28.0 | 8.00 | 6.14 | 47.0 | 7.97 | 278 | 9.40 | 572 | 0.28 |
| P3 | | 27.0 | 7.16 | 2.16 | 23.0 | 8.29 | 310 | 5.70 | 637 | 0.31 |
| P4 | | 27.0 | 7.09 | 1.41 | 31.0 | 8.07 | 316 | 5.64 | 650 | 0.32 |
| P5 | | 26.0 | 6.50 | 1.30 | 33.0 | 8.17 | 327 | 2.11 | 672 | 0.33 |
| P1 | February 2014 | 27.0 | 6.11 | 5.60 | 175 | 7.87 | 424 | 14.4 | 778 | 0.31 |
| P2 | | 25.0 | 6.20 | 4.31 | <10 | 7.16 | 430 | 13.7 | 530 | 0.25 |
| P3 | | 25.0 | 6.01 | 0.97 | <10 | 8.94 | 325 | 8.90 | 489 | 0.25 |
| P4 | | 24.5 | 6.56 | 1.40 | 18.0 | 8.61 | 311 | 7.22 | 579 | 0.21 |
| P5 | | 24.0 | 6.91 | 2.15 | 36.0 | 8.75 | 242 | 4.50 | 780 | 0.27 |
| P1 | March 2014 | 27.0 | 6.16 | 7.44 | 42.0 | 7.99 | 299 | 18.5 | 615 | 0.30 |
| P2 | | 26.5 | 6.27 | 6.98 | 1.00 | 8.50 | 230 | 12.1 | 477 | 0.23 |
| P3 | | 26.0 | 6.14 | 1.63 | 35.0 | 7.64 | 230 | 24.1 | 476 | 0.23 |
| P4 | | 23.0 | 6.09 | 2.11 | 68.0 | 3.28 | 278 | 17.3 | 578 | 0.28 |
| P5 | | 21.0 | 6.33 | 2.87 | 14.0 | 7.68 | 307 | 9.12 | 631 | 0.31 |
| P1 | April 2014 | 25.0 | 7.99 | 7.71 | 34.0 | 7.97 | 234 | 6.32 | 474 | 0.24 |
| P2 | | 25.0 | 7.18 | 7.65 | 26.0 | 6.42 | 225 | 5.41 | 466 | 0.22 |
| P3 | | 24.5 | 6.02 | 2.16 | <10 | 7.35 | 273 | 6.21 | 562 | 0.27 |
| P4 | | 22.0 | 6.24 | 0.48 | 34.0 | 7.00 | 290 | 4.36 | 597 | 0.29 |
| P5 | | 22.0 | 6.23 | 1.91 | 30.0 | 7.29 | 295 | 1.16 | 606 | 0.29 |
| P1 | May 2014 | 25.0 | 8.92 | 2.40 | 269 | 7.79 | 360 | 11.9 | 737 | 0.36 |
| P2 | | 21.0 | 8.56 | 7.56 | <10 | 8.15 | 275 | 9.48 | 566 | 0.27 |
| P3 | | 22.0 | 7.75 | 0.76 | <10 | 8.47 | 293 | 6.79 | 602 | 0.29 |
| P4 | | 21.5 | 7.95 | 2.99 | <10 | 8.51 | 302 | 7.89 | 621 | 0.30 |
| P5 | | 22.0 | 7.76 | 6.21 | <10 | 8.83 | 311 | 8.10 | 639 | 0.31 |
| P1 | June 2014 | 20.0 | 9.04 | 12.4 | 72.0 | 9.46 | 388 | 3.32 | 655 | 0.36 |
| P2 | | 20.0 | 8.87 | 6.74 | 6.00 | 8.77 | 301 | 3.68 | 650 | 0.29 |
| P3 | | 21.0 | 6.52 | 3.22 | <10 | 6.09 | 269 | 5.05 | 425 | 0.26 |
| P4 | | 20.0 | 6.30 | 4.01 | <10 | 7.76 | 284 | 5.80 | 672 | 0.31 |
| P5 | | 21.0 | 8.40 | 4.12 | 2.00 | 8.00 | 312 | 4.16 | 691 | 0.33 |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **P1** | **July 2014** | 20.0 | 8.98 | 1.93 | 31.0 | 8.57 | 426 | 12.1 | 869 | 0.43 |
| **P2** | | 19.0 | 8.05 | 8.41 | 19.0 | 5.66 | 267 | 6.18 | 552 | 0.27 |
| **P3** | | 18.0 | 7.20 | 3.10 | 30.0 | 9.17 | 287 | 9.79 | 592 | 0.29 |
| **P4** | | 19.0 | 7.14 | 3.18 | 32.0 | 8.19 | 297 | 7.96 | 611 | 0.30 |
| **P5** | | 19.0 | 6.99 | 5.92 | 30.0 | 8.58 | 314 | 5.98 | 646 | 0.31 |
| **P1** | **August 2014** | 21.0 | 8.16 | 9.27 | 42.0 | 6.42 | 267 | 8.48 | 549 | 0.27 |
| **P2** | | 21.0 | 8.22 | 5.47 | 37.0 | 4.84 | 233 | 8.71 | 484 | 0.23 |
| **P3** | | 20.0 | 6.29 | 3.21 | 27.0 | 5.21 | 298 | 8.07 | 626 | 0.30 |
| **P4** | | 19.0 | 6.11 | 2.92 | 28.0 | 6.88 | 290 | 7.23 | 596 | 0.29 |
| **P5** | | 19.0 | 7.12 | 3.07 | 24.0 | 8.08 | 291 | 4.26 | 599 | 0.29 |
| **P1** | **September 2014** | 20.0 | 7.20 | 10.8 | 27.0 | 7.35 | 334 | 62.4 | 684 | 0.33 |
| **P2** | | 21.0 | 7.01 | 10.3 | <10 | 5.95 | 233 | 16.4 | 482 | 0.23 |
| **P3** | | 19.5 | 6.92 | 4.62 | <10 | 6.64 | 279 | 12.5 | 574 | 0.28 |
| **P4** | | 18.0 | 6.68 | 3.04 | 71.0 | 6.89 | 301 | 12.1 | 619 | 0.30 |
| **P5** | | 21.0 | 8.76 | 3.28 | <10 | 6.76 | 316 | 8.57 | 650 | 0.32 |

Turbidity and $BOD_5$ showed significant difference ($p < 0.01$) to each other while COD showed a low positive correlation ($r = 0.293$; $p < 0.023$) to E.C. The DO content depicted a weak positive correlation ($r = 0.320$; $p < 0.013$) to E.C. measurements at all sampling months and points. Salinity showed low positive correlations ($r = 0.297$; $p < 0.021$ and $r = 0.282$; $p < 0.029$) to pH and COD, respectively. Furthermore, a strong positive correlation was observed between E.C. and salinity ($r = 0.778$; $p < 0.000$).

### 2.3.2 Bacterial indicator analysis

Total heterotrophic bacteria (THB) were enumerated at all sampling months and points along the Umhlangane River (Figure 2.2). The general trend for THB proliferation was the sudden increase in population numbers during December, January and February (summer months) followed by a progressive monthly decrease as the colder conditions came about (lowest during June, July and August). A subsequent increase in THB populations was observed in September. The highest and lowest THB counts were detected at P1 (Phoenix industrial) and P3 (natural wetlands) with values ranging from 14.9 x $10^6$ cfu/100mℓ in January 2014 to 1.3 x $10^6$ cfu/100mℓ in July 2014, respectively. Significant difference ($p < 0.000$) between the THB populations and the sampling months was observed while no significant difference ($p > 0.05$) was seen between THB counts and the sampling points.

Presumptive *E. coli* (EC), total coliform (TC) and faecal coliform (FC) counts fluctuated at all sampling points and months along the Umhlangane River and are depicted in Figure 2.3 a, b and c. The monthly EC populations depicted significantly different ($p < 0.01$) variations ranging from 6.1 x $10^3$ cfu/100mℓ (January 2014; P3) to 0.87 x $10^3$ cfu/100mℓ (August 2014; P4). The TC and FC counts ranged from 5.9 x $10^3$ cfu/100mℓ (January 2014;

P1) to 0.63 x $10^3$ cfu/100mℓ (August 2014; P3) and 6.6 x $10^3$ cfu/100mℓ (December 2013; P1) to 0.36 x $10^3$ cfu/100mℓ (September 2014; P3), respectively. Significant differences ($p < 0.01$) between the TC and FC populations and the sampling months were observed. No significant difference ($p > 0.05$) was seen between all 3 indicators (EC, TC and FC) and the sampling points along the Umhlangane River.

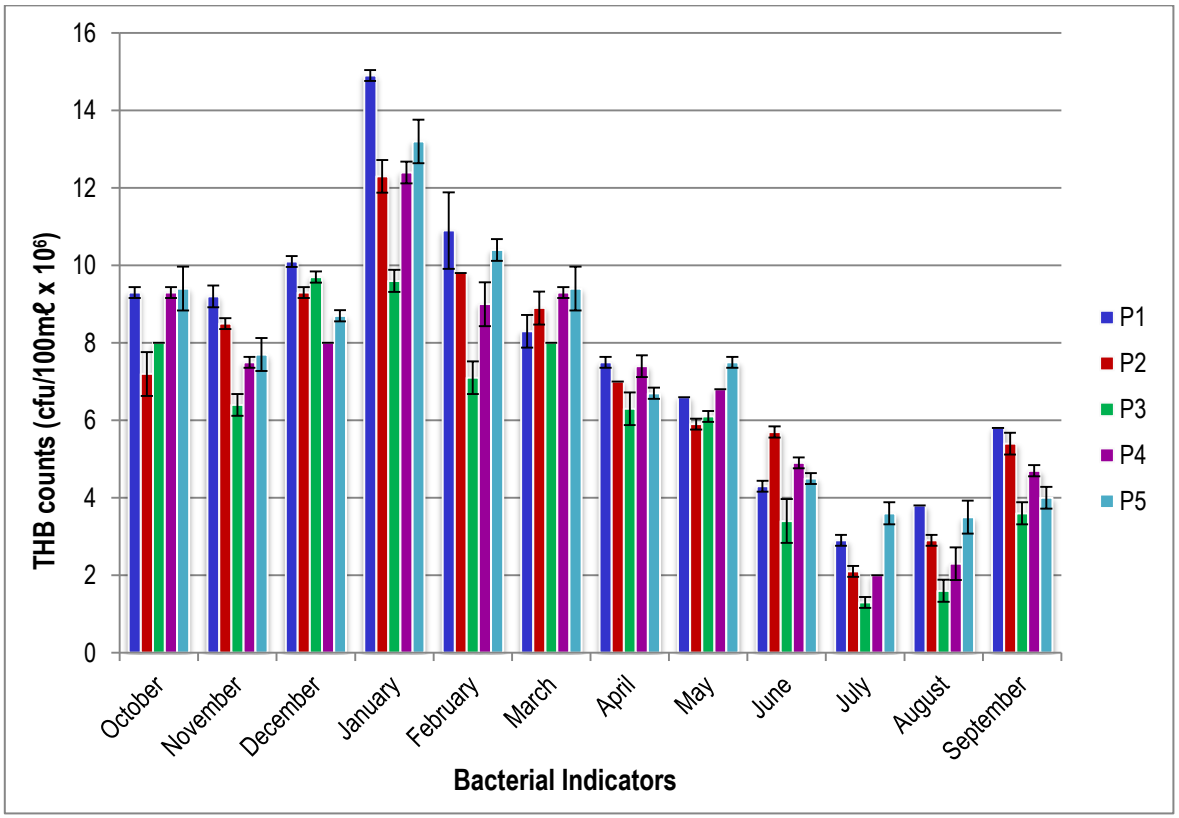


**Figure 2.2** Total heterotrophic bacterial (THB) populations at the five sampling points along the Umhlangane River from October 2013 to September 2014. Bars indicate the averages ($n = 2$) with standard deviation depicted by the error bars.

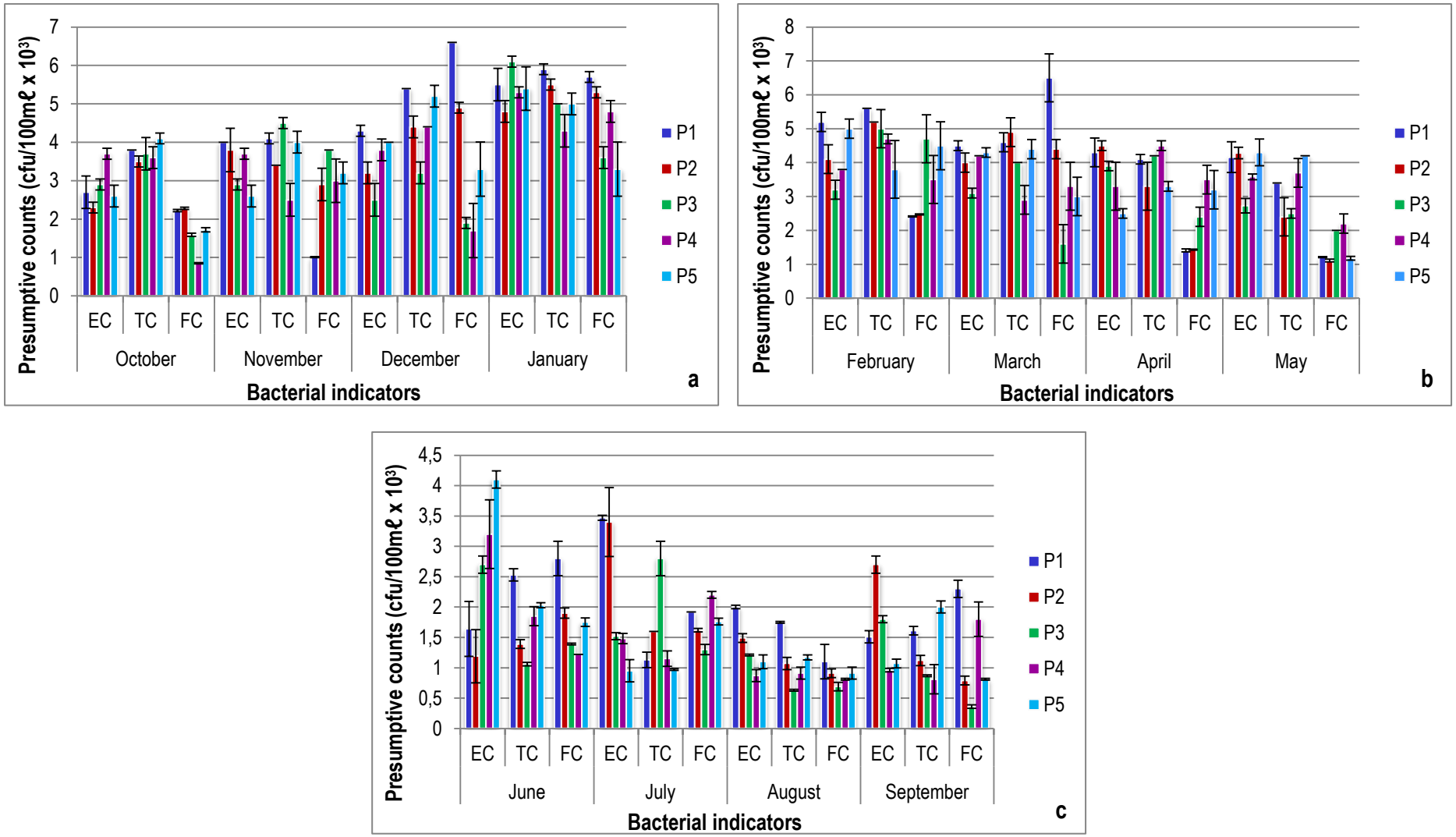


**Figure 2.3** Presumptive *E. coli* (EC), total coliform (TC) and faecal coliform (FC) counts at the five sampling points along the Umhlangane River from October 2013 to September 2014. Bars indicate the averages ($n$ = 2) with standard deviation depicted by the error bars.

Presumptive faecal streptococci (FS), *Vibrio* spp. (VIB), *Salmonella* spp. (SAL) and *Shigella* spp. (SHIG) were enumerated at all sampling months and points (Figure 2.4 a, b, c) alongside the Umhlangane River. Significant difference ($p < 0.01$) between the FS populations and the sampling months was observed and ranged from 0.4 x $10^3$ cfu/100mℓ (September 2014; P3) to 7.5 x $10^3$ cfu/100mℓ (April 2014; P1). The VIB counts depicted its lowest and highest values at point P3 (natural wetlands) in March and February 2014 with values ranging from 0.5 x $10^3$ cfu/100mℓ to 7.2 x $10^3$ cfu/100mℓ. Significant ($p < 0.05$) differences were observed with the monthly VIB populations in the river water samples. The SAL and SHIG counts ranged from 0.27 x $10^3$ cfu/100mℓ (August 2014; P4) to 6.5 x $10^3$ cfu/100mℓ (April 2014; P2) and 0.43 x $10^3$ cfu/100mℓ (August 2014; P5) to 5.5 x $10^3$ cfu/100mℓ (January 2014; P1), respectively. No significant difference ($p > 0.05$) was seen between the SAL and SHIG populations and the sampling points and months.

The FS, VIB, SAL, and SHIG showed a general trend where high counts were enumerated during the warmer months (October 2013 – March 2014) while gradual decreases were observed as the conditions became colder (May – August 2014). The same trend was seen for the EC, TC and FC populations in the river water samples. Bacterial counts recorded during September indicated a slight increase in the number of enumerated indicator bacteria. Moreover, higher bacterial counts were observed at the industrial sites (P1, P2 and P5).

Positive significant correlations were observed amongst all 8 bacterial indicators as depicted in Table 2.3. The THB populations showed strong correlations with EC ($r = 0.753$; $p < 0.000$) and TC ($r = 0.843$; $p < 0.000$) populations while strong correlations was seen between TC and EC ($r = 0.745$; $p < 0.000$). Moderate to weak correlations was seen between the other indicators and no correlation ($r = 0.190$; $p > 0.145$) was observed between SHIG and VIB populations.

**Table 2.3** Correlation coefficients ($r$) for the 8 bacterial indicators at all months and points.

| Indicators | THB | EC | TC | FC | FS | VIB | SAL | SHIG |
|---|---|---|---|---|---|---|---|---|
| **THB** | 1.000 | | | | | | | |
| **EC** | 0.753** | 1.000 | | | | | | |
| **TC** | 0.843** | 0.745** | 1.000 | | | | | |
| **FC** | 0.627** | 0.514** | 0.662** | 1.000 | | | | |
| **FS** | 0.549** | 0.534** | 0.618** | 0.471** | 1.000 | | | |
| **VIB** | 0.344** | 0.260* | 0.366** | 0.289* | 0.258* | 1.000 | | |
| **SAL** | 0.405** | 0.439** | 0.343** | 0.349** | 0.358** | 0.285* | 1.000 | |
| **SHIG** | 0.496** | 0.468** | 0.533** | 0.423** | 0.300* | 0.190 | 0.278* | 1.000 |

* Correlation significant at $p < 0.05$; ** correlation significant at $p < 0.01$

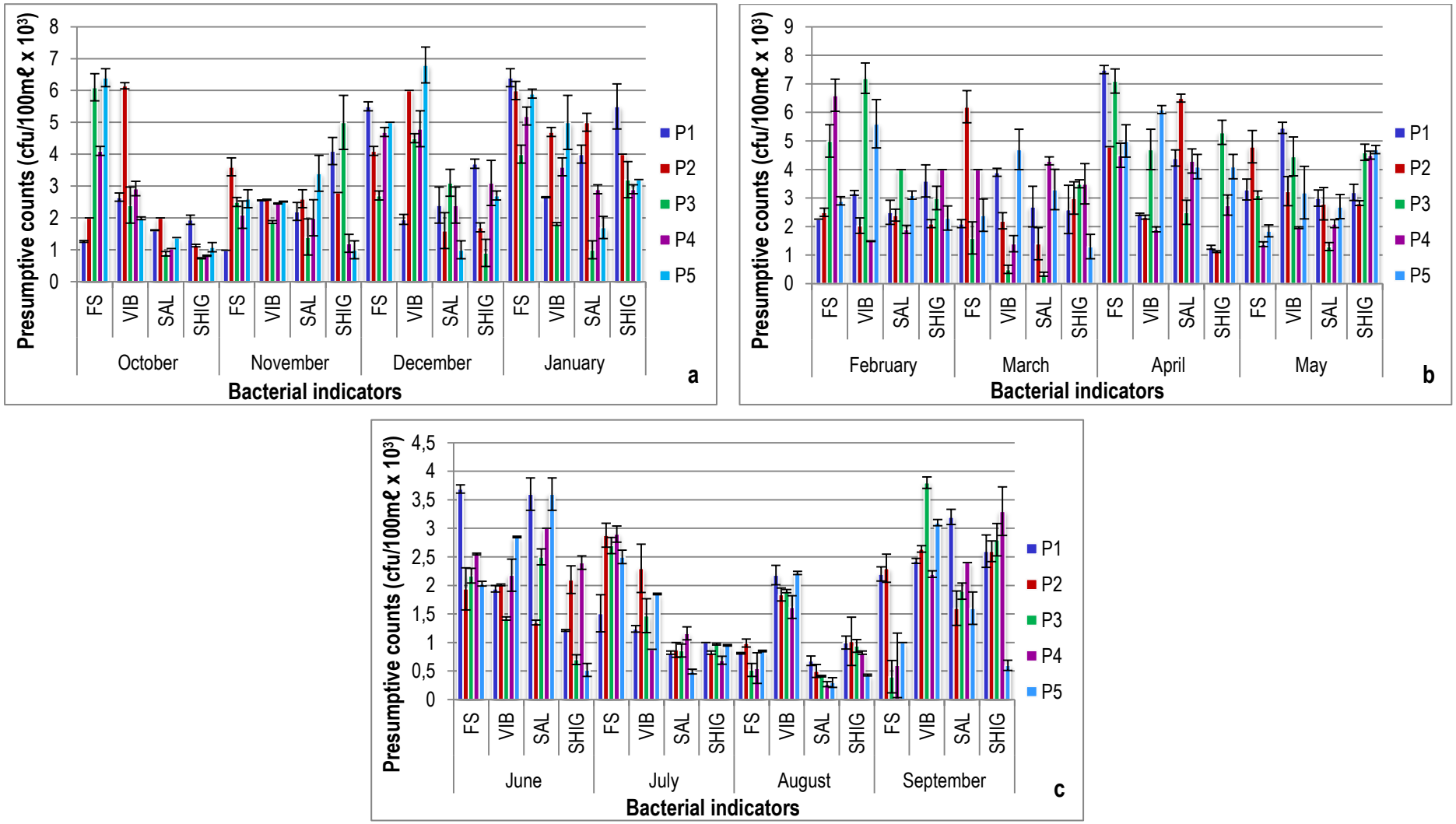


**Figure 2.4** Presumptive faecal streptococci (FS), *Vibrio* spp. (VIB), *Salmonella* spp. (SAL) and *Shigella* spp. (SHIG) counts at the five sampling points along the Umhlangane River from October 2013 to September 2014. Bars indicate the averages ($n$ = 2) with standard deviation depicted by the error bars.

The average FC/FS ratios for all sampling months are depicted in Table 2.4 and ranged from 0.41 and 1.19 in April and August, respectively. Three months showed strong evidence for animal pollution while the remaining months were predominantly domestic wastes.

**Table 2.4** Average faecal coliform (FC) to faecal streptococci (FS) ratio from October 2013 to September 2014.

| Sampling months | FC/FS ratio | Sampling months | FC/FS ratio | Sampling months | FC/FS ratio |
|---|---|---|---|---|---|
| October | 0,44[a] | February | 0,91[b] | June | 0,73[b] |
| November | 1,18[b] | March | 1,15[b] | July | 0,70[a] |
| December | 0,84[b] | April | 0,41[a] | August | 1,19[b] |
| January | 0,83[b] | May | 0,53[b] | September | 0,93[b] |

[a] Pollution is of animal origin
[b] Pollution is predominantly domestic wastes in mixed pollution

### 2.3.3 Canonical correspondence analysis

Canonical correspondence analysis (CCA) was used to evaluate the relationships between all the tested water quality parameters (physico-chemical and bacterial indicators) amongst the sampling months and points along the Umhlangane River. Figure 2.5 represents the ordination plot for the physico-chemical and bacterial indicators during the sampling months and points. The plot revealed a strong relationship between FC, TC and temperature while a moderate relationship was seen between TDS, E.C., TC and FC populations. The THB populations showed correlations with salinity, TDS and E.C. Turbidity, BOD and SHIG populations depicted close relationships with pH and COD showing more closeness to the EC populations. The plot also revealed that SAL, VIB and FS populations were not strictly associated with the physico-chemical parameters or other bacterial indicators. The variance of species data for CCA axis 1 was found to be 8.6% and the species – environment relation was 72.2%. This suggests strong variance between the physico-chemical and bacterial data compared to the species data alone. The eigenvalues for both axes were significant ($p < 0.05$) while moderate positive correlations was observed for both axes.

The sampling points over the 12 month period seemed to be mostly clustered around the origin although a few points are scattered throughout the ordination diagram as depicted in Figure 2.6. According to the CCA plot most sampling points from April to September was in close proximity to pH. Temperature, salinity, TDS and E.C. showed closer relationships to most of the sampling points during the warmer months (January – March) thus impacting the

bacterial indicators. Sampling points found closer to the origin suggest that all physico-chemical parameters had an impact on the bacterial populations.

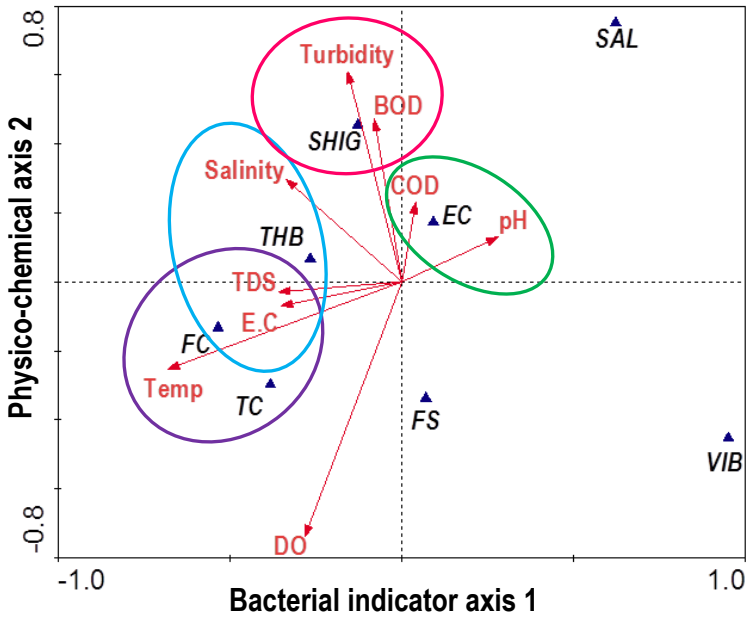


**Figure 2.5** CCA ordination diagram for the physico-chemical parameters and bacterial indicators at the five sampling points from October 2013 to September 2014.

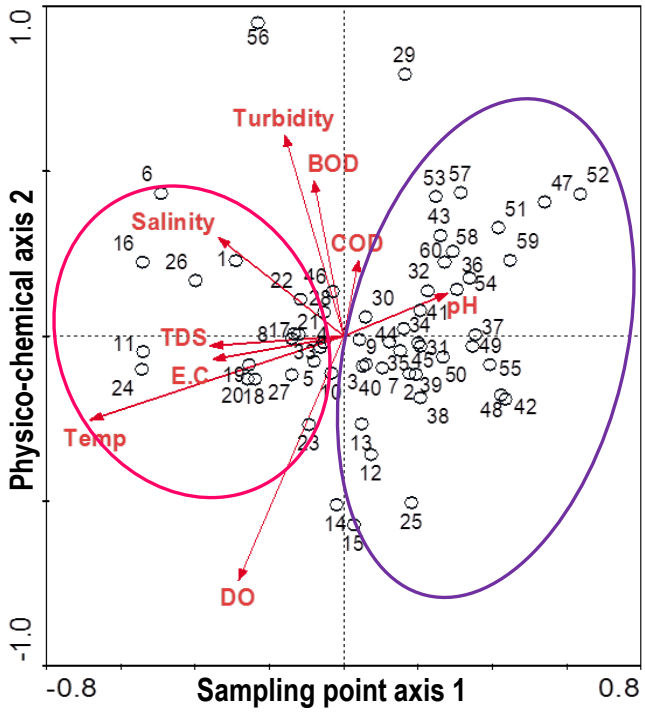


**Figure 2.6** CCA ordination diagram for the physico-chemical and bacterial indicators at the five sampling points from October 2013 to September 2014. Numbers 1-60: sampling points represented at each month.

### 2.3.4 Presence-absence coliphage test

The somatic and F$^+$RNA coliphages were detected using the formation of plaques on a lawn of their respective bacterial hosts. The presence of the somatic coliphages (Table 2.5) were highly prevalent in the Umhlangane showing positive plaque formation for all sampling sites and months with the exception of P3 (natural wetlands) in August 2014. The F$^+$RNA coliphages (Table 2.3) were found absent from P3 and P4 (July 2014), P3 and P4 (August 2014) as well as P3 and P5 (September 2014).

**Table 2.5** Presence-absence spot test for the somatic and F$^+$RNA coliphages during the 12 month sampling period.

| Sampling points | | Somatic | F$^+$RNA | Sampling points | | Somatic | F$^+$RNA | Sampling points | | Somatic | F$^+$RNA |
|---|---|---|---|---|---|---|---|---|---|---|---|
| October | P1 | + | + | February | P1 | + | + | June | P1 | + | + |
| | P2 | + | + | | P2 | + | + | | P2 | + | + |
| | P3 | + | + | | P3 | + | + | | P3 | + | + |
| | P4 | + | + | | P4 | + | + | | P4 | + | + |
| | P5 | + | + | | P5 | + | + | | P5 | + | + |
| November | P1 | + | + | March | P1 | + | + | July | P1 | + | + |
| | P2 | + | + | | P2 | + | + | | P2 | + | + |
| | P3 | + | + | | P3 | + | + | | P3 | + | – |
| | P4 | + | + | | P4 | + | + | | P4 | + | – |
| | P5 | + | + | | P5 | + | + | | P5 | + | + |
| December | P1 | + | + | April | P1 | + | + | August | P1 | + | + |
| | P2 | + | + | | P2 | + | + | | P2 | + | + |
| | P3 | + | + | | P3 | + | + | | P3 | – | – |
| | P4 | + | + | | P4 | + | + | | P4 | + | – |
| | P5 | + | + | | P5 | + | + | | P5 | + | + |
| January | P1 | + | + | May | P1 | + | + | September | P1 | + | + |
| | P2 | + | + | | P2 | + | + | | P2 | + | + |
| | P3 | + | + | | P3 | + | + | | P3 | + | – |
| | P4 | + | + | | P4 | + | + | | P4 | + | + |
| | P5 | + | + | | P5 | + | + | | P5 | + | – |

*Plaque formation: +: positive plaques; –: no visible plaques

### 2.3.5 Somatic and F$^+$RNA enumeration

Enumeration of the somatic and F$^+$RNA coliphages using the double-overlay plaque assay for the duration of the study is depicted in Figure 2.7. Moderate correlations were seen between the somatic ($r$ = -653; $^{**}p < 0.000$) and F$^+$RNA ($r$ = -643; $^{**}p < 0.000$) coliphages and the time of sampling. Interesting, the somatic and F+RNA coliphages depicted the lowest counts at P3 (natural wetlands) in August 2013 and the highest at P1 (Phoenix industrial) in

January 2014 ranging from 24.5 x $10^2$ pfu/mℓ to 765 x $10^2$ pfu/mℓ and 10 x $10^2$ pfu/mℓ to 585 x $10^2$ pfu/mℓ, respectively. No significant difference ($p > 0.01$; 0.05) was seen between both phages and the sampling points.

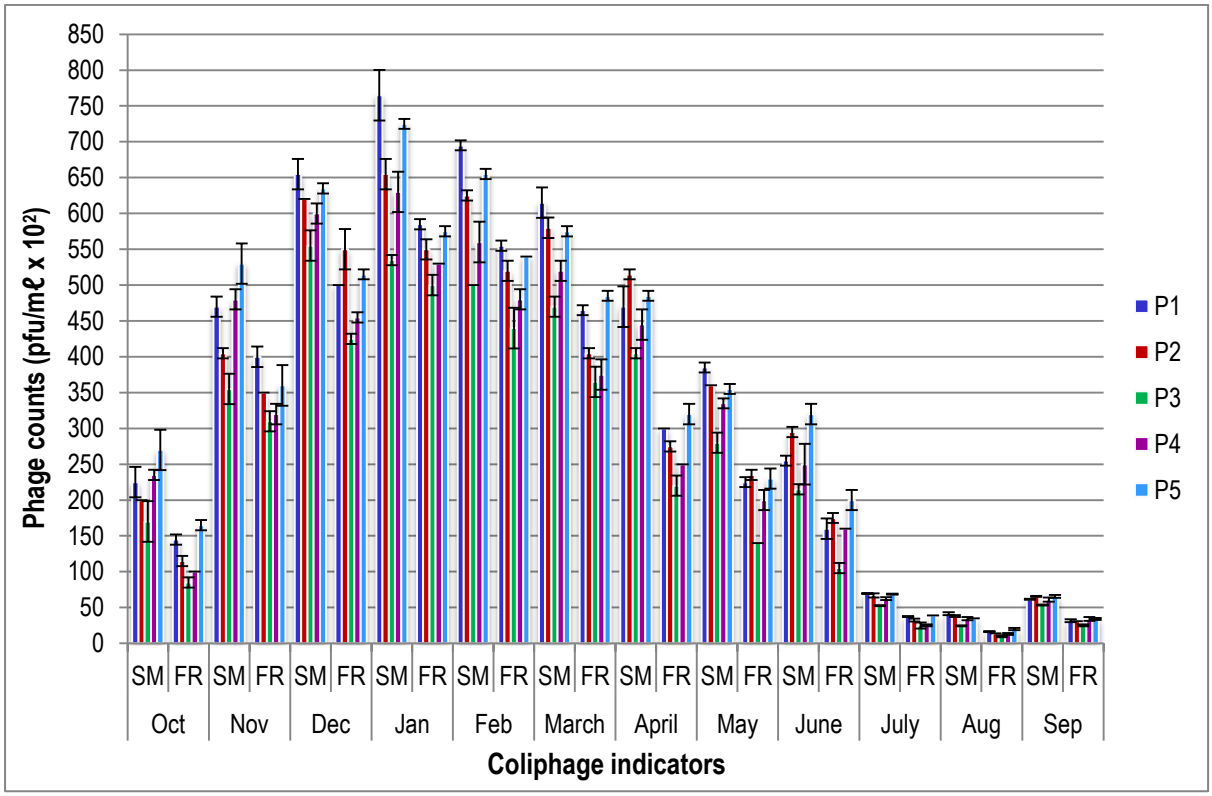


**Figure 2.7** Somatic and F$^+$RNA coliphage counts for the five sampling points along the Umhlangane River from October 2013 to September 2014. Bars indicate the averages ($n = 2$) with standard deviations depicted by the error bars. Abbreviations: SM: somatic phage and FR: F$^+$RNA phage.

The somatic and F$^+$RNA coliphages displayed a similar trend observed to that of the bacterial indicators (Figures 2.3 and 2.4 a, b, c) where higher counts were noted during the warmer months compared to when colder conditions were experienced. A strong positive correlation ($r = 0.977$; $p < 0.000$) was seen between the somatic and F$^+$RNA coliphage populations.

The CCA bi-plot (Figure 2.8) revealed no direct relationship between the somatic (pink circle) and F$^+$RNA (purple circle) coliphages and weak relations was observed between the physico-chemical and phage communities. All physico-chemical parameters spanned from the origin to lie relative to each other across axis 1 (blue circle). This shows that in spite of no

direct relationship observed between the phage and physico-chemical parameters it did have some impact on the coliphage populations.

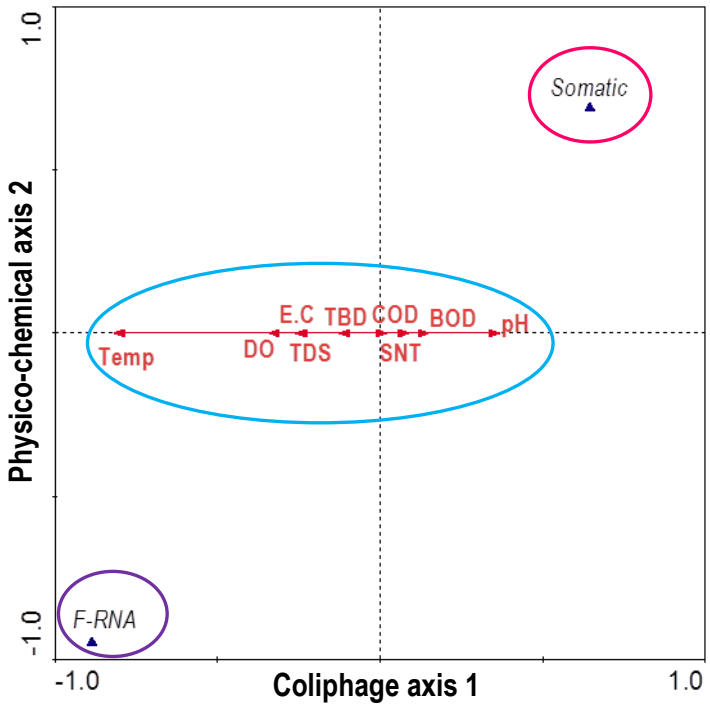


**Figure 2.8** CCA ordination plot for the somatic and F$^{+}$RNA coliphages and physico-chemical parameters at the five sampling points from October 2013 to September 2014. Abbreviations: TBD: turbidity and SNT: salinity.

A similar relationship was observed between the coliphage, physico-chemical parameters, sampling months and points (Figure 2.9). Sampling points during January and March were found to have stronger relations with turbidity, E.C., temperature, DO and TDS while most sampling conducted during the latter, colder months was found in close proximity to COD, salinity, pH and BOD. However, most points were found scattered and not clustered as seen with the bacterial population sampling points and months. The sampling points at each sampling month had a stronger relationship with the physico-chemical parameters rather than with the phage itself.

The relationship between the bacterial and coliphage populations is depicted in Figure 2.10. The ordination bi-plot revealed strong associations between SAL, EC, THB, SHIG, TC and the coliphage populations (purple circle). This indicates that these bacterial communities contributed to the variance and prevalence of phage in the river water. The FS, FC and VIB spp. did not impact the somatic and F$^{+}$RNA coliphages. The total variance percentage of species data was 29% and the cumulative variance of species−environment relation

accounted for 95.1%. This suggests a close relation between the bacterial and phage communities. The eigenvalues for both axes were found to be significant (Appendix ii).

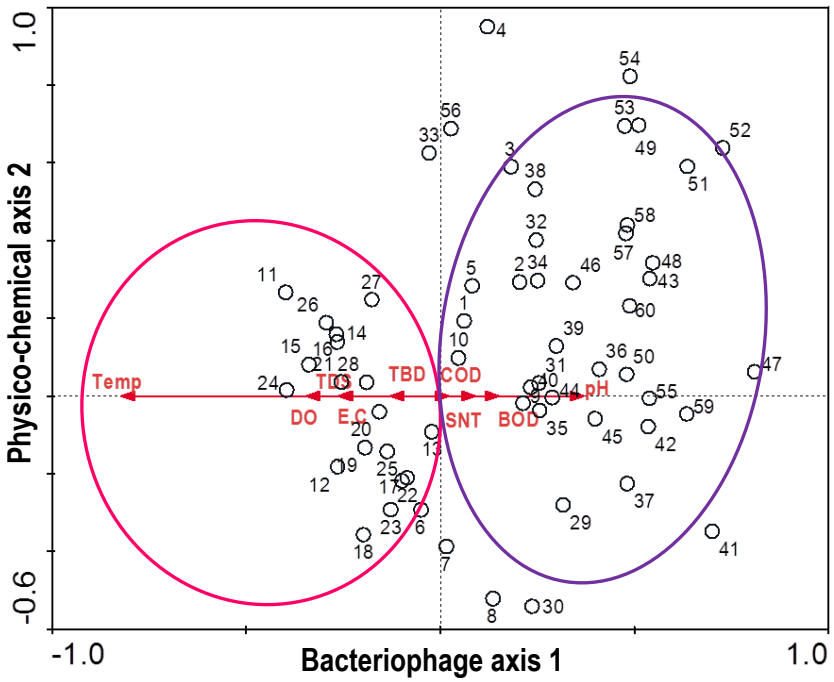


**Figure 2.9** CCA ordination plot for the somatic and F$^+$RNA bacteriophages and physico-chemical parameters at the 5 sampling points during October 2013 to September 2014. Abbreviations: TBD: turbidity and SNT: salinity. Numbers 1-60: sampling points at each month.

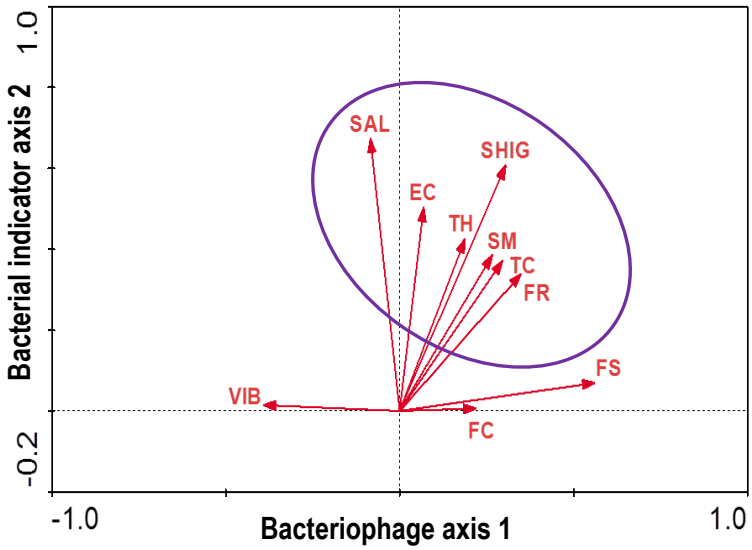


**Figure 2.10** CCA ordination plot for the coliphage and bacterial indicators at the 5 sampling points during from October 2013 to September 2014. Abbreviations: TH: total heterotrophs, SM: somatic phage and FR: F$^+$RNA phage.

## 2.4 Discussion

This study evaluated the physico-chemical properties of the Umhlangane River over a 12 month period at five sampling points of different land use zones. Temperature fluctuated through the sampling period with the highest recorded temperatures in December, January and February and the lowest during June, July and August. This increase and decrease in temperature measurements corresponds with the fluctuating monthly bacterial growth observed during the sampling period (Figures 2.4; 2.5 a, b, c and 2.7). The increase or decrease in temperature affects the chemical and physical properties of water (Papafilippaki *et al*., 2008).

According to the South African water quality guidelines for recreational use pH was within the recommended limit of 6.5 to 8.5 with the exception of P1 and P2 in May and June, P1 in July and P5 in September. Natural biological processes and anthropogenic influences such as nutrient cycling and the discharge of industrial effluents, respectively as well as alien plant or algal growth, runoffs (urban or agricultural) and microbial activity affect the fluctuations of pH range within surface water bodies (DWAF, 1996a). The $BOD_5$ test was used to estimate the amount of oxygen required to degrade the organic matter present in the river while COD was used to estimate the total of all inorganic and organics in the river water. High levels of BOD and COD have been shown to affect both the taste and odour of water sources (Kolawole *et al*., 2011). Currently, the DWA does not stipulate a recommended limit for BOD in recreational or industrial water use (DWAF, 1996b; c). Davies (2009) stipulated that water bodies with a BOD measurement of <4 mg $O_2/\ell$ is considered unpolluted and an acceptable range. The BOD measurements for P1 and P2 in all months except December, May and July as well as P2, P5 in May and July and P3 in October, November and September did not fall within this limit. The correlation between pH and BOD indicates that as BOD increased so did the pH as can be seen with the predominantly high pH and BOD values at P1 and P2. The COD content of the river water samples was found to exceed the recommended water quality limit of 0 – 10 mg $O_2/\ell$ for industrial use with the exception of P5 in November, P4, P5 in December, P2, P3 in February, P2 in March, P3 in April, P2 – P5 in May, P3 – P5 in June and P2, P3 and P5 in September. This high amount of organics may exist within the river in either an autochthonous or allochthonous form. Autochthonous organic matter spontaneously arises within the water through the death and growth of aquatic organisms (Nebbioso and Piccolo, 2012). The latter form of organics originates from an external environment. Agricultural, pasture, urban and industrial wastes contribute significantly to the accumulation of organic wastes in water bodies (DWAF,

1996b). Therefore, the highly differentiated land use zones and their respective wastes surrounding the Umhlangane River may have contributed to the high amount of organic matter detected by the COD test. Furthermore, the elevated COD levels coupled with the fluctuating DO content, particularly lower during August and September (5.21 mg $O_2$/ℓ to 5.29 mg $O_2$/ℓ, respectively) compared to the other sampling months could reiterate the discharge of organic pollution into the river at that time (Caraco *et al*., 2000). Moreover, the different land activities may have allowed for the fluctuations in the BOD and COD levels. This can be seen particularly at the industrial sites in Phoenix, Springfield and Riverhorse Valley where BOD and COD levels are high. The $BOD_5$/COD ratio of biodegradability revealed no trend amongst the sampling points or months. Most sites had a ratio of <0.2 indicating that the substances present in the river is not biodegradable. This could be due to the discharge of industrial effluent at the time of sampling (Kumar *et al*., 1998). However, th ratio of some water samples of P3 and P5 were in the range of 0.4 – 0.5 (average) or >0.5 (easily biodegradable). Since P3 is a natural wetland it is possible that most of the matter present may be animal faecal matter whereas P5 is downstream of the Northern WWTP which may allow for the discharge of domestic effluents to enter the river.

The total rainfall data (October 2013: 89 mm; November: 62 mm; December: 191 mm January 2014: 105 mm; February: 47 mm; March: 151 mm; April: 19 mm; May: 0 mm; June: 0 mm; July: 0 mm; August: 8 mm and September: 20 mm) for the sampling period was obtained (Accuweather, 2014). The negative correlation observed between turbidity and the sampling points may be due to the discharge of different waste effluents and changing anthropogenic activities at each of these tested points along the river (Salmore *et al*., 2006). Although no temporal variations were observed with turbidity fluctuating climatic conditions may have influenced the level of turbidity measured in the water samples. Turbidity increases during periods of increased precipitation due to the washing out of various waste effluents and debris into rivers (Zamxaka *et al*., 2004; Neves *et al*., 2014). Thus, the higher values recorded during the rainy months, particularly March may have permitted for the increase in turbidity. All TDS and E.C. values exceeded the permissible limit of 0 – 100 mg/ℓ and 0 – 15 mS/m for industrial use, respectively (DWAF, 1996c). However, the slight decrease in TDS and E.C. at P3 and P4 may be due to self-purification processes along the river (Kolawole *et al*., 2011). Furthermore, downstream points P3 and P4 may contain minerals that might contribute to the conductivity levels while upstream points P1 (Phoenix industrial), P2 (residential, upstream KwaMashu WWTP) and downstream point P5 (Springfield industrial, downstream NWWTP) might be influenced by highly charged industrial effluent (Mahazar *et*

*al*., 2013). The overall variations in TDS and E.C. measurements throughout the sampling period and points may be due to changes in human activities and climatic changes (Kolawole *et al*., 2011). Increased E.C. and TDS can be linked to the high temperature values at the upstream points in Phoenix and Springfield industrial compared to the lower temperatures seen at P3 and P4. Similar observations were observed by Mahazar *et al*. (2013). Salinity is the measurement of water to successfully conduct and electrical current and can therefore be related to TDS and E.C. (Kadhem, 2013). No direct correlation between salinity and TDS was observed however the low positive correlations between salinity, pH and COD are indicative of inorganic or organic pollution. Furthermore, the strong positive correlation between salinity and E.C. means that as one variable increased so did the other.

The rapid change in bacterial enumeration amongst the five sampling points could be due to changing anthropogenic activities during the period of analysis (Kolawole *et al*., 2011). The detection of THB populations is an estimation of the general quality of water thus higher values are indicative of poor water quality (DWAF, 1996a). The high enumeration of THB during the warmer months (November 2013 to March 2014) may be attributed with the warm conditions, supporting bacterial growth and the increased rainfall as surface effluents are consequently drained into the river (Saha *et al*., 2009; Edun *et al*., 2012). The significant difference observed between THB and all other indicators tested in this study shows that THB populations did influence the prevalence of the other indicators.

Faecal indicator bacteria are usually employed for the assessment of pathogenic microorganisms, faecal pollution and the inherent risk of transmissible waterborne infections (Kishinhi *et al*., 2013). The occurrence of TC and FC populations in the water suggests that faecal contamination is entering the river (Saha *et al*., 2009; Kolawole *et al*., 2011). These contaminations are frequently manifested mainly through diarrhoea and on occasion fever and other secondary complications (Antony and Renuga, 2012). Irregular counts of both indicators were noted throughout the study and this was similar to findings reported by Chandra *et al*. (2006), Kolawole *et al*. (2011) and Sengupta *et al*. (2014). However, seasonal incidences of both indicators were noted as also described by Bezuidenhout *et al*. (2002). The enumeration of EC populations in the river water samples indicates the presence of faecal contamination originating from warm-blooded animals. Bacterial pathogens including *Shigella* spp. *Salmonella* spp., *Campylobacter jejuni*, *Yersinia enterocolitica*, *Vibrio cholerae* and pathogenic *E. coli* are indicated by FC populations (DWAF, 1996b). Faecal coliforms and EC populations are usually examined together for the estimation of faecal pollution (Kolawole *et al*., 2011). According to the South African water quality guidelines for

recreational use the permissible limit for negligible risk to FC populations should be 0 – 130 cfu/100mℓ (DWAF, 1996b) where all counts exceeded this limit. The fluctuating prevalence of FS is an indication of both animal and human faecal pollution entering the river through urban and wastewater discharges, agricultural effluent or surface runoffs from informal settlements (Tallon *et al*., 2005).

*Vibrios* have been associated with domestic sewage and can cause illness in both animals and humans if contaminated food and water has been consumed (Igbinosa *et al*., 2009). The VIB spp. enumerated from the collected water samples may be predominantly of animal origin since most VIB spp. found in water are due to animal faeces (Keshav *et al*., 2010; Asplund, 2013; Takemura *et al*., 2014). The entry of animal faecal matter in this study could be from the cattle that regularly pass through the river at the Phoenix industrial area and the goats that are kept by the low cost residential farmers in KwaMashu and Phoenix. Most VIB bacteria exist in the viable but non-cultivable state (VBNC) in water environments which may have allowed for the low estimation of these populations in the water samples (du Preez *et al*., 2010). The presence of *Salmonella* spp. and *Shigella* spp. in the Umhlangane is major health concern. Previous studies have shown that meteorological conditions, anthropogenic activities and the defecation of infected individuals are the main drivers of contamination into surface waters (Kinge and Mbewe, 2010; Levantesi *et al*., 2012). A study conducted by Levantesi *et al*. (2012) stated that 57% of all SAL occurrences in river water are due to pasture and agricultural runoffs as well as the inflow of animal and human faecal matter. The relationship observed between EC, COD and pH indicates that the proliferation of EC was dependent on the amount of organic wastes and nutrients that may be present in the river water (Mishra *et al*., 2009) while the prevalence of SHIG spp. could have been predominantly influenced by the amount of oxygen and suspended matter present. The CCA graph also revealed that salinity had an impact on the THB populations in the river water. The presence of coliforms is not highly impacted by salinity however it has known to have some effect on bacterial communities (Ortega *et al*., 2009). Thus, a stronger relation was observed with the THB populations.

Interestingly, a distinct relationship was seen between the physico-chemical, bacterial indicators and the sampling months and points. Bacterial community structure has been shown to be affected by various factors such as light intensity (Sigee, 2005), topographical environment (Zhang *et al*., 2011), temperature (Hall *et al*., 2008), available nutrients (Pomeroy and Wiebe, 2001) and pH (Yannarell and Triplett, 2005). The TDS content, E.C., salinity and temperature showed a stronger relationship to the sampling conducted during the

warmer, rainy conditions (Figure 2.6). Singh *et al*. (2013) reported a similar observation where conductivity and TDS were higher in the summer, rainy seasons. The cyclic and seasonal TDS pattern may indicate that the dissolved materials have an allochthonous origin which may have entered the river through surface run-offs (Singh *et al*., 2013). Moreover, the TDS and E.C. was found to run parallel to each other exhibiting the same amount of pressure on the indicators. A similar observation was reported by Johnson (1988) where the author concluded that TDS content proportionally enhanced the conductivity in water and ran analogous to each other. Khalil *et al*. (2014) reported a greater relationship of salinity to the summer months. This may be due to the low water levels and the increase in evaporation (Bahgat, 2011). However, this study reported lower salinity in summer due to decreased rainfall. Since temperature affects the physical and chemical properties of water it would have influenced the biological properties allowing for increased bacterial growth at the time of sampling (Govindarajan *et al*., 2012; Hamaidi-Chergui *et al*., 2013). In addition, turbidity would have been an important factor in microbial proliferation as the increased rainfall would have allowed heavy loads of slit into the river (Singh *et al*., 2013). The relationship between pH and the remaining sampling points could be due to effluent from the surrounding communities that could influence the chemical properties of the river water. This is owing to the leaching of minerals from the nearby infrastructure (Mahazar *et al*., 2013). Additionally, the growth of aquatic plants may also contribute to pH changes (Sabae *et al*., 2014) thus reducing the growth of bacterial indicators. The negative relationship between these sampling points in the CCA graph (Figure 2.6) reinforces the influence on microbial proliferation imposed on by temperature (Kaiser *et al*., 2009; El-Sherbiny *et al*., 2011). However, the reduction in bacterial loads may also be due to the settling of these organisms along the river as sediments have been noted as reservoirs of *E. coli* and other coliforms (Salmore *et al*., 2006).

Coliphages have been employed as microbial indicators of faecal pollution due to their resemblance to enteric viruses in water environments (Leclerc *et al*., 2000). The presence of bacteriophages in water environments is dependent on the survival of their respective bacterial hosts (Chaturongakul and Ounjai, 2014). This was evident by the similar growth or replication trend observed for both the coliphage and bacterial indicator populations throughout the sampling period. Moreover, the obvious abundance of somatic phage in comparison to that of the $F^{+}$RNA phages obtained in this study further reiterates that somatic phages outnumber $F^{+}$RNA phages by a factor of 5 in water environments (Cimenti *et al*., 2007). However, the somatic phage numbers may be over-estimated as these phages may

have replicated in the river water (Scott *et al*., 2002). The DWA (recreational use) recommended limit of 0 – 20 pfu/mℓ was exceeded by all tested river water samples (DWAF, 1996b).

Although no direct relationship was observed between any of the physico-chemical and phage communities, an indirect impact may have played a role in phage variance and survival. The physico-chemical parameters had a greater impact on the bacterial indicators (Figure 2.8) influencing their survival which in turn influenced the coliphages. Coliphage prevalence could be due to the sporadic inflow of faecal contamination from the surrounding areas (Scott *et al*., 2002) whereas the decrease in phage populations could be due to sunlight inactivation (Sinton *et al*., 1999) and the decrease in bacterial populations. Bacterial-dependent phage persistence was restated by the relationship observed between the coliphages and the bacterial indicators (EC, TC, SAL, SHIG and THB) as depicted in Figure 2.10. Furthermore, fluctuating anthropogenic activities may be one of the main drivers of the coliphage prevalence in the Umhlangane River (Levy *et al*., 2012).

Since not only seasonal variations but also anthropogenic activities have been shown to influence water quality (Markich and Brown, 1998; Farnsworth and Milliman, 2003; Wang *et al*., 2007), this study evaluated the river water at various land use zones at different months that simultaneously captured the dry and rainy periods. The main findings of this study showed that elevated bacterial and phage populations was observed during the warmer months with temperature, turbidity, TDS, E.C. and salinity mainly influencing these communities. Furthermore, the change in phage and bacterial enumeration along the sampling points demonstrates that the complexity (mainly animal pollution) of the pollution at each land use zone played a pertinent role in microbial proliferation.

The quality of South African water resources is an essential point to consider as the depleting water resources, expanding economy and growing population cannot be relied solely on the sources currently exploited by the country. Therefore, methods to quarantine impeding pollution from industries and the accumulation of unsolicited faecal pollution needs to be dealt with in an organized manner. This study highlighted the importance of monitoring water resources flowing through different land use zones. Water quality monitoring at the 5 sampling points showed great fluctuations in the microbial and physico-chemical constituents. Domestic and industrial regions (Phoenix and Springfield) presented greater inclinations to pollution as compared to the natural wetlands. Considering the worsening water quality of the Umhlangane River, better efforts in wastewater and effluent (industrial or

agricultural) management and/or disposal should be re-assessed to improve the status of the river water.

# Chapter *three*

# Presence and diversity of enteric pathogenic viruses in the Umhlangane River

## 3.1 Introduction

Treated and untreated polluted waters harbouring microbial pathogens poses a major health risk to the public. Despite the advent in advanced wastewater and water treatment processes, waterborne diseases still persevere. On an annual scale, these pathogens infect around 250 million people ultimately resulting in 280 000 – 520 000 deaths (WHO, 2014). Gastroenteritis associated with waterborne viral pathogens contributes to great health issues in developing nations. Despite the low mortality rates caused by viral gastroenteritis, it‟s related economic and morbidity implications are noteworthy (Kang *et al*., 2013). Currently, over 100 different types of viruses are present in faecal matter that causes a variety of diseases annually (Kiulia *et al*., 2010). Apart from gastroenteritis, some of the other sicknesses include meningitis, rash and hepatitis. However, only a small subset of these pathogenic viruses is waterborne transmitted (Okoh *et al*., 2010). These viruses enter water systems through agricultural and urban runoffs, septic systems, sewage outfall and wastewater discharge which are then transmitted through the faecal-oral route to finally replicate in the gastrointestinal tract (Coudray-Meunier *et al*., 2013). The World Health Organization (WHO) estimates an average of approximately 2.2 million deaths reported each year due to the use of unsafe water, hygiene and sanitation problems (WHO, 2008).

A number of enteric viruses have been reported in aquatic environments (Wong *et al*., 2012) some of which include hepatitis A viruses (Dongdem *et al*., 2009), adenoviruses (Chigor and Okoh, 2012) and polyomaviruses (Haramoto *et al*., 2010). Hepatitis A viruses (HAVs) cause approximately 40% of severe hepatitis yearly with HAV infections being 3-fold more infective than hepatitis B viruses (Redwan and Abdullah, 2012). Numerous HAV outbreaks have been reported on a global scale (Arankalle *et al*., 2006; Craig *et al*., 2007; Frank *et al*., 2007; Robesyna *et al*., 2009). Adenoviruses (AdVs) cause a variety of clinical symptoms with many outbreaks related to recreational water exposure (Mena and Gerba, 2009; Vieira *et al*., 2012). Moreover, the persistence and stability of adenoviruses in environmental water far exceeds other enteric viruses (Tong and Lu, 2011). Human polyomaviruses BK and JC cause chronic infections in humans and are frequently defecated in municipal sewage and urine. These viruses have been linked to other important disease in immune-compromised individuals and also some types of human cancer such as colorectal cancer (Hundesa *et al*., 2006). Even though hepatitis C viruses (HCV) are not waterborne but fluid related (blood-borne) they are important etiological agents of non-A, non-B hepatitis in people (Aslanzadeh *et al*., 1996). East and Central Asia as well as Northern Africa are regions mostly affected by HCV infections (WHO, 2014). Beld *et al*. (2000) frequently

detected HCVs in the defecation of chronically infected patients. Therefore, speculation of their possible presence in water through surface runoffs could be made. Moreover, studies involving the transmission of HCV in injection drug users have demonstrated the presence of HCVs in water containers (Doerrbecker *et al*., 2013) and rinse water (Thorpe *et al*., 2002).

Current water quality monitoring and guidelines employ the sole use of bacterial indicators which has been found to be poorly correlated with the presence of enteric viruses (Wyn-Jones *et al*., 2011; Lee *et al*., 2014). Wastewater discharge as well as recreational and drinking water sources does not require the detection of viruses owing to the absence of an appropriate concentration method that is rapid, easy and sensitive (Li *et al*., 2010). Enteric viruses have a low infectious dose (Hamza *et al*., 2011) and the Centre of Disease Control and Prevention estimated that approximately 50% of all gastrointestinal diseases might be caused by viruses (Jiang *et al*., 2007). Thus, the importance of waterborne viruses should not be overlooked (Kiulia *et al*., 2010). The low virus numbers on water has pushed for the development of concentration methods that can be used to isolate a number of different viruses from environmental waters (Verheyen *et al*., 2009). Tangential flow filtration has been used to concentrate enteric viruses from water environments with high recovery rates (John *et al*., 2011).

Electron microscopy has long since been used in the visualization of virus-particles particles (VLPs) (Goldsmith and Miller, 2009). Considering the current drawbacks of using plaque counts for the estimation of viruses in water direct enumeration methods involving epifluorescence microscopy and an adequate dye has been adopted (Ortmann and Suttle, 2009). This method allows the accurate estimation of virus-like particle abundance (Budinoff *et al*., 2011; Diemer *et al*., 2012). Conversely, transmission electron microscopy can be used in environmental virological studies to presumptively identify viruses based on the morphology and size (Vale *et al*., 2010).

The most accurate confirmation to investigate viral infectivity relies on its ability to replicate in its natural host. However, viruses are considered infectious if they are capable of entering the cell and expressing at least one gene or show replication (Hamza *et al*., 2011). Therefore, tissue culture has been employed to investigate infectious virus particles from environmental samples (Coudray-Meunier *et al*., 2013). The use of multiple cell lines may allow for the detection of a variety of viral groups in environmental samples rather than the infectivity of one (Lee *et al*., 2004).

Currently, the polymerase chain reaction (PCR) is the most commonly applied method of choice for virus detection from environmental samples (Katayama *et al*., 2002). This

method has been modified to also include nested-PCR reactions (van Heerden *et al*., 2005) where additional primers are used for a second round to increase the throughput of the initial reaction (Gajardo *et al*., 1995; Nainan *et al*., 2006).

This study investigated the presence of enteric viruses in the Umhlangane River using an ultrafiltration concentration technique, namely tangential flow filtration coupled with various electron microscopies (transmission electron microscopy and epifluorescence microscopy), tissue culture and nested polymerase chain reactions to evaluate the presence of 4 different viral groups.

## 3.2 Materials and methods

### 3.2.1 Sample collection for viral analyses

Twenty litres of river water was collected at each sampling point (P1 to P5) over a 6 month period commencing in April 2014 to conclude in September 2014 for virus concentration and analysis. Samples were collected in 25 ℓ plastic drums (previously disinfected with 70% (v/v) alcohol and rinsed with deionized water). At each sampling site the drums were rinsed with river water prior to sample collection (Olaniran *et al*., 2012). Water was collected using the grab sampling method with a bucket and rope for every sampling point along the Umhlangane River (Hadlow, 2011). All samples were kept on ice and transported to the Discipline of Microbiology, University of KwaZulu-Natal (Westville campus) for virus concentration.

### 3.2.3 Filter installation

The complete TFF system (Merck-Millipore Corp.) contained a number of parts that required assembly before its first use and the order in which these parts were assembled (Figure 3.1) is as follows: (i) bottom manifold plate with feed, permeate, retentate ports and 2 pressure gauges (white); (ii) bottom and top acrylic pressure plates with holder compression assemblies, washers and nuts (black); (iii) two inter-cassette gaskets (blue); (iv) top acrylic pressure plate (green) and; (v) the Millipore 100 kDa filter (pink).

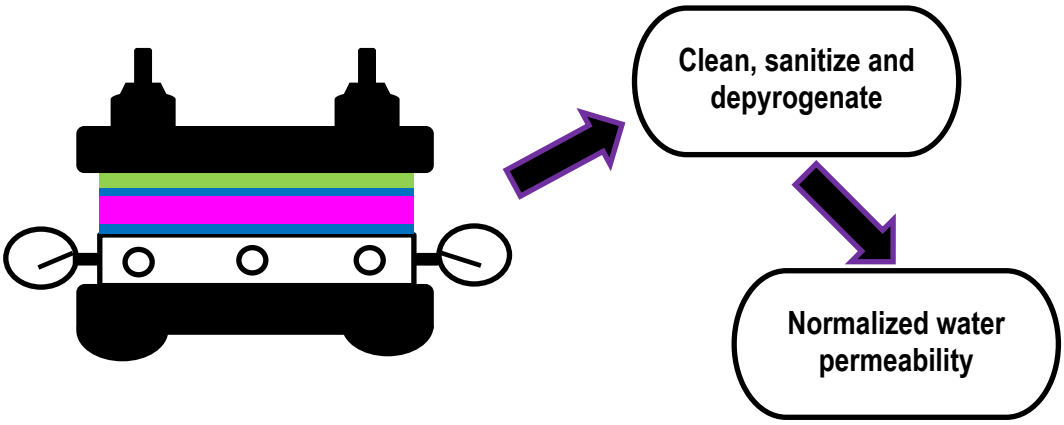


**Figure 3.1** System setup and subsequent maintenance procedures for an optimized TFF system (Merck-Millipore, 2013).

#### 3.2.2.1 Calculation of normalized water permeability

The normalized water permeability (NWP) was calculated prior to and after filtration to ensure filter integrity (Appendix ii). The initial NWP (prior to first use of the filter) was used as a benchmark against all subsequent water permeability measurements. The NWP measurements were established according to the following formula:

$$NWP = \frac{\mathbf{R*F}}{\mathbf{A*}\left(\frac{\mathbf{P(in)+P(out)}}{\mathbf{2}}\right)\mathbf{-Pp}}$$

R = Permeate flow rate in ℓ/hour

P(in) = Feed pressure in bar

P(out) = Retentate pressure in bar

T = Water temperature in °C

Pp = Permeate pressure (if non-zero) in bar

A = Total filter area in $m^2$

F = Temperature correction factor

### 3.2.3 Tangential flow filtration for primary virus concentration

Tangential flow filtration (TFF) was used to concentrate viruses from the collected water samples along the Umhlangane River (Figure 3.2). Twenty litres of river water was pre-filtered through a sediment filter (0. 45 µm, Merck-Millipore Corp.) at 130 mℓ/min to remove large debris and solids that could clog the filters. Virus concentration then involved 2

separate steps. Firstly, the pre-filtered water was filtered through a 142 mm diameter, 0.22 µm membrane filter (Merck-Millipore Corp.) at 330 mℓ/min to remove all bacteria. The second step further concentrated the viruses from the 0.22 micron concentrate through a 100 kDa (molecular weight cut-off) cartridge filter. The resulting retentate was then re-circulated through the system until approximately 500 mℓ of sample remained (Rosario *et al*., 2009; Wommack *et al*., 2010; Ganesh *et al*., 2014).

### 3.2.4 Secondary concentration of viruses by ultracentrifugation

Re-concentration of the TFF samples was carried out using ultracentrifugation according to a procedure described by Colombet *et al*. (2007) with some modifications. Six tubes of 28 mℓ retentate were ultracentrifuged for 2.5 hours at 130 000 x *g* (i.e. 29 000 revolutions per minute (rpm); 4°C) in a SW-32 *Ti* rotor (Optima L-100 XP, Beckman Coulter) for each of the previously concentrated samples. Each tube containing the viral pellet was re-suspended in 500 µℓ phosphate buffered saline (PBS; pH 7.2), pooled together (final volume of 3 mℓ) and stored at -20°C until further analyses (Pusch *et al*., 2005; Wyn-Jones *et al*., 2011).

### 3.2.5 Epifluorescence microscopy for direct VLP enumeration

Epifluorescence microscopy and SYBR Gold staining was used to determine the VLP numbers in the water samples (Rosario *et al*., 2009; Thurber *et al*., 2009). After primary concentration 1000 µℓ of the retentate (100 kDa concentrate) was fixed with 40 µℓ of 2% paraformaldehyde for 10 minutes at room temperature and filtered onto 0.02 µm anodisc filters (Whatman, Maidstone, Kent, UK) using a vacuum presume under 20 kPa. The filters were removed from the glass filtration unit (Merck-Millipore Corp.) while the vacuum was on to remove excess liquid on the bottom part of the filter. Any remaining solution was additionally wicked on paper towel as this allows good staining to be obtained. The filters were then placed on a drop of freshly prepared 1 x SYBR Gold stain for 15 minutes in the dark. All filters were wicked to remove the excess stain, air dried and mounted onto glass slides with 5 – 10 µℓ glycerol. Finally, cover slips were placed over the filters and visualized using a NIKON Eclipse (80*i*) fluorescence microscope. Four fields of view were examined to count the VLPs at 1000 x magnification under a blue-green light excitation (480 – 495 nm) for each water sample (Chen *et al.,* 2001). The images obtained from the microscope were used to count the VLPs (depicted as green spots against a dark screen) using the NIS-D Elements (D 3.2) and iTEM software.

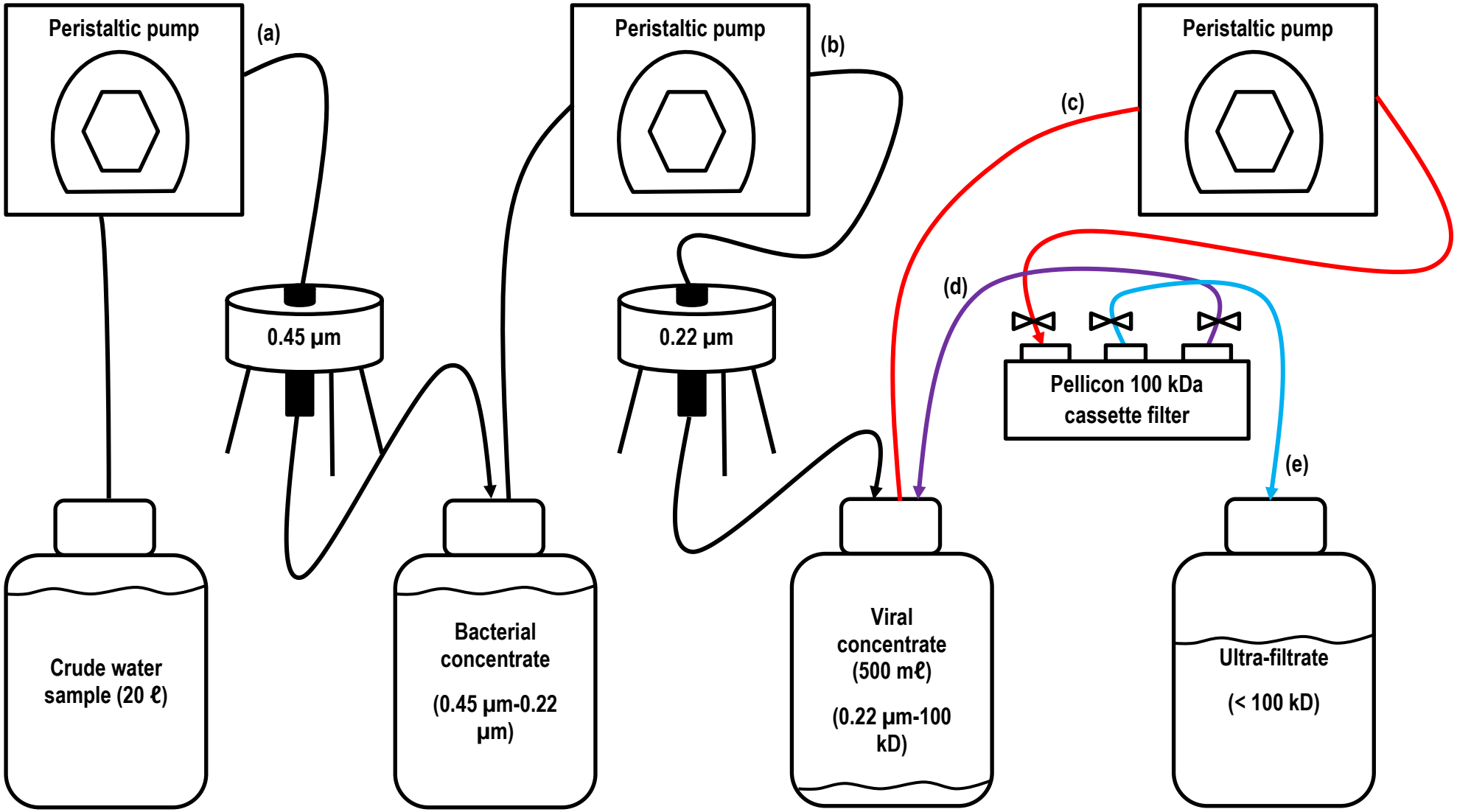


**Figure 3.2** Schematic diagram of TFF procedure to concentrate viral particles from the river water samples (adapted from Ganesh *et al*. (2014) with some modifications). **(a)** Debris removal and bacterial concentrate; **(b)** removal of bacteria; **(c)** feed; **(d)** retentate and **(e)** permeate.

### 3.2.6 Tissue culture experiments

Cell culture was used to determine the infectious nature of the virus-like particles in the river water. Three different cell lines was used including human hepatocellular carcinoma (Hep-G2), African green monkey kidney cells (Vero) and human embryonic kidney cells (HEK293). Six 250 mℓ tissue culture flasks (Corning, USA) for each of the five sampling points and 1 control flask were prepared. Dulbecco‟s modified essential medium (10%) supplemented with 10% foetal calf serum containing a 1:1:1 penicillin/streptomycin/fungizone mix (v/v/v) was used to grow the appropriate cell lines until semi-confluent to confluent monolayers were achieved. Once the cells were grown, the media was removed from all flasks and 200 µℓ of the 100 kDa concentrate was inoculated onto the respective monolayers. The flasks were placed into the incubator (37°C and 5% $CO_2$) for 1 hour, shaking every 15 minutes to allow for initial virus attachment and adsorption. Thereafter, 5 mℓ of Dulbecco‟s media was added to the 6 flasks, parafilmed and incubated for 5 to 7 days at 37°C and 5% $CO_2$. The cells were monitored daily using an inverted microscope (Olympus) at 400 x magnification for the production of cytopathic effect (CPE) indicating positive virus infectivity (EPA, 2001).

### 3.2.7 Transmission electron microscopy for viral diversity

Viral morphology and diversity was examined using transmission electron microscopy (TEM) according to a procedure described by Vale *et al*. (2010). After secondary concentration, 1 drop of each water sample was placed onto a carbon-coated grid (Electron Microscopy Sciences, Fort Washington, Pa) for 2 minutes, stained with 4% uranyl acetate solution for 30 s, rinsed with deionized water for 10 s and air dried prior to visualization with a high resolution TEM (JEOL 2100). Electron micrographs of the virus particles were taken between 250 000 to 500 000 x magnification. Bacteriophage and virus particles were measured for size and compared to known viruses for presumptive identification.

### 3.2.8 Molecular detection of human enteric viruses in the Umhlangane River

#### 3.2.8.1 Viral nucleic acid extraction

The total viral RNA and DNA were extracted separately from 1 mℓ sample each using the High Pure Viral RNA Kit (Roche Diagnostics, Germany) and the High Pure Viral Nucleic Acid Large Volume Kit (Roche Diagnostics, Germany), respectively according to manufacturer‟s instructions. The quantity and quality of both the DNA and RNA extracts was

measured using the NanoDrop 2200 spectrophotometer (Thermo Scientific, Finland) and stored at -80°C until further downstream processes.

#### 3.2.8.2 cDNA synthesis

First strand synthesis was accomplished using the DyNamo$^{TM}$ cDNA Synthesis Kit (Finnzymes, Thermo Fischer Scientific, Finland). All RNA extracts were standardized to 1 µg/mℓ with RT-PCR grade water in the reaction tubes. Each reaction contained 2x RT buffer, M-MuLV RNaseH$^{+}$ reverse transcriptase, 300 ng/µℓ random hexamer primer set, nuclease-free water and 3-7 µℓ RNA template. The reaction mixtures were placed in a PCR thermocycler (BIORAD, South Africa) and incubated for 10 minutes at 25°C, 40 minutes at 37°C and 5 minutes at 85°C. The cDNA concentration and quality was checked using a NanoDrop 2200 spectrophotometer (Thermo Scientific, Finland) and stored at -20°C.

#### 3.2.8.3 Nested PCR/RT-PCR for virus populations

Four different virus groups were detected using sequence-specific primer sets and are depicted in Table 3.1. The PCR/RT-PCR was carried out according to Symonds *et al*. (2009) for human adenoviruses, McQuaig *et al*. (2006) for human polyomaviruses, Pina *et al*. (2001) for hepatitis A viruses and Hu *et al*. (2003) for hepatitis C viruses. Main stocks (100 µM) of each primer (Inqaba Biotech, South Africa) were prepared by adding the appropriate amount of molecular grade water (Ambion) to the lyophilized oligo pellets. Working stocks were then made to a final concentration of 10 µM using the molecular grade water and stored at -20°C. All reactions were a final volume of 25 µℓ and contained 5 µℓ of template DNA or cDNA, 12.5 µℓ of 2 x PCR master-mix (0.05 U/_L *Taq* DNA polymerase, reaction buffer, 0.4 mM of each dNTP (dATP, dCTP, dGTP and dTTP) and 4 mM MgCl2) (Thermo Fischer Scientific Inc.), 1 µM of each primer and PCR grade water.

##### 3.2.8.3.1 Human adenoviruses

Nested PCR was used to amplify the hexon gene of 47 different adenovirus serotype genomes (Allard *et al*., 1992). Five microlitres of the PCR product from the first round was used as a template for the second PCR round. Both rounds contained an additional 0.4 mM $MgCl_2$. The first and second rounds were amplified under the same conditions: 4 min at 94°C, 40 cycles of 92°C for 30 sec, 60°C for 30 sec and 72°C for 1 min. Final elongation was 72°C for 5 min. A positive control (cell cultured adenovirus) and negative control (distilled water) was included for all reactions.

**Table 3.1** Primer sequences for the PCR amplification of viruses from four viral groups.

| Primer | Primer sequence (5' − 3') | Amplicon size | Reference |
|---|---|---|---|
| Adenoviruses (A-F) | | | |
| AV-A1 | GCCGCAGTGGTCTTACATGCACATC | 300 bp | |
| AV-A2 | CAGCACGCCGCGGATGTCAAAGT | | (Allard *et al*., 1992) |
| AV-B1[a] | GCCACCGAGACGTACTTCAGCCTG | 143 bp | |
| AV-B2[a] | TTGTACGAGTACGCGGTATCCTCGCGGTC | | |
| Polyomaviruses | | | |
| P1 | GTATACACAGCAAAGGAAGC | 630 bp | |
| P2 | GCTCATCAGCCTGATTTTGG | | (McQuaig *et al*., 2006) |
| P3[a] | AGTCTTTAGGGTCTTCTACC | 173 bp | |
| P4[a] | GGTGCCAACCTATGGAACAG | | |
| Hepatitis A viruses | | | |
| HHA1 | TGCAAATTAYAAYCAYTCTGATGA | 532 bp | |
| HHA2 | TTTCTGTCCATTTYTCATCATTC | | (Pina *et al*., 2001) |
| HHA3[a] | TTYAGTTGYTAYTTGTCTGT | 436 bp | |
| HHA4[a] | TCAAGAGTCCACACACTTC | | |
| Hepatitis C viruses | | | |
| HCV1 | ACTGTCTTCACGCAGAAAGCGTCTAGCCAT | 271 bp | |
| HCV2 | CGAGACCTCCCGGGGCACTCGCAAGCACCC | | (Hu *et al*., 2003) |
| HCV3[a] | ACGCAGAAAGCGTCTAGCCATGGCGTTAGT | 255 bp | |
| HCV4[a] | TCCCGGGGCACTCGCAAGCACCCTATCAGG | | |

[a] Nested primers

#### 3.2.8.3.2 Human polyomaviruses

Nested PCR was used to amplify the human-specific BK and JC polyomaviruses genomes from the collected water samples according to McQuaig *et al*. (2006) with some modifications. Both rounds contained 5 µℓ of template and were run under the same conditions as follows: 94°C for 2 min, 45 cycles of 94°C for 20 s, 55°C for 20 s, 72 for 20 s. Final elongation was 72°C for 2 min. A negative control comprising distilled water was included in all reactions. Both rounds had an additional 0.5 mM $MgCl_2$.

#### 3.2.8.3.3 Hepatitis A viruses

Nested PCR was used to amplify the VP1/VP2 region of the hepatitis A virus genomes (Pina *et al*., 2001). The reaction mixtures contained 5 µℓ of cDNA and both rounds was run under the same conditions: 95°C for 3 min, 30 cycles of 95°C for 60 s, 42°C for 60 s and 72 for 60 s. Final extension was 72°C for 5 min. A negative control of distilled water was also prepared. Both rounds contained an additional 0.5 mM $MgCl_2$.

#### 3.2.8.3.4 Hepatitis C viruses

Nested PCR was used to amplify the 5' untranslated (5' UTR) region of the hepatitis C virus genomes according to Hu *et al*. (2003) with some modifications. The first and second rounds contained 5 µℓ templates and were run under the following conditions: 95°C for 3 min, 30 cycles of 95°C for 60 s, 50°C for 60 s and 72 for 60 s. Final extension was 72°C for 5 min. The negative control containing distilled water was also included.

### 3.2.8.4 Visualization and sequencing of the PCR products

Five microlitres of the PCR products were visualized on 1.5% or 2% (w/v) agarose (Seakem®LE Agarose, BioWhittaker Molecular Applications, Rockland, ME, USA) gels electrophoresis in 1 x Tris-Acetate-EDTA (TAE) running buffer at 80 V for 60 min. A ready-to-use GeneRuler DNA ladder mix (Thermo Fischer Scientific Inc.) was used to estimate the amplicon sizes. Thereafter, the gels were stained in ethidium bromide (1 µg/mℓ) and visualized with the Chemi Genius$^2$ BIO Imaging System and Gene Snap software (Syngene, UK).

To confirm the identities of some of the products, random nested-PCR products were sequenced with their primer sets by Inqaba Biotech (Pretoria, South Africa). The sequences obtained for each product was analysed by BLAST (Basic Local Alignment Search Tool) programme on the NCBI (National Centre for Technology Control, NIH, USA) website: http://www.ncbi.nlm.nih.gov/BLAST, in the nucleotide sequence database (nucleotide BLAST; nr/nt) to acquire the identity of the PCR products.

## 3.2.9 Quality control

The probability of sample contamination due to DNA amplicons or cross-contamination was reduced through the practice of standard molecular preparation protocols. Separate areas were used to prepare the reagents and manipulate the amplified samples. Negative controls were included in all reactions and a positive control was also used where available. All RNA samples were manipulated in a separate RNA room (DNase/RNase-free zone) that contained PCR pipettes, filter tips, centrifuges, etc. specifically designated for RNA work only. Furthermore, master-mixes for the PCR reactions were prepared in the laminar flow to reduce contamination.

### 3.2.10 Statistical analysis

Bivariate analysis between the sampling months, points, coliphages, VLPs, physico-chemical (obtained from Chapter 2, section 2.3.1) and bacterial indicators (obtained from Chapter 2, section 2.3.2) was compared over a 6 month period (April to September 2014). Pearson‟s correlation test (Students *t*-test) was used to evaluate the correlations between all tested indicators using SPSS programme version 22 (SPSS Inc., Illinois). The level of significance was set at $p < 0.05$ and $p < 0.01$ (Olaniran *et al*., 2012).

Multivariate comparative analysis between all microbial and physico-chemical indicators from April to September 2014 was evaluated using CCA. Physico-chemical indicators are represented as arrows emanating from the origin in the bi-plot with the species and sample data scattered according to the magnitude of its relation to each other. Four hundred and ninety-nine permutations generated by the Monte Carlo permutation test were used to estimate the significance of the exes. Canoco for Windows version 4.5 was used to determine the CCA bi-plots (ter Braak, 1994; ter Braak and Verdonschot, 1995).

## 3.3 Results

### 3.3.1 Direct VLP enumeration

The VLP counts from April to September 2014 are depicted in Figure 3.3 in comparison to the somatic and F-specific RNA counts during that sixth month period. The highest recorded VLP count of 105 VLP/mℓ was at the Phoenix industrial site in April while the lowest was at P2 (upstream KwaMashu WWTP; Phoenix residential) in August with 12.6 VLP/mℓ. In comparison to the VLP counts, the somatic and $F^+$RNA coliphages also depicted the highest and lowest counts in April and August, respectively. The phage counts enumerated by the plaque assays were substantially higher than the VLP counts with the highest and lowest average phage counts being 417.5 pfu/mℓ and 17.3 pfu/mℓ, respectively. As previously described in Chapter 2 of section 2.4.5, the somatic and F-specific RNA coliphages showed a significant difference with the sampling months. Similarly, the VLP populations showed a significant difference ($p < 0.000$) and a strong negative correlation ($r = -0.909$) with the sampling months. No significant difference ($p > 0.01$; 0.05) was observed between the sampling points and the VLP counts. The VLPs had strong positive correlations with the somatic ($r = 0.971$) and $F^+$RNA ($r = 0.965$) coliphages, THB ($r = 0.847$), TC ($r = 0.832$), EC ($r = 0.747$), FS ($r = 0.787$) and SAL ($r = 0.744$) populations. The remaining bacterial indicators showed weak to moderate correlations with the VLPs (Appendix ii).

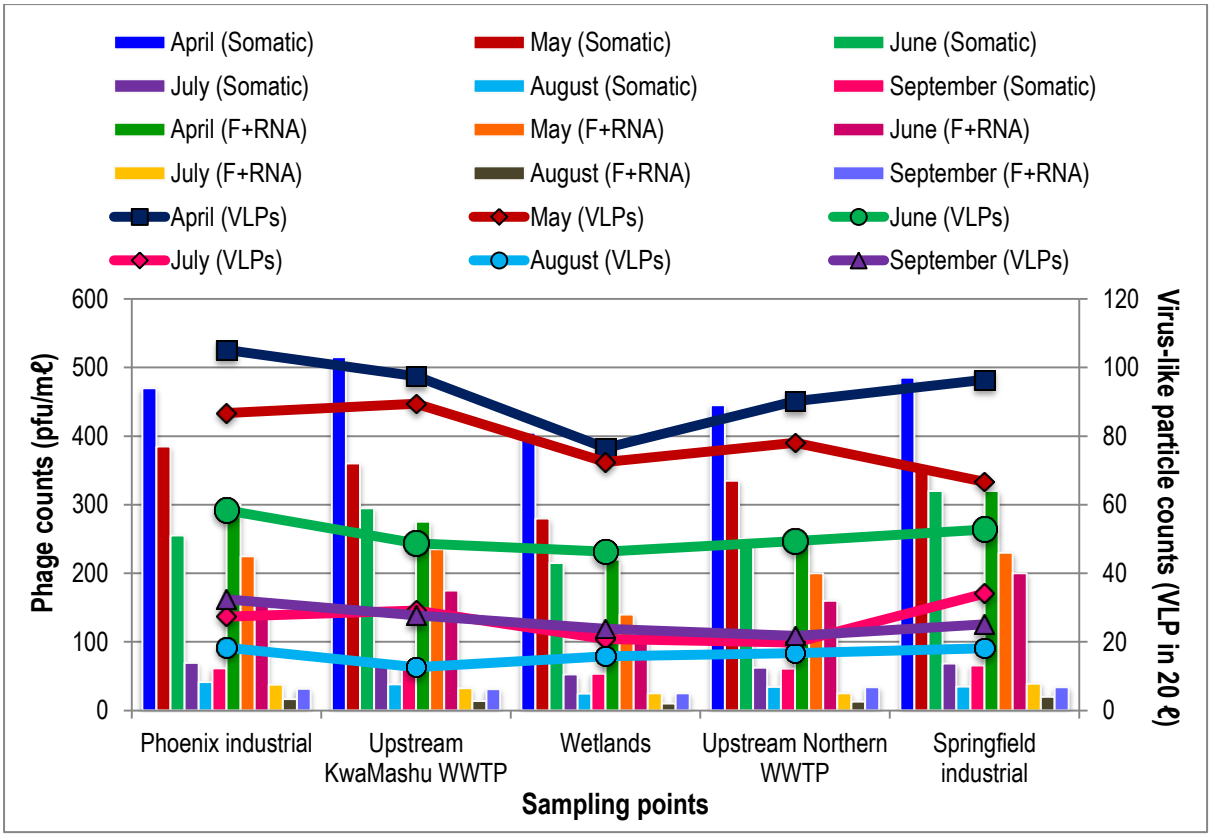


**Figure 3.3** Coliphage and virus-like particle (VLP) counts from April to September 2014 at all sampling locations. Bars indicate coliphage averages ($n$ = 2), line graph for VLPs ($n$ = 4) and error bars are standard deviation.

### 3.3.2 Canonical correspondence analysis

The direct gradient analysis was used to evaluate the relationships between the phage, VLP, physico-chemical parameters and the sampling points over the 6 month period. The relationship between the physico-chemical indicators, coliphage and VLP populations are depicted in Figure 3.4. The ordination plot revealed no direct relationship between the somatic (pink circle), $F^{+}$RNA (blue circle) and the VLP communities (purple circle). The overall inertia (species variance) is 0.025 and the amount of total variation owing to the explanatory variables (physico-chemical) is 0.014. This means that the general variance in phage and VLP populations was not gregariously dependent on the environmental variables. Interestingly, apart from all the other physico-chemical parameters, turbidity seemed to have a much stronger relationship with the VLP populations. However, the percentage of the total species variance for CCA axis 1 was 53.7% and the percentage of the species−environment relation was 100% suggesting a correlation may be present between the virus (phage and VLPs) and the environmental parameters.

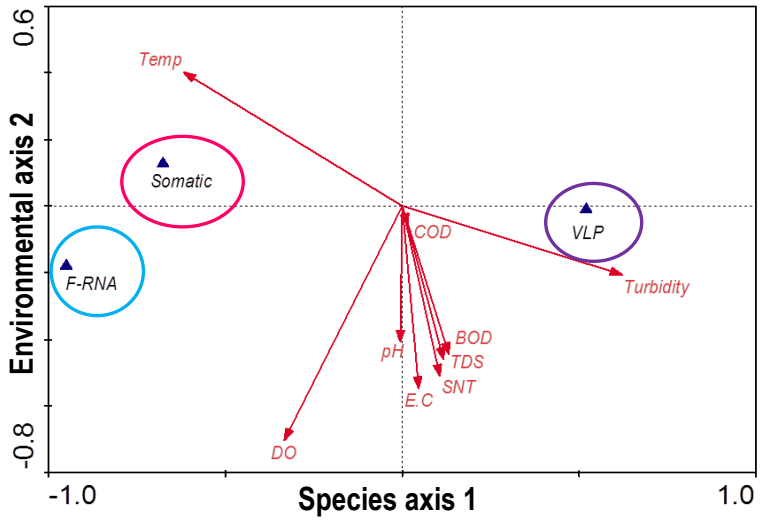


**Figure 3.4** CCA bi-plot for the coliphage, VLP populations and the physico-chemical parameters from April to September 2014.

The CCA ordination plot (Figure 3.5) revealed that temperature, DO and turbidity were the most influential variables during the time of sampling (longest arrows). Some of the sampling points during May, June, August, September and all during July were found to be correlated with DO, turbidity, E.C., salinity, TDS, BOD and to a lesser extent pH and COD (green circle). Interestingly, a distinct separation of sampling points P2 to P5 during April (orange circle) with a strong association to temperature was seen.

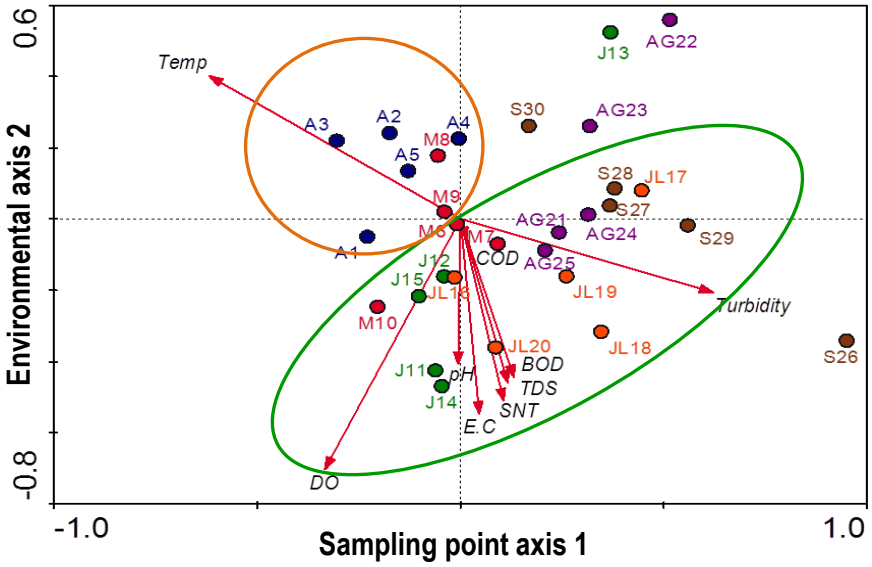


**Figure 3.5** CCA bi-plot for the coliphage, VLP and physico-chemical parameters at the five sampling points from April to September 2014. Abbreviations: A: April; M: May; J: June; JL: July; AG: August and S: September. Numbers 1-30: sampling points at each month.

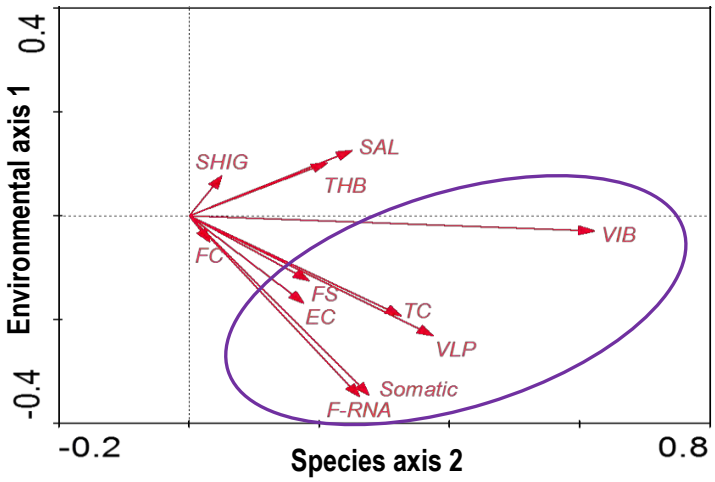


**Figure 3.6** CCA bi-plot for the bacterial, coliphage and virus populations at all sampling points during April to September 2014.

A strong relationship between the VLP, TC and the coliphage populations was revealed by the CCA plot in Figure 3.6 (purple circle). In addition, the CCA plot also revealed that the VIB populations had a positive relationship with the VLP populations in the Umhlangane River while the FS and EC communities had a lower rate of change (shorter arrows) and therefore had a lesser impact on the VLPs. The remaining bacterial indicators (SHIG, SAL, THB and FC) did not greatly influence the VLP populations (Figure 3.6).

### 3.3.3 Virus-like particle infectivity

The CPE of the VLPs was based on the morphological change of the cells, the visibility of granulated, elongated cells, vacuole production and the loss of cell adherence to each other and the wall of the flask. The un-infected monolayers are depicted in Figure 3.7 a, b and c. The appearance of a circular shape and the overall deformity in cell morphology is seen in Figure 3.7 d, e and f.

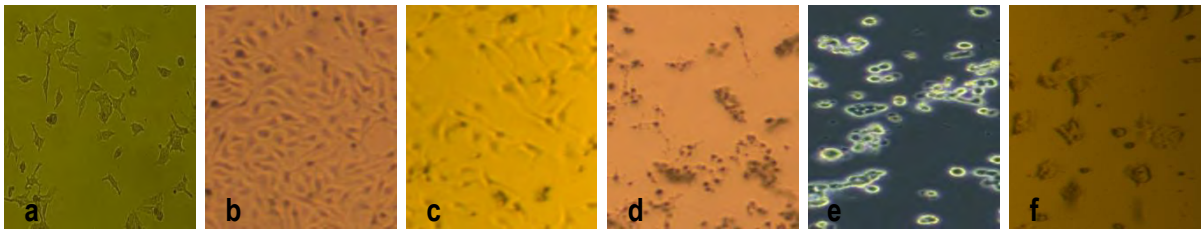


**Figure 3.7** Microscope images of the different cells lines and the CPE production. (a) Un-infected Vero, (b) un-infected HEK293 and (c) un-infected Hep-G2 cell lines. CPE production on (d) Vero, (e) HEK293 and (f) Hep-G2 cell lines. Images captured at 400 x magnification.

The positive or negative CPE of the concentrated VLPs from the Umhlangane River on the 3 cell lines are depicted in Table 3.2. Most of the viral concentrate was capable of causing infectivity to the 3 cell lines with the exception of P4 and P5 (Hep-G2), P4 (HEK293) and P3, P4 and P5 (Vero). The human adenovirus 9 VR-1086 was able to produce a positive CPE on all 3 cell lines. However, the human hepatitis A VR-1402 was not able to cause infectivity to the HEK293 cell line.

**Table 3.2** Cytopathic effect (CPE) of the virus concentrate from the Umhlangane River.

| SAMPLE | | POSITIVE OR NEGATIVE CPE | | |
|---|---|---|---|---|
| | | HEP-G2 | HEK293 | VERO |
| Adenovirus 9 VR-1086 | | + | + | + |
| Hepatitis A VR-1402 | | + | - | + |
| TRIAL | P1 | + | + | + |
| | P2 | + | + | + |
| | P3 | + | + | - |
| | P4 | + | - | - |
| | P5 | + | + | + |
| TEST SAMPLES | P1 | + | + | + |
| | P2 | + | + | + |
| | P3 | + | + | + |
| | P4 | - | + | + |
| | P5 | - | + | - |

Cytopathic effect (CPE): + positive CPE; - negative CPE (no cell death)

### 3.3.4 Visualization and presumptive identification of the virus-like particles

Transmission electron microscopy revealed a number of virus-like particles in the Umhlangane River (Figure 3.8). All images (Figure 3.8 a – e) depicted a variety of enveloped and non-enveloped viruses as well as bacteriophages.

Throughout the study, a number of different phage morphotypes was observed at all sampling locations. The bacteriophages were identified according to the International Committee on the Taxonomy of Viruses (ICTV) classification scheme as described by Ackerman and Eisenstark (1947). Bacteriophages belonging to the *Caudovirales* order consisting of morphotypes A1 (*Myoviridae* – contractile tails), B1 (*Siphoviridae* – short capsid, non-contractile and long tail), B2 (*Siphoviridae* – long capsid, non-contractile and long tail) and C1 (*Podoviridae* – short tail) were identified in the Umhlangane River (Figures 3.9 to 3.13). Members of the *Siphoviridae* and *Myoviridae* families were highly abundant in the river water samples while members of the *Podoviridae* family were much scarcer.

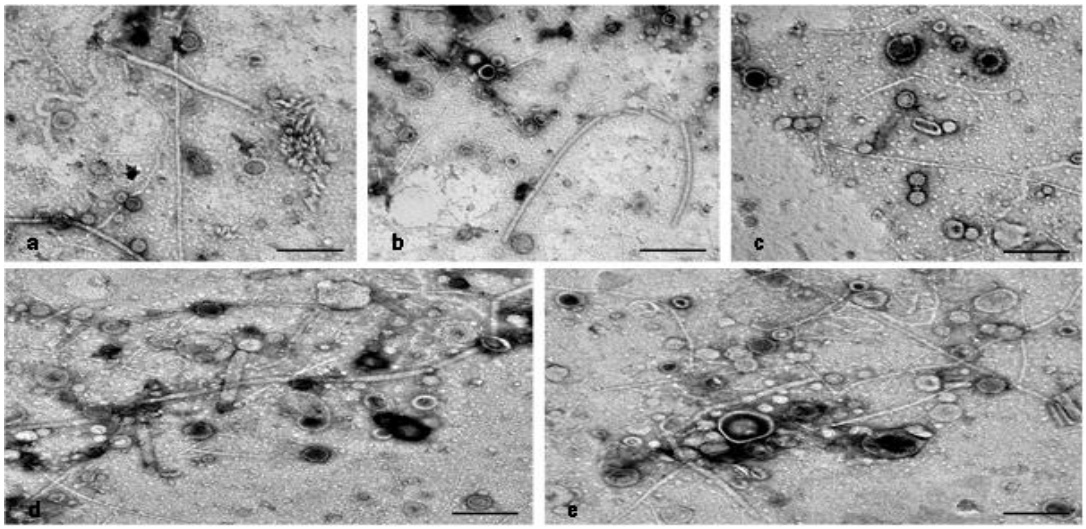


**Figure 3.8** TEM images of various viruses present in the Umhlangane River at all sampling points. Images captured at 60 000 to 150 000 x magnification. Scale bar 500 nm.

The outline of the bacteriophage heads had a regular hexagonal outline (Figure 3.10 a and h) with the tails attached to one of the angles while some phages were irregular (Figure 3.9 c, f and g). The discrimination between an octahedral, icosahedral and dodecahedral shape could not be determined for certain due to the insufficient nature of the current data. Furthermore, the tail fibres, neck and baseplate could be visualized in all the images except in Figure 3.10 b (tail fibres and neck), h (neck and baseplate). The observation of a *Myoviridae* bacteriophage after tail contraction was also seen (Figure 3.12 g).

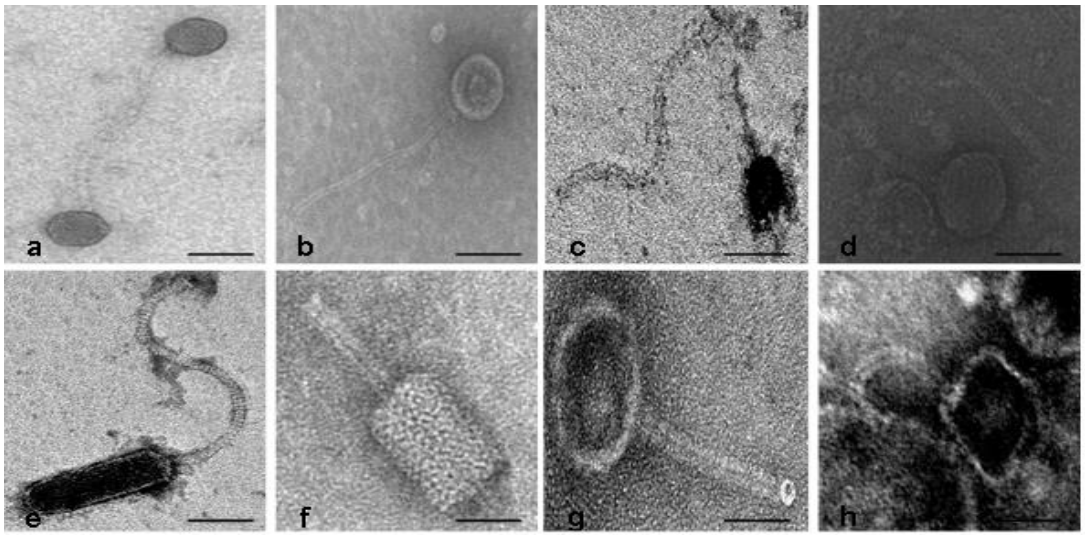


**Figure 3.9** TEM images of various bacteriophages in the Umhlangane River at all sampling points from April 2014 to September 2014. Images captured at 250 000 to 500 000 x magnification. Scale bar 100 nm.

Members of the B1 and B2 *Siphoviridae* family bear similar resemblance to Figure 3.9 a and c while Figure 3.10 b and g closely resemble members of the *Myoviridae* and *Podoviridae* family.

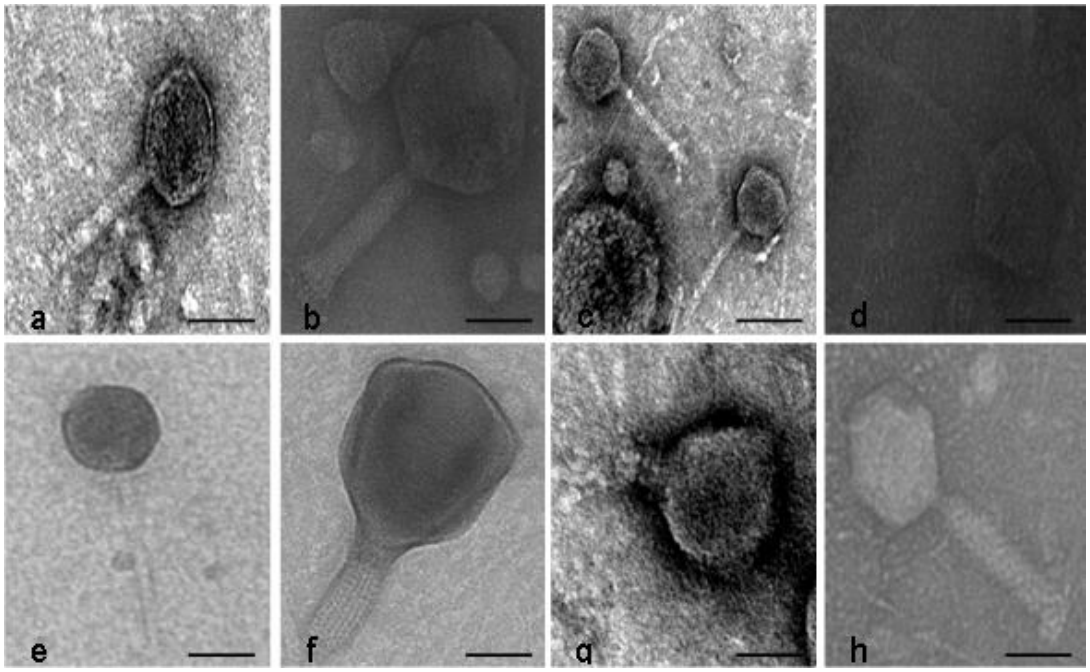


**Figure 3.10** TEM images of different phage morphotypes present in the Umhlangane River at all sampling points from April to September 2014. Images captured at 250 000 to 500 000 x magnification. Scale bar 100 nm.

Figures 3.11, 3.12 and 3.13 represent phages identified in the Umhlangane River at the five sampling points that resemble some known bacteriophages. These phages include CP6-1 *Serratia quinivorans* phage (Ashelford *et al*., 2003), T4-like *Vibrio parahaemolyticus* phage (Ackermann and Heldal, 2010), *Mycobacterium* Babsiella phage (*Mycobacterium* Database, 2014), phage T4 (Ackermann and Heldal, 2010) (Figure 3.11 b, d, f, h), *Mycobacterium* D29 phage (*Mycobacterium* Database, 2014), VvAWI *Vibrio vulnificus* phage (Nigro *et al*., 2012), Frp2 *Bacillus anthracis* phage, Wip5 *Bacillus anthracis* phage (Schuch and Fischetti, 2009) (Figure 3.12 b, d, f, h) and PSA *Listeria* phage (Figure 3.13 b).

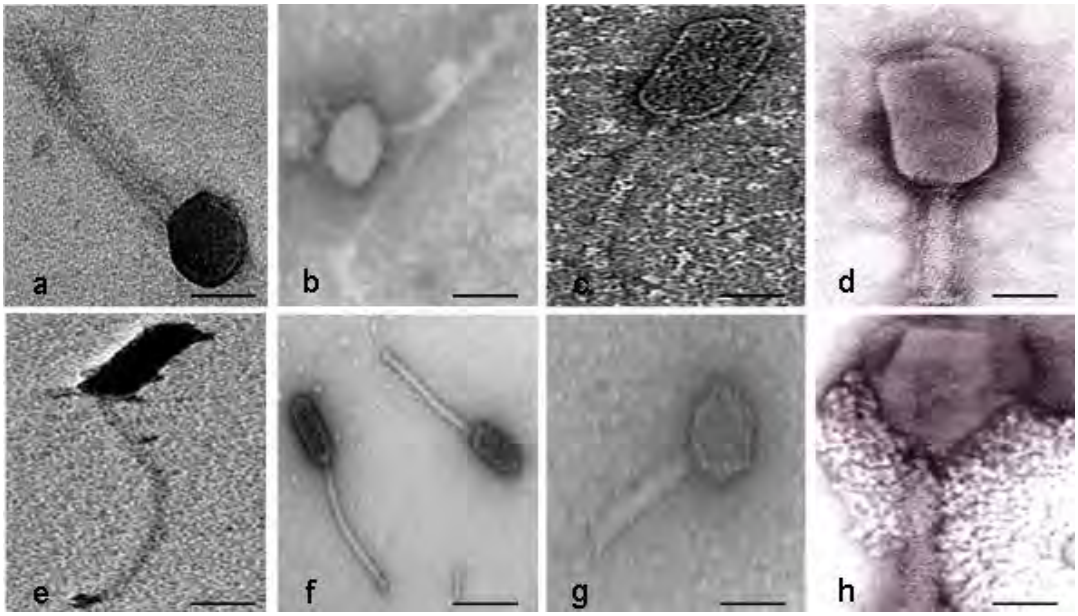


**Figure 3.11** Electron micrographs of different phage morphotypes (a, c, e, g) present in the Umhlangane River at the five sampling points from April to September 2014. (b) Known CP6-1 *Serratia quinivorans* phage (Ashelford *et al*., 2003), (d) known T4-like *Vibrio parahaemolyticus* phage (Ackermann and Heldal, 2010), (f) known *Mycobacterium* Babsiella phage (*Mycobacterium* Database, 2014) and (h) known phage T4 (Ackermann and Heldal, 2010). Images captured at 250 000 to 500 000 x magnification. Scale bar 100 nm.

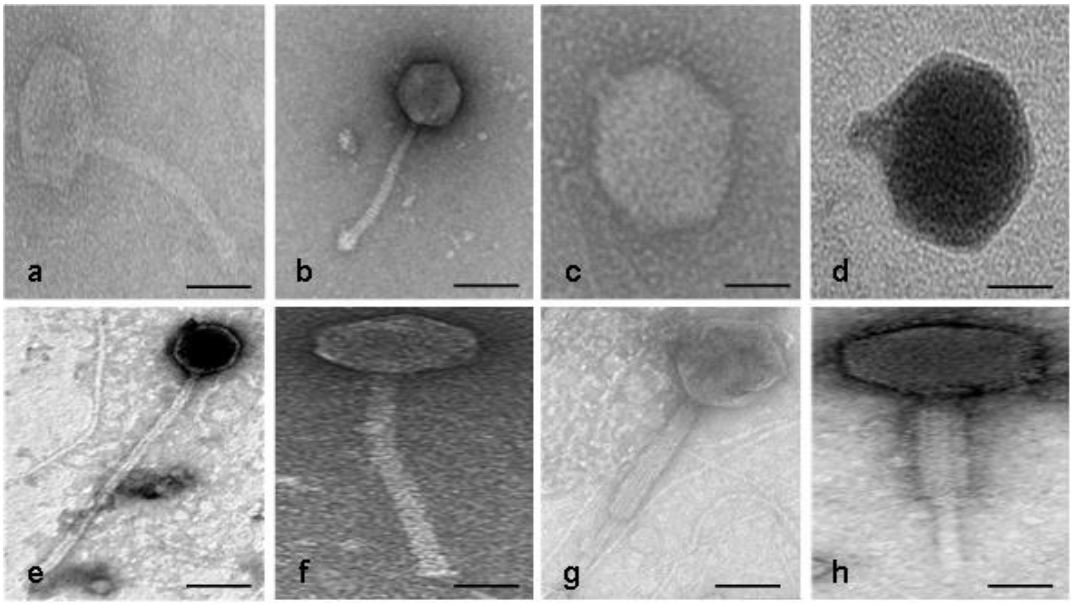


**Figure 3.12** Electron micrographs of different phage morphotypes (a, c, e, g) present in the Umhlangane River at the five sampling points from April to September 2014. (b) Known *Mycobacterium* D29 phage (*Mycobacterium* Database, 2014), (d) known VvAWI *Vibrio vulnificus* phage (Nigro *et al*., 2012), (f) known Frp2 *Bacillus anthracis* phage and (h) known Wip5 *Bacillus anthracis* phage (Schuch and Fischetti, 2009). Images captured at 250 000 to 500 000 x magnification. Scale bar 100 nm.

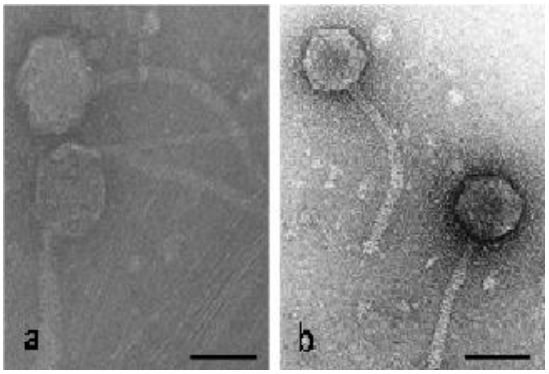


**Figure 3.13** Electron micrograph of bacteriophages present in the Umhlangane River (a) and (b) known PSA *Listeria* phage. Images captured at 250 000 to 500 000 x magnification. Scale bar 100 nm.

The presence of various presumptive viruses visualized in the Umhlangane River at all sampling points from April to September 2014 are depicted in Figures 3.14 to 3.18. The presumptive classification of all viruses was done according to size measurements and comparative structure similarities to known viruses found in literature. The identification of presumptive naked enterovirus-like particles with sizes ranging from 25.92 – 27.46 nm (a – f) and known coxsackieviruses (20 – 30 nm) are depicted in Figure 3.14.

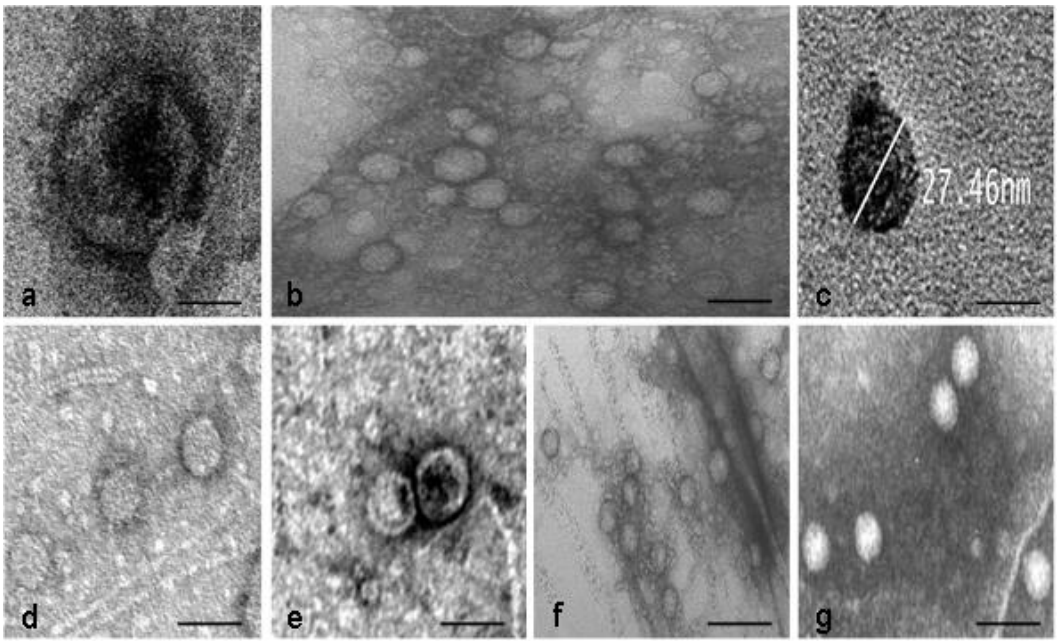


**Figure 3.14** TEM images of presumptive *Picornaviridae*-like particles (a – f) present in the Umhlangane River at all sampling points from April to September 2014. (g) Known coxsackievirus (Schramlová *et al*., 2010). Images captured at 250 000 to 500 000 x magnification. Scale bar 100 nm.

Figure 3.15 depicts the TEM images of presumptive naked *Adenoviridae*-like particles (a – g) and (h) known adenovirus (70 – 90 nm). These VLPs ranged from 67.29 to 78.11 nm. Presumptive *Polyomaviridae*-like particles and *Reoviridae*-like particles were visualized in the Umhlangane River and are depicted in Figure 3.16 a – c and e – g, respectively. These viruses were compared to known polyomaviruses (40 – 50 nm; Figure 3.16 d) and known rotaviruses (Figure 3.16 h), respectively. Figure 3.17 depicts the presumptive *Herpesviridae*-like particles (a – c), (d) known herpes virus (120 – 200 nm), presumptive *Orthomyxoviridae*-like particles (e – g) and (h) known influenza viruses. Presumptive *Coronaviridae*-like particles were also detected in the Umhlangane River (Figure 3.18 a – c) and were compared to a known coronavirus as depicted in Figure 3.18 d. Many presumptive enveloped viruses (Figure 3.18 e – h) were seen in the Umhlangane River. However, a comparison between these viruses and known viruses could not be made due to the similar structure of these viruses to many different viruses.

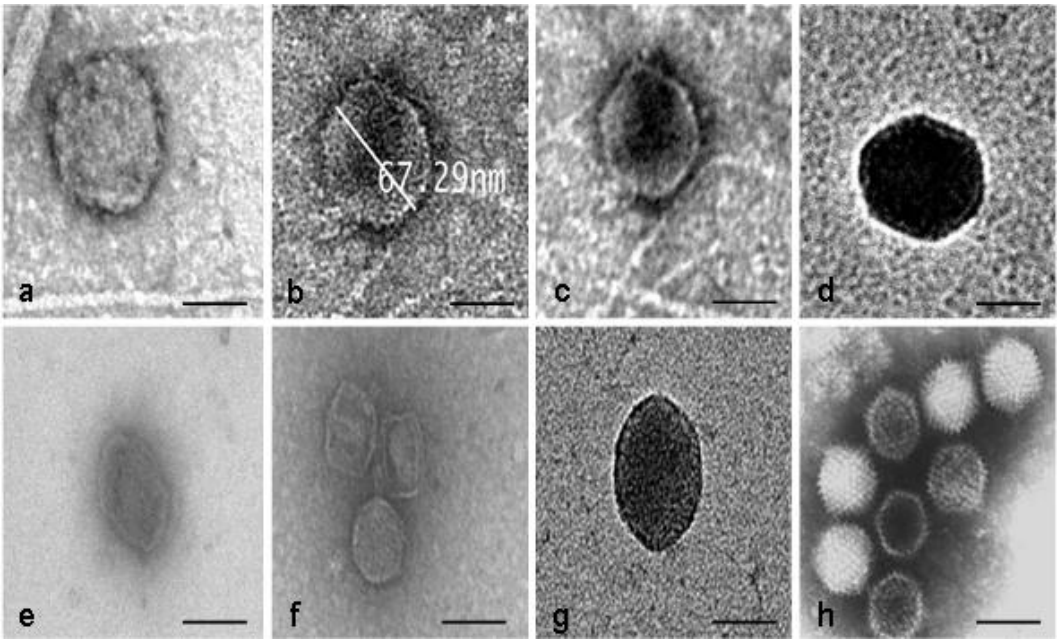


**Figure 3.15** TEM images of presumptive *Adenoviridae*-like particles (a – g) present in the Umhlangane River at all sampling points from April to September 2014. (h) Known adenovirus (Li *et al*., 2013). Images captured at 250 000 to 500 000 x magnification. Scale bar 100 nm.

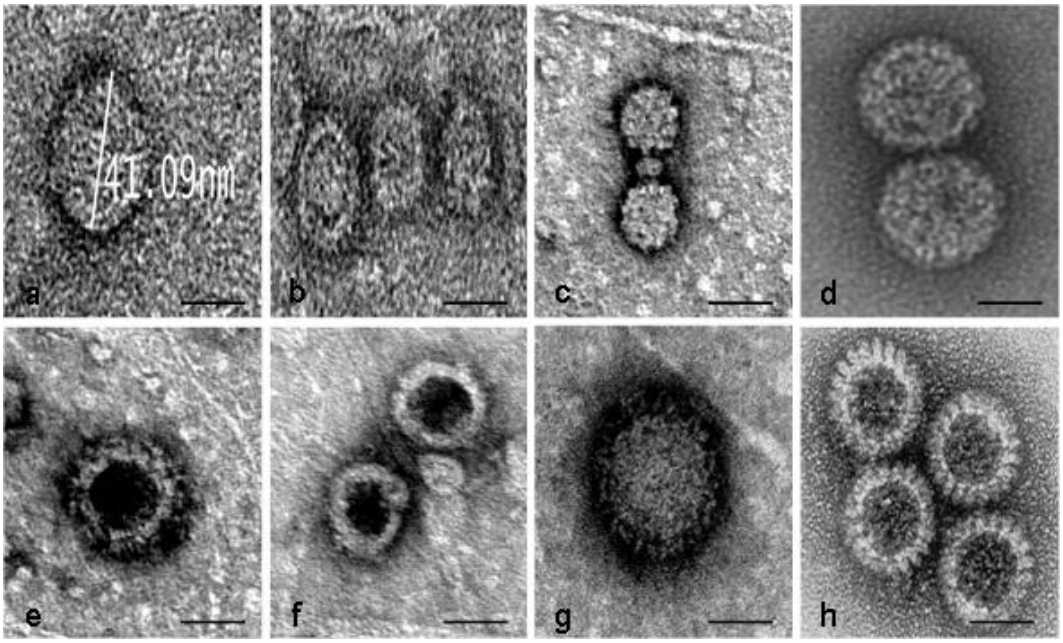


**Figure 3.16** Electron micrographs of presumptive *Polyomaviridae*-like particles (a – c), (d) known polyomavirus (Broekema and Imperiale, 2012), presumptive *Reoviridae*-like particles (e – g) and (h) known rotaviruses (Zeng *et al*., 1996). Images captured at 250 000 to 500 000 x magnification. Scale bar 100 nm.

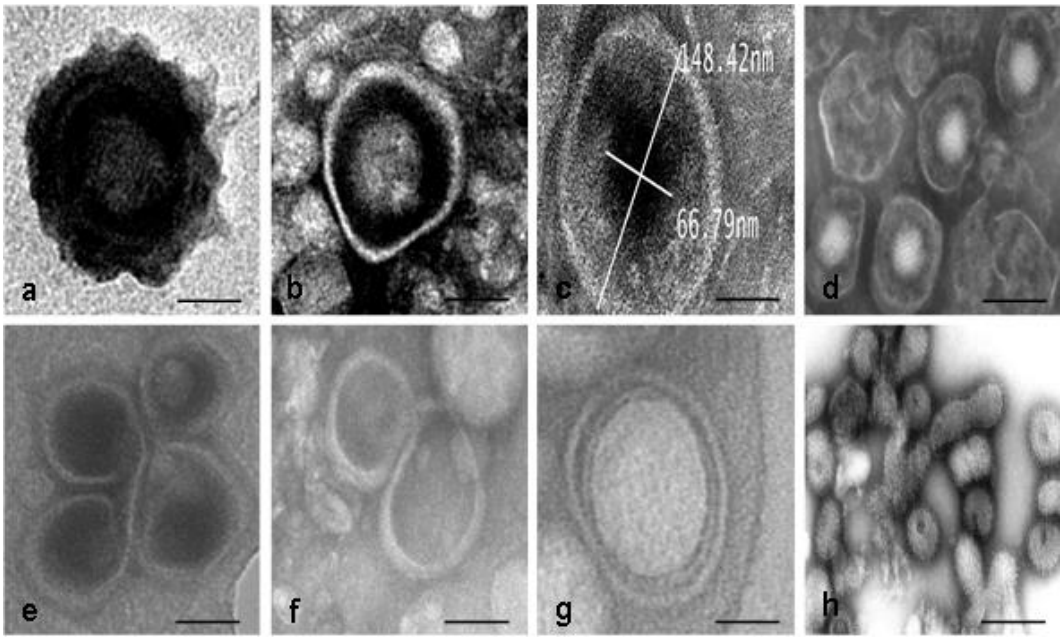


**Figure 3.17** Electron micrographs of presumptive *Herpesviridae*-like particles (a – c), (d) known herpes virus (Goldsmith and Miller, 2009), presumptive *Orthomyxoviridae*-like particles (e – g) and (h) known influenza virus (Schramlová *et al*., 2010). Images captured at 250 000 to 500 000 x magnification. Scale bar 100 nm.

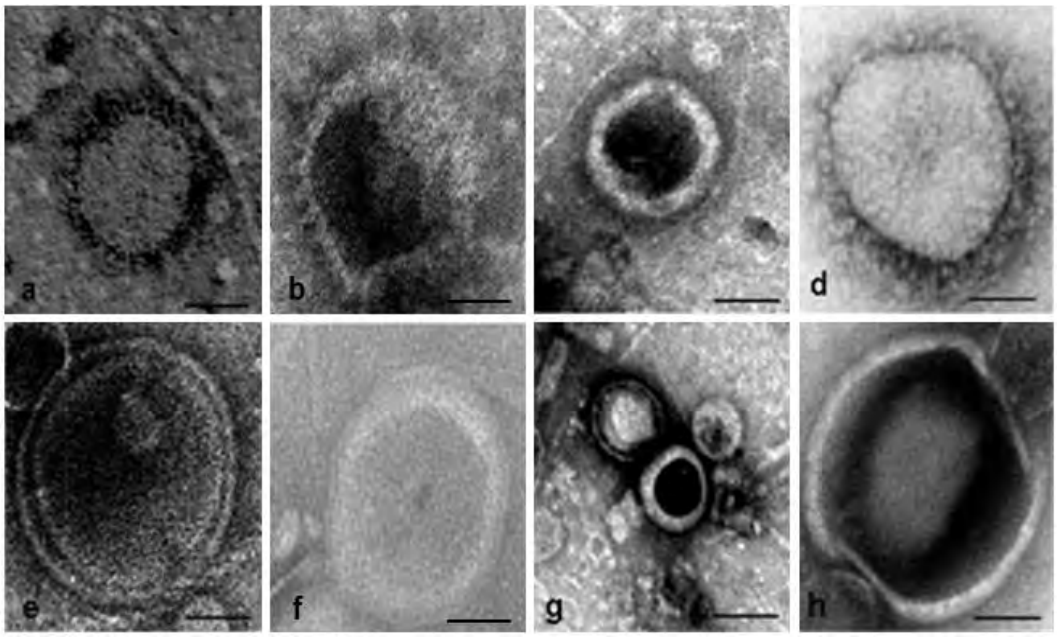


**Figure 3.18** Electron micrographs of presumptive *Coronaviridae*-like particles (a – c), (d) known coronavirus (Schramlová *et al*., 2010) and presumptive enveloped virus-like particles (e – h). Images captured at 250 000 to 500 000 x magnification. Scale bar 100 nm.

### 3.3.5 Primer-specific nested PCR/RT-PCR

The presence of adenoviruses, polyomaviruses, hepatitis A viruses and hepatitis C virus genomes were determined using nested PCR/RT-PCR reactions are depicted in Figures 3.19, 3.20, 3.21 and 3.22. Figure 3.19 a and b represents the PCR amplification of adenovirus genomes in the Umhlangane River. The sensitivity of the target numbers of the primers were 100 and all sampling points during the six month period (April – September 2014) produced a positive PCR product of 143 bp which corresponded to the adenovirus control.

The presence of human polyomaviruses BK and JC virus genomes are illustrated in Figure 3.20 a and b. Positive human polyomaviruses genomes were detected in 60% ($n$ = 30) of the tested samples yielding 173 bp products which was the expected product size according to the sequence-specific primers. All samples tested during June (Figure 3.20 a; lanes 11 to 15) was positive for the human polyomaviruses while only P1 (Phoenix industrial) during September depicted a positive PCR product (Figure 3.20 b; lane 26).

The PCR amplification of the VP1/VP2 and 5' UTR regions of hepatitis A and C viruses are shown in Figures 3.21 and 3.22, respectively. Positive hepatitis A viruses was detected in 70% ($n$ = 30) of the samples yielding 436 bp products. The expected product size of 255 bp for positive hepatitis C viruses was seen in 3 samples (July and upstream KwaMashu WWTP – P2; September; Riverhorse Valley – P4 and Springfield – P5).

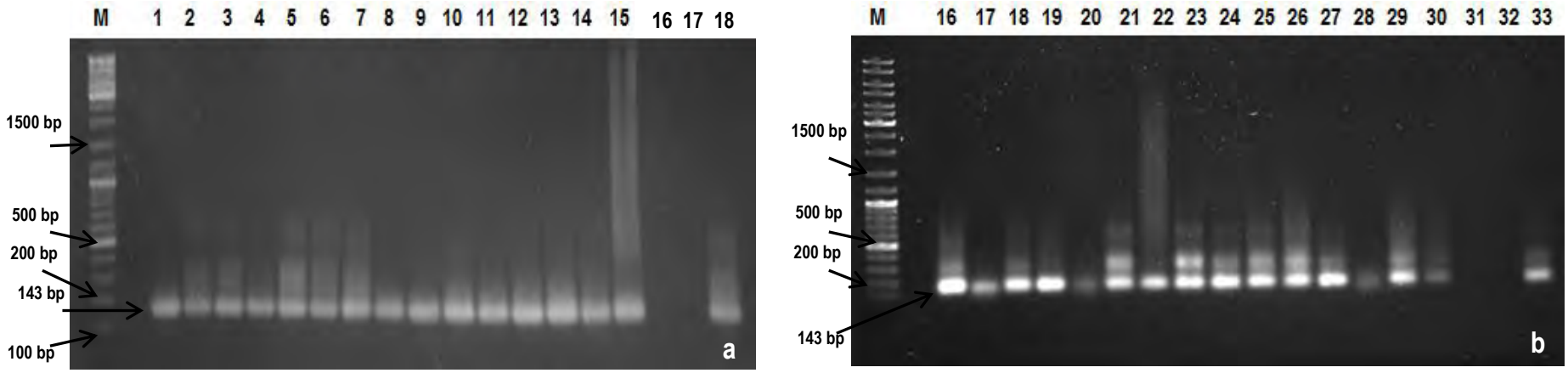


**Figure 3.19** Nested PCR amplification of the hexon gene of 41 adenovirus serotypes. (a) M: molecular weight markers, L1 – L5: April, L6 – L10: May, L11 – L15 June points, L16 blank well, L17: negative control and L18: positive control. (b) M: molecular weight markers, L16 – L20: July, L21 – L25: August, L26 – L30: September, L31: blank well, L32: negative control and L33: positive control.

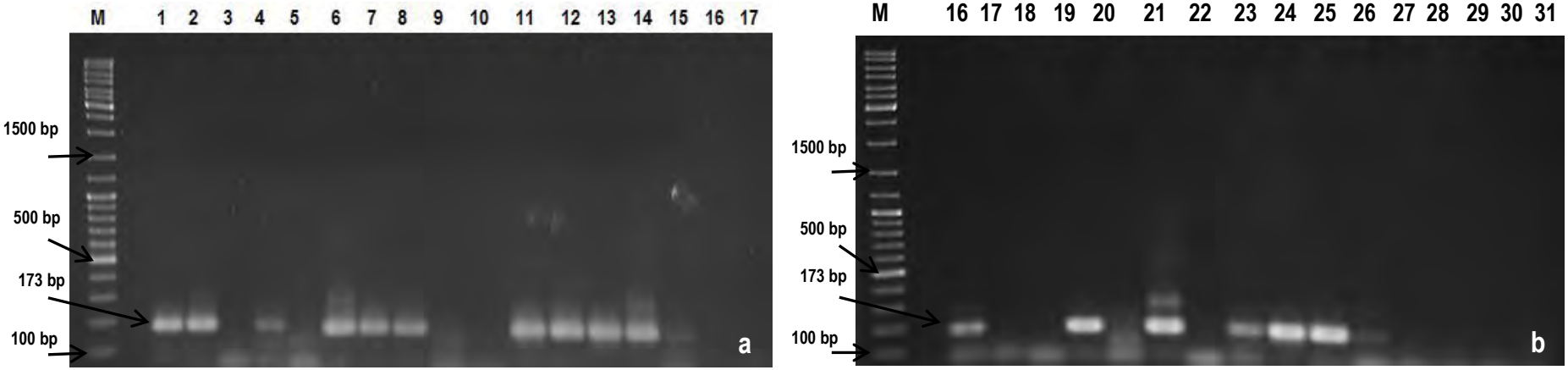


**Figure 3.20** Nested PCR amplification of BK and JC polyomaviruses. (a) M: molecular weight markers, L1 – L5: April, L6 – L10: May, L11 – L15 June points, L16 blank well, L17: negative control. (b) M: molecular weight markers, L16 – L20: July, L21 – L25: August, L26 – L30: September, L31: negative control.

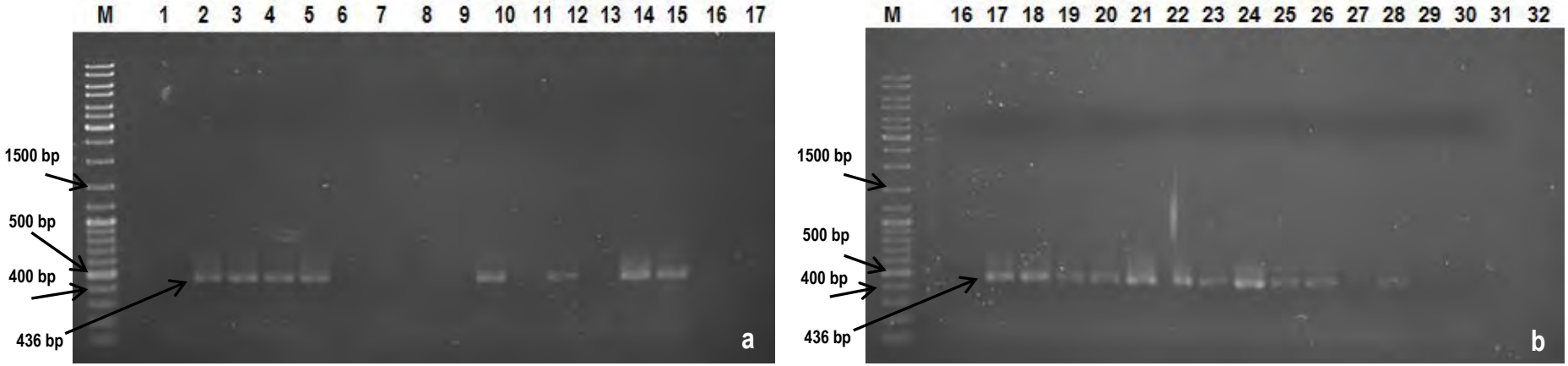


**Figure 3.21** Nested PCR amplification of the VP1/VP2 region of hepatitis A viruses. (a) M: molecular weight markers, L1 – L5: April, L6 – L10: May, L11 – L15 June points, L16 blank well, L17: negative control. (b) M: molecular weight markers, L16 – L20: July, L21 – L25: August, L26 – L30: September, L31: blank well, L32: negative control.

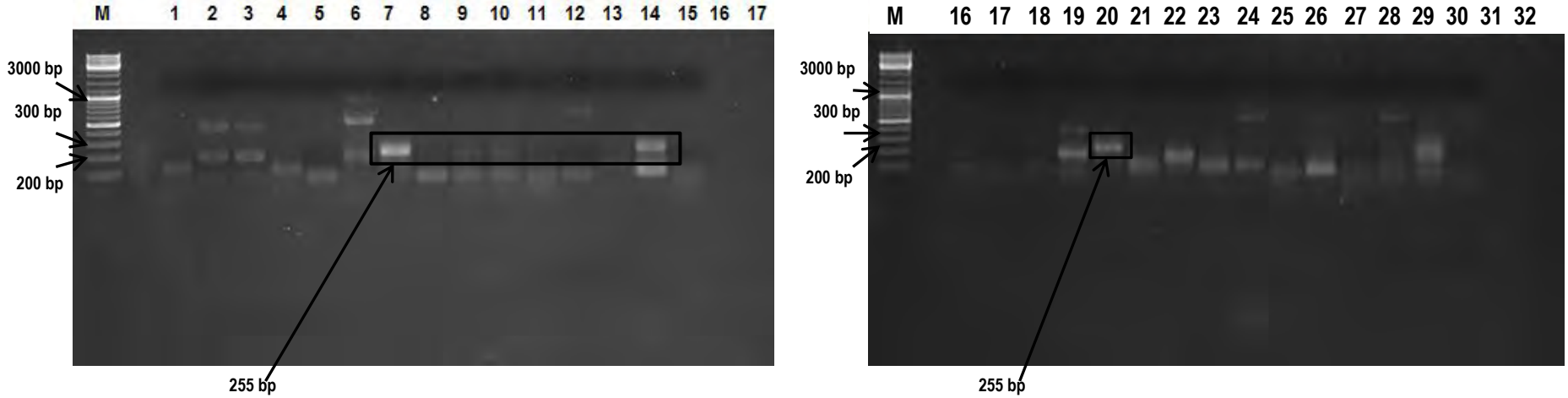


**Figure 3.22** Nested PCR amplification of the 5' untranslated region of hepatitis C viruses. (a) M: molecular weight markers, L1 – L5: April, L6 – L10: May, L11 – L15 June points, L16 blank well, L17: negative control. (b) M: molecular weight markers, L16 – L20: July, L21 – L25: August, L26 – L30: September, L31: blank well, L32: negative control.

The comparison between the environmental samples and the reference sequences in GenBank revealed confirmed adenoviruses, polyomaviruses and hepatitis A and C viruses present in the Umhlangane River. The percentage maximum identity and E-values obtained during the BLAST analysis are depicted in Table 3.3. The sequence identities varied from 86% to 100% to their known complements on the GenBank database. Amongst the sequenced data, human adenovirus C strain, JC polyomavirus isolate GCN8, hepatitis A virus strain CFH and hepatitis C virus isolate Ind-MN19 was identified.

**Table 3.3** BLAST analysis showing the maximum identity and E-values for the tested samples.

| Accession | Description | E-value | Maximum Identity (%) |
|---|---|---|---|
| KF268310.1 | Human adenovirus c strain | 2e-28 | 89 |
| KM205587.1 | Adenovirus 2 isolate AAU4 | 1e-26 | 86 |
| KM225765.1 | JC polyomavirus isolate GCN8, complete genome | 9e-53 | 98 |
| AB081021.1 | JC virus DNA, isolate ME-5 | 4e-61 | 99 |
| HQ246217.1 | Hepatitis A virus CFH-HAV, complete genome | 3e-177 | 95 |
| FJ687513.1 | Hepatitis A isolate 9 polyprotein | 4e-166 | 96 |
| EF473252.1 | Hepatitis C isolate Ind-MN19 5' UTR | 8e-05 | 100 |

## 3.4 Discussion

The contamination of water bodies with diverse microbial communities is a major concern to public safety (Croci *et al*., 2000). Diarrhoeal outbreaks caused by waterborne pathogens is an alarming concern with most cases reported in developing countries due to the lack of proper drinking water infrastructure and sanitation (Hofstra, 2011). Amongst these pathogens the greatest cause for concern comes from the prevalence of enteric viruses in water (Croci *et al*., 2000). Apart from the socio-economic implications of waterborne virus-related sicknesses in both developing and developed nations, the extent of the burden and impact of viral disease is far concentrated in regions with enormous environmental contaminations (Rodríguez-Díaz *et al*., 2009). This study assessed the presence of two coliphages, namely the somatic and F-specific RNA coliphages as well as the prevalence of enteric viruses using various analytical and molecular techniques.

The use of epifluorescence microscopy and SYBR Gold staining provided a cost effective and rapid method for the detection of VLPs in the samples of the Umhlangane River. Moreover, SYBR Gold efficiently stains both RNA and DNA viruses providing a stable and standard enumerative technique (Pavlov *et al*., 2011; Pollard, 2012). Temperature

values varied during the sampling months and points ranging from 18°C (P4 – September) to 25°C (P1, P2 – April; P1 – May) as described in Chapter 2 of section 2.4.1. The high and low direct VLP and indirect coliphage counts observed during April and August, respectively may be due to the change in temperature. The increased viral and bacterial abundances during warmer conditions and lower during colder weather was similar to findings reported by Jiang and Paul (1994), Rinta-Kanto *et al*. (2004) and He *et al*. (2009). The total rainfall data (April: 19 mm; May – July: 0 mm; August: 8 mm and September: 20 mm) was obtained (Accuweather, 2014). Higher temperatures coupled with the washing out of faecal contaminations from the surrounding areas may have also contributed to the high VLP and phage counts observed in April as seen in Figure 3.4 of the CCA diagram. The sudden increase in precipitation during September may have allowed for the sudden increase in the coliphage and VLP populations. Furthermore, changing anthropogenic activities during the time of sampling may have allowed for variations in the virus counts (Eifan, 2013). Although the physico-chemical parameters did not strongly influence the virus population variations, it may have had some impact on the prevalence of coliphages and VLPs in the water. Moreover, a larger impact may have been imposed on the bacterial populations (Rajiv *et al*., 2012) thus affecting the virus populations.

Bacterial proliferation followed the same trend as observed for the coliphage and VLP counts during the sixth month sampling period where higher counts was seen in April, lowest in August and a sudden increase seen in September (Chapter 2, section 2.2.2). The equivalent trend seen for the bacterial and viral indicators may imply that most of the VLPs found in the Umhlangane River may be bacteriophages (Barros *et al*., 2010). Therefore, the high bacterial abundance may allow for greater phage productions since it was observed that phage infectivity increases with bacterial abundance (Mathias *et al*., 1995; Jin *et al*., 2012). The introduction of faecal contamination through anthropogenic activities or runoff effluents could allow for the added presence of bacterial hosts in the water (Pandey *et al*., 2014). Furthermore, the persistence of the bacterial hosts relies on the diversity of virus-host relationships that promote evolution and species diversity (Matteson *et al*., 2011). Considering that most of the enumerated VLPs may be bacteriophages their presence must be vulnerable to the spatial and temporal environmental variations influencing the bacterial populations in the water (Huszar and Reynolds, 1997). Therefore, the TDS content, E.C., turbidity, salinity and DO may have indirectly affected the VLP populations through the direct effect on the bacterial communities. However, considering all the factors revolving around virus enumeration it is difficult to determine if virus abundance is definitively

controlled by bacterial-dependent lysis (Barros *et al*., 2010). The variation between coliphage and VLP numbers may be due to the plaque count anomaly. Bacteriophages in the water samples may exhibit great diversity thus making indirect enumeration for virus counts inefficient as compared to direct counts where all virus particles are stained and enumerated (Shapiro and Kushmaro, 2011).

The low concentration of enteric viruses in environmental waters requires extensive concentration methods to attain a sufficient amount of virus populations for further experimental objectives (Symonds and Breitbart, 2015). Ultrafiltration of the river water samples employing tangential flow filtration (TFF) efficiently concentrated the viruses from the collected water samples. The TFF procedure produces clear concentrates with yielded viral densities in the recycled output retentate (Wommack *et al*., 2010). The two step TFF procedure coupled with the debris and solids removal step provided clear concentrates with most viruses reaming intact and no bacteria.

The detection of infectious enteric viruses in water has long employed cell culture methods (Calgua *et al*., 2011) and is the only method approved by the United States Environmental Protection Agency (USEPA) for the environmental detection of enteric viruses (Jiang, 2006). The method involves the production of cytopathic effect (CPE) imposed on the cells by the viruses particles. Any observable morphological change is considered CPE positive and positive for infective viruses (Lee *et al*., 2004). Since most of the VLPs may have been bacteriophages, tissue culture was used to evaluate if any of the virus particles present in the Umhlangane River were enteric viruses that could be infective to humans or animals.

The production of positive CPE on 50% ($n$ = 10) of the tested virus concentrates shows that infectious enteric viruses are present at various sampling points along the Umhlangane River. Water from sampling points P1 (Phoenix industrial) and P2 (upstream KwaMashu WWTP; tributary from KwaMashu) showed positive CPE on all cell lines. This may be due to the influx of effluents containing human or animal faecal matter harbouring some enteric viruses (La Rosa *et al*., 2012). Cytopathic-based tissue culture assays are considered quantal as they are more sensitive rather than quantitative (EPA, 2001). Quantal assays are simple as the effect is either present or not (Zivin and Waud, 1992) and in tissue culture only one infectious particle is enough to produce a successful CPE (EPA, 2001). However, the efficacy of viral replication on various cell lines depends on the serotypes of the viruses present (Jiang, 2006). Therefore not all viruses or serotypes are susceptible to all cell lines (Lee *et al*., 2004). Furthermore, some enteric viruses are slow growing (Jiang *et al*., 2009) or produce

unclear or no CPE (Calgua *et al*., 2011). Consequently, the negative CPE produced by some of the tested samples may be due to the diversity of the viral population"s present, thus showing negative propagation on the cell monolayers or they may have produced little or no CPE. Moreover, studies have shown that the testing of total environmental VLPs which may contain viruses that do produce CPE on cell lines may also be inhibited due to interference by groups of other viruses (Carducci *et al*., 2002). The present study evaluated the infectious nature of the VLPs using the Hep-G2, HEK293 and Vero cell lines. The Hep-G2 and HEK293 carcinoma cell lines are quite common and can support the growth of many viruses (Leland and Ginocchio, 2007). Hep-G2 cells are highly sensitive to hepatitis A, B and C viruses (WHO, 2008) while HEK293 cells show great sensitivity to human adenovirus types 40 and 41(Jiang *et al.,* 2009). However, certain viruses such as hepatitis viruses are difficult to propagate as seen by the negative CPE of the negative hepatitis A control on the HEK293 cell line. However, the African Green Monkey Kidney (Vero) cell line can harbour the growth of hepatitis viruses (Konduru and Kaplan, 2006) as well as measles viruses, rubella viruses and arboviruses (Osada *et al*., 2014) and was therefore used to increase the sensitivity of viruses in this study.

The high VLP counts and infectious nature of the virus concentrates observed in this study motivated for the use of transmission electron microscopy (TEM) to evaluate the phage and enteric viruses present in the Umhlangane River. The Umhlangane River revealed an enormous abundance of viruses containing both enteric viruses and bacteriophages (Figure 3.8). This indicates that a large amount of organic or faecal contamination is present in the river which is a cause for concern. The classification of bacteriophages allows the simplification in identifying bacterial viruses and facilitates a comparative understanding of these viruses in the environment. Moreover, many bacteriophages possess novel features that can be applied in environmental biotechnological and medical applications (Ackermann, 2011).

Bacteriophages belonging to the *Myoviridae*, *Siphoviridae* and *Podoviridae* families were morphologically diverse showing great abundance. Previous studies reported similar findings where 96% of the total detected phages possessed tails with isometric heads (Demuth *et al*., 1993). Diversity exhibited among the bacteriophages corresponds with the colossal dynamics and diversity observed in their bacterial counterparts and vice versa (Demuth *et al*., 1993; Beckett and Williams, 2013; Williams, 2013). The diversity seen between the phage and bacterial populations were further reiterated by the comparisons of known phages to phages concentrated from the river water samples (Figures 3.11 – 3.13).

*Mycobacteria* that do not cause tuberculosis are environmental *Mycobacteria* and may be found in water, soil, animals and humans which subsequently allows for their phages to be present (Pedulla *et al*., 2003). However, the possibility of some of the phages observed may originate from an allochthonous source (Hewson *et al*., 2012) such as wild animals, waterfowl or anthropogenic practices near the river and cannot be overlooked since a selection of bacteriophages exist in ruminants, sewage and the faeces from birds (Demuth *et al*., 1993).

A number of diverse enteric viruses observed in the Umhlangane River are indicative of a significant amount of faecal contaminations entering the catchment (Cantalupo *et al*., 2011). The source of this faecal pollution and consequent viral contamination may be from surface runoff, recreational activities, storm water discharge, sewage discharge and overflows as well as other humanized practices (Olaniran *et al*., 2009). Many of the detected viruses were found upstream in P1 and P2 (Phoenix residential/industrial; upstream KwaMashu WWTP/tributary from KwaMashu) and downstream in P4 and P5 (Riverhorse Valley business estate/upstream Northern WWTP; Springfield industrial/downstream Northern WWTP). These areas are densely populated comprising a variety of anthropogenic practices with the additional input of animal faecal matter from the surrounding farms (Phoenix areas) and possible effluent originating from informal settlements (Riverhorse Valley). This may have contributed to the viruses observed in the river water samples.

Many presumptive enteric viruses belonging to the *Polyomaviridae*, *Coronaviridae*, *Adenoviridae*, *Picornaviridae* (enterovirus), *Herpesviridae*, *Reoviridae* (rotavirus) and *Orthomyxoviridae* (influenza virus) was visualized along with other unassigned enveloped viruses. Viruses containing nucleocapsids (contains nucleic acids) are protected by an envelope while naked viruses contain an inflexible capsid that can withstand harsh conditions (Ackermann and Heldal, 2010). These morphological characteristics allow enteric viruses to persist in the environment for long periods of time and most often than not survive many treatment processes (Steyer *et al*., 2011). The negative staining allowed for a variety of structures to be visible such as the double-layered rotaviruses (Figure 3.16 g), the *Coronaviridae* "setting-sun" appearance or projections studding the outer membrane called peplomers (Figure 3.18 b), the rough polyomavirus capsids (Figure 3.16 c) and the envelopes and nucleocapsids of many viruses. Moreover, negative staining also allows for the visualization of incomplete viruses as they appear white if incomplete or black if complete. However, since these viruses were concentrated from water their morphological structures

may have faulted through degradation effects (Eifan, 2013) or via the concentration method (Karim *et al*., 2004).

The visualization of a diverse array of enteric viruses in the Umhlangane River drove the motivation for the molecular detection of some of the enteric viruses that may be present in the river water samples. To the best of our knowledge this is the first study done to evaluate the presence of enteric viruses in the Umhlangane River of Durban, South Africa. The application of PCR-based methods to study the presence and diversity of enteric viruses in aquatic environments has greatly advanced over the years (Jiang, 2009) and is the most common technique applied for the detection and identity of viruses in water (Fongaro *et al*., 2013). Conventional PCR has proved to be effective, specific and sensitive to detect low virus concentrations present in water (Girones *et al*., 2010). Nested-PCR has been adopted to further increase the sensitivity of detection for viruses from environmental samples employing two rounds of PCR using two sets of primers (van Heerden *et al*., 2005).

This study demonstrates the effectiveness of ultrafiltration coupled with nested-PCR for the investigation of human enteric viruses from 20 ℓ of river water samples as also concluded by Jiang *et al*. (2001). The nested-PCR detection for the human adenovirus, polyomavirus and hepatitis A and C virus populations was investigated in this study. Many of the first round PCR amplifications using the outer primer sets did not produce bands for some (adenovirus), many (polyomavirus) or even all of the tested samples (hepatitis A and C viruses). Moreover, some of the gels contained many other PCR products that were not the bands of interest. However, the second round of PCR using the inner primers and primer templates from the first rounds provided positive PCR products for the viruses in question. Similar findings was observed by Puig *et al*. (1994) where 12 samples produced negative results for adenoviruses in the first round but appeared positive in the second round of PCR.

The detection of human adenoviruses was observed in 100% ($n$ = 30) of the tested water samples (Figure 3.19 a and b). A number of adenovirus serotypes have been found to infect a variety of human and animal species with a total of 51 *Adenoviridae* serotypes infecting humans (Hundesa *et al*., 2006). Serotypes 40 and 41 are the second leading cause of gastroenteritis in children next to rotaviruses (Jiang *et al*., 2001). The detection of adenoviruses can be done quite easily and their numbers far exceed hepatitis A and enteroviruses in many aquatic habitats. Furthermore, adenoviruses show greater stability to chlorination and UV irradiation than enteroviruses (Hundesa *et al*., 2006) allowing them to persist in the environment for much longer time periods. This study corroborated with these statements showing high prevalence of adenoviruses in the Umhlangane River. A similar

finding was observed by van Heerden *et al*. (2005) where adenoviruses were detected in many river water samples in South Africa.

Human polyomaviruses (BKV and JCV) was identified in 18/30 river water samples during April to September 2014 (Figure 3.20 a and b). Polyomaviruses BK and JC are exclusive to humans and cause asymptomatic viruria (Polo *et al*., 2004). These viruses are excreted from the faeces and urine of humans (Hundesa *et al*., 2006). The high occurrence of human polyomaviruses present in urban sewage may come from urine since JCVs has been found in 20 – 80% of adult urine samples (Kitamura *et al*., 1990). These viruses are protected when ingested with food and are stable at acidic pH (Bofill-Mas *et al*., 2001).

Hepatitis A virus was detected in 70% ($n$ = 30) of the river water samples (Figure 3.21 a and b). The hepatitis A virus is the number one cause for gastroenteritis worldwide. Outbreaks of hepatitis A related sickness in many countries is noteworthy (Bloch *et al*., 1990; de Serres *et al*., 1999; Utaipiboon *et al*., 2002). These viruses have been successfully isolated from various water sources including dams (Taylor *et al*., 2001), rivers (Pina *et al*., 2001) and groundwater (Borchardt *et al*., 2003). Hepatitis A viruses have been found to persist in groundwater for a number of months (Rzeżutka and Cook, 2004; El-Senousy *et al*., 2014; La Rosa *et al*., 2012) and shows resistance to common disinfectants (Li *et al*., 2002b; Bigliardi and Sansebastiano, 2006). Furthermore, these viruses can survive exposure 20% ether, acidity (pH 1.0 for 2 hours) and heating to 60°C for 1 hour (Kocwa-Haluch, 2001).

Hepatitis C viruses was detected in 10% ($n$ = 30) of the collected water samples (Figure 3.22 a and b). These viruses infect approximately 150 to 200 million people worldwide (Maier and Wu, 2002). These viruses cause chronic and acute hepatic diseases and due to the high mutation rates of the virus therapeutic or prophylactic vaccines are currently not available (Doerrbecker *et al*., 2013).

The nucleotide sequences of the adenoviruses, polyomaviruses and hepatitis A and C viruses confirmed using BLAST analysis revealed different virus strains including adenoviruses C strains, JC polyomaviruses and strain CFH and isolate Ind-MN19 for hepatitis A and C viruses, respectively. Not only does the BLAST analysis confirm the presence of these four viral groups in the river water but also indicates the diversity of these enteric viruses residing the Umhlangane River. This solidifies the fact that enteric viruses, particularly RNA viruses have high mutation rates (Schrag *et al*., 1999; Hryniszyn *et al*., 2013).

In conclusion, this study investigated the presence of viral populations in the Umhlangane River using TFF and subsequent analytical and molecular methods. The TFF

concentration of viruses from 20 ℓ of river water did not require sample manipulation such as pH adjustment or the use of PCR inhibitors (beef extract) for elution (Jiang *et al*., 2001). Epifluorescence microscopy showed the apparent difference in virus enumeration (105 vlp/mℓ; April) in comparison to indirect plaque assays (417 pfu/mℓ; April) which was coincidentally found to be infective to Hep-G2, HEK293 and Vero cell lines. Furthermore, the use of TEM allowed the visualization of many bacterial and eukaryotic viruses (*Picronaviridae*, *Adenoviridae*, *Herpesviridae*, *Polyomaviridae*, *Orthomyxoviridae*, *Reoviridae* and *Coronaviridae* families) present in the catchment. Molecular detection further identified specific viral groups, namely human adenoviruses, polyomaviruses and hepatitis A and C viruses that are present in the Umhlangane which may impose a great health risk to individuals who may directly or indirectly utilize this water source. The findings presented in this study reiterate the potential danger of enteric viruses in water environments. Furthermore, land use activities and poor management in effluent disposal is a lesson not just for organizations dealing with the Umhlangane River but for all organizations managing any water resource, globally.

# Chapter *four*

# Research in perspective and future recommendations

## 4.1 Research in perspective

The accessibility to ample and safe sanitation and water is a basic human need and is indispensable to human wellbeing (Kanyerere *et al*., 2012). Surface water quality is a very delicate subject that deserves the attention it most often does not receive. Many anthropogenic impacts including agricultural and industrial exploits, urbanization and the routinely annual increase in water sources for consumption are decreasing water quality.
In addition, natural effects such as erosion, oscillating geographical precipitation and the weathering of crustal materials are also contributing to the declining surface water quality.
These effects can impair water sources for recreational, agricultural and drinking purposes (Simeonov *et al*., 2003). Water-scarce countries like South Africa rely on rivers for the nation‟s supply of water intended for various societal purposes (Chigor and Okoh, 2012). Apart from the role of rivers in the assimilation of municipal wastes and runoffs (industrial and agricultural) (Wang *et al*., 2007), rivers are also important water sources (Yu and Shang, 2003). Thus, it is imperative to control and possibly prevent river pollution with the continuous availability of reliable information on water quality for accurate management purposes (Wang *et al*., 2007). Moreover, endemic diseases are much higher in developing countries (Dhara *et al*., 2013) where wastewaters containing high indices of waterborne pathogens are rarely identified which is also true for most cases in developed countries (Ashbolt, 2004).

Pathogenic microorganisms are ubiquitous components of every ecosystem. However, the introduction of faecal pollution through anthropogenic practices has majorly contributed to the on-going declining surface water quality (Wang *et al*., 2007; Páll *et al*., 2013). Animal and human enteric pathogenic microorganisms that are transmissible through soil, water, sediment and agricultural environments are imperative pollutants (Bonetta *et al*., 2011). Groundwater and surface water quality assessment remains one of the main objectives in developed parts of the world (Páll *et al*., 2013). There is a persistent plea for water quality monitoring in terms of pathogenic microorganisms (Pekárová *et al*., 2009) thus indicating the cause for concern over human and animal health protection (Fey *et al*., 2004).

Currently, many water quality monitoring programmes employ bacterial indicators such as intestinal enterococci and faecal coliforms (Mattioli *et al*., 2014). However, bacterial indicators do not provide the accurate assessment on the presence of waterborne enteric viruses (Straub *et al*., 2003; Rodríguez *et al*., 2008; Sibanda and Okoh, 2013). In addition, bacterial indicators are (i) difficult to detect when faecal pollution is low (Stewart *et al*., 2008), (ii) can reproduce in the environment (Hot *et al*., 2003) and (iii) are quickly removed

by treatment processes (Rodríguez *et al*., 2008). Thus, the use of bacterial viruses (somatic and $F^{+}$RNA coliphages) were later proposed as indicators of faecal contamination due to their survival characteristics and cost-effective detection methods (Leclerc *et al*., 2000). However, the use of somatic coliphages as surrogates to enteric viruses may not be applicable in all scenarios due to the ecology and origin of enteric viruses while also displaying unsuccessful indication of the integrity of distribution systems even when the problem may be faecal contamination. The male F-specific RNA coliphages may also be an untrustworthy indicator of enteric viruses as they have been known to replicate in the environment when a suitable host has been introduced (Figueras and Borrego, 2010).

Enteric viruses are a public health threat which can cause disease with 1 – 50 infectious virus particles. Apart from the overbearing socio-economic impact caused by viruses their severity of viral disease is greatly present in societies engrossed in high pollution levels (Rodríguez-Díaz *et al*., 2009). Enteric viruses are found in high numbers in human or animal excrement which are then transmitted via the faecal-oral route (Lee and Jeong, 2004). Thus, water environments, irrespective of the source (recreational or drinking) are important vectors for virus transmission not only due to the uncertainty on acceptable virus levels but the resistance exhibited by enteric viruses to disinfection processes (Dongdem *et al*., 2009). To date over 100 types of enteric viruses have been identified in water and wastewater sources that can be transmitted to humans (Bosch *et al*., 2011). From a health perspective the most noteworthy waterborne enteric viruses include adenoviruses, rotaviruses, nonoviruses, enteroviruses and hepatitis A and E viruses (Okoh *et al*., 2010). These viruses may emerge to cause sporadic outbreaks that could endanger public health and wellbeing. For instance, a severe rotavirus outbreak in Durban in 2013 (South Africa) unexpectedly infected 48 (confirmed positive) people most of which were children under the age of 5. The origin of the outbreak was unknown but could have been indirectly related to a potential contaminated water source (Data provided by the eThekwini Municipality). The first step for virus detection from environmental waters is the initial concentration from large volumes of water (Lambertini *et al*., 2008; Ikner *et al*., 2011). Thereafter, a variety of methods can be employed to detect enteric viruses in aquatic environments (Hamza *et al*., 2011).

Apart from an extensive microbiological examination of the Umgeni River (Ganesh *et al*., 2014) very few studies have evaluated surface water quality in Durban. Considering the implications of faecal pollution entering rivers through natural and anthropogenic practices containing both bacterial and viral pathogens, this study attempted to investigate the quality of the Umhlangane River (tributary to Durban‟s drinking water catchment) over a 12 month

period. Sampling points were chosen upstream and downstream of various land use zones to evaluate the effects of human activities on the water quality. Moreover, the 12 month experimental design allowed the investigation of temporal dynamics that may be influencing the survivability of these pathogens in the river water.

Physico-chemical properties of the river water samples fluctuated at all sampling points throughout the sampling period. Accordingly, most of the measured varied significantly with the sampling points which may be due to the anthropogenic influences at the sampling points. Moreover, many of the sampling points at different months exceeded the DWA guidelines for either recreational or industrial use depicting the water as public safety or work hazard.

High bacterial counts were observed for all indicators throughout the sampling period and sampling points. As expected, increased bacterial proliferation was observed during the summer and rainy months compared to the cold and dry seasons. No trend was observed in the sampling points as conditions changed at every sampling location each sampling month. The fluctuations between the indicators at the sampling points could be due to changing anthropogenic activities at the time of sampling. The CCA statistical assessment provided evidence of relationships between the bacterial indicators and the physico-chemical parameters.

The somatic and $F^{+}$RNA coliphages followed a similar trend to that observed for the bacterial indicators. This was expected since bacteriophages are dependent on the bacterial hosts to replicate in the environment. Moreover, as previously reported in literature the somatic coliphage count was substantially higher than $F^{+}$RNA phages. The VLPs observed under epifluorescence microscopy was found to be infectious with many presumptive enteric viruses visualized under TEM thus showing that these viruses may pose a risk to human and animal health. However, it should be noted that while TEM may be useful to the visualization of viruses in water, it cannot be the sole method towards the exact identification of pathogens as not all images are clear enough to conclude on. The confirmation of adenovirus, polyomaviruses, hepatitis A and hepatitis C virus genomes in the river further reiterates the point of the Umhlangane River as a health hazard.

The hypothesis of the current study postulated the presence microbial pathogens (bacteria, coliphages and enteric viruses) with particular interest in enteric viral pathogens residing in the Umhlangane River of Durban, South Africa. The outcomes gathered in this study supported the aforementioned hypothesis showcasing an abundance of bacterial and viral pathogens existent in the Umhlangane River. Moreover, these results undoubtedly demonstrate the poor water quality indicating the drainage of organic pollution in the river.

Therefore, the current study recognizes that this water source is not safe for any societal purpose without the continuous admission of proper treatment or monitoring systems set in place. Additionally, the overbearing existence of enteric viruses in the river shows that regular monitoring systems of virus populations should not be overlooked in an attempt to safeguard human and animal health as virus persistence is implicit and not at all overstated.

## 4.2 Future recommendations

Current water quality monitoring programmes have been extensively standardized, extending from the sampling methods to the eventual pathogen enumeration and detection techniques. However, the probability that any particular technique or indicator can be reliably used in water quality monitoring or in the detection of faecal pollutions is fundamentally unlikely. Many drawbacks hold true for the constant reuse of bacterial indicator and coliphage analyses. The possible use of enteric viruses as indicators of faecal contaminations should be implemented in regular water quality monitoring programmes. However, second world counties such as South Africa would not be able to establish such a system for monitoring viruses. These types of equipment are expensive and require specialized technicians for operation. Thus, proper detection using general chemical and microbial analyses should be conducted with proper management on waste disposal.

Considering the difficulty in virus concentration and detection methods from environmental samples, a joint project between water research professionals at research and academic based institutions and government organizations should be established to disseminate a successful water quality programme and available guideline inclusive of bacterial and viral indicators. Furthermore, the motivation for virus-bacterial population dynamics and enteric virus resilience properties to harsh conditions could sprout new and advanced wastewater and drinking water treatment processes. The possible advancement in wastewater treatments that guarantees a 100% removal of enteric viruses may drive the consolidated use of wastewater as a possible drinking or recreational water resource thus partially thwarting water scarcity problems in developing countries and drought-filled countries. The significance of enteric pathogenic viruses in surface water cannot be overstated and should not only be taken into account when an outbreak endangering human and animal has intermittently occurred.

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# Appendix i

# Preparation of reagents, media and TFF maintenance solutions

# 1 Reagent preparations

## 1.1 Calcium-glucose solution

Dissolve 3 g of calcium chloride dihydrate ($CaCl_2.2H_20$ – Merck-Millipore) and 10 g glucose (Sigma) into 100 mℓ distilled water. Filter sterilize and store in the dark at 4°C.

## 1.2 1% Triphenyltetrazolium chloride solution

Dissolve 1 g of 2,3,5 triphenyltetrazolium chloride (Sigma) into 70 mℓ double distilled water. Dissolve the contents and then top up to 100 mℓ. Filter sterilize, cover the bottle in foil and maintain at 4°C.

## 1.3 1 M NaOH solution

Dissolve 4 g NaOH pellets (Merck-Millipore) in 100 mℓ distilled water.

## 1.4 Nalidixic acid solution

Suspend 1.25 g nalidixic acid (Sigma) in 10 mℓ NaOH solution and 40 mℓ of distilled water and mix well. Filter sterilize the solution, cover in foil and store at 4°C.

## 1.5 1 M Sodium carbonate solution

Suspend 10.6 g sodium carbonate (Merck-Millipore) in 100 mℓ autoclaved distilled water and mix well to homogenize the solution.

## 1.6 1 M Magnesium chloride solution

Dissolve 20.3 g magnesium chloride (Merck-Millipore) in 100 mℓ autoclaved distilled water and mix well.

## 1.7 1 X Phosphate buffered saline (PBS)

Dissolve 2 PBS pellets (Sigma) in 200 ml of distilled water and autoclave. Adjust pH with 1 M sodium hydroxide solution if necessary.

# 2 Indicator bacteria media preparations

## 2.1 Nutrient Agar

Suspend 31 g in 1 ℓ demineralized water. Boil whist stirring until completely dissolved. Autoclave at 121°C for 15 minutes. Cool to 45-50°C, mix and pour plates.

## 2.2 m-ENDO Agar

Suspend 51 g in 1 ℓ demineralized water. Add 20 ml ethanol and stand for 10 minutes. Boil until dissolved and pour into plates. Store away from light at 4°C. DO NOT AUTOCLAVE/OVER HEAT. (pH 7.2).

## 2.3 m-FC Agar

Dissolve 50 g in 1 ℓ demineralized water. Boil whilst stirring until dissolved. If desired add 10 ml 1% solution of rosolic acid in 0.2 N NaOH. Continue heating for 1 minute. Cool to 45-50°C, mix well and pour plates. DO NOT AUTOCLAVE/OVER HEAT. (pH 7.4).

### 2.4 FS (KF – *Streptococcus* Agar)

Suspend 76 g in 1 ℓ distilled water and dissolve by boiling. Dispense into 100 ml portions and sterilize by autoclaving at 121°C for 10 minutes. Cool to 50°C and add 1 ml of 1% triphenyltetrazolium chloride solution per 100 ml. DO NOT OVER HEAT. (pH 7.2).

### 2.5 TCBS Agar

Dissolve 88 g in 1 ℓ demineralized water and bring to the boil. Cool and pour plates. DO NOT AUTOCLAVE. (pH 8.6).

### 2.6 SS Agar

Suspend 60 g in 1 ℓ demineralized water by heating in a boiling water bath or in a current of stream. DO NOT AUTOCLAVE. Cool rapidly and pour plates. (pH 7).

### 2.7 Chromocult Agar

Suspend 26.5 g in 1 ℓ demineralized water by heating in a boiling water bath or a current of stream. Stir until dissolved. DO NOT AUTOCLAVE. Cool to 45-50°C and pour plates. (pH 6.8).

## 3 Bacteriophage media

### 3.1 Bottom agar for somatic coliphages

| | |
|---|---|
| Bacteriological agar | 14 g |
| Tryptone (Pancreatic Digest) | 13 g |
| Sodium chloride | 8 g |
| Glucose | 1.5 g |

Dissolve all components in 1000 mℓ distilled water and autoclave for 15 minutes at 121°C. Cool the media to 50°C and add 1 mℓ nalidixic acid solution. Pour plates and allow solidifying.

### 3.2 Top agar for somatic coliphages

| | |
|---|---|
| Bacteriological agar | 4 g |
| Tryptone (Pancreatic Digest) | 5 g |
| Sodium chloride | 4 g |
| Glucose | 1.5 g |

Dissolve all components in 500 mℓ distilled water and autoclave for 15 minutes at 121°C. Cool the media to 50°C and add 2.5 mℓ sodium carbonate solution, 0.5 mℓ magnesium chloride solution and 0.5 mℓ nalidixic acid solution. Pour plates and allow solidifying.

### 3.3 Bottom agar for F-RNA coliphages

| | |
|---|---|
| Trypticase peptone | 10 g |
| Yeast extract | 1 g |
| Sodium chloride | 8 g |
| Bacteriological agar | 12 g |

Dissolve all components in 1000 mℓ distilled water and autoclave for 15 minutes at 121°C. Cool the media to 50°C and add 10 mℓ calcium-glucose solution. Pour plates and allow solidifying.

### 3.4 Top agar for F-RNA coliphages

| | |
|---|---|
| Trypticase peptone | 5 g |
| Yeast extract | 0.5 g |
| Sodium chloride | 4 g |
| Bacteriological agar | 3.3 g |

Dissolve all components in 500 mℓ distilled water and autoclave for 15 minutes at 121°C. Cool the media to 50°C and add 5 mℓ calcium-glucose solution and 2 mℓ nalidixic acid solution. Pour plates and allow solidifying.

## 4 TFF maintenance and storage solutions

### 4.1 0.4 N Sodium hydroxide solution

Dissolve 160 g NaOH pellets (Merck-Millipore) in 10 ℓ distilled water. Mix and allow the solution to heat between 30-50°C.

### 4.2 Chlorine solution

Dissolve 1 chlorine pellet (reverse osmosis chlorine pellets, Sigma) in 15 ℓ distilled water.

### 4.3 0.1 N Sodium hydroxide solution (depyrogenation and storage solution)

Dissolve 20 g NaOH pellets (Merck-Millipore) in 10 ℓ distilled water. Mix and allow the solution to heat between 30-50°C.

## 5 Buffers for molecular work

### 5.1 0.5 M Disodium ethylenediaminetetraacetate (EDTA)

| | |
|---|---|
| EDTA (Saarchem) | 186.12 g |
| Distilled water | 1000 mℓ |
| Sodium hydroxide pellets (pH adjustment) | pH 8 |

### 5.2 50 X Tris-acetate EDTA buffer (TAE)

| | |
|---|---|
| Tris base (Merck) | 242 g |
| 0.5 M EDTA (pH 8) | 100 mℓ |
| Glacial acetic acid (Merck) | 57.1 mℓ |
| Sodium hydroxide pellets (pH adjustment) | pH 8 |

### 5.3 Phosphate buffered saline (PBS)

Dissolve 5 PBS pellets (Sigma) in 1000 mℓ distilled water. Adjust pH to 7 using hydrochloric acid if necessary. Autoclave for 15 minutes at 121°C.

5.4 Ethidium bromide stain

| | | |
|---|---|---|
| Ethidium bromide (Sigma) | 50 | μℓ |
| Distilled water | 500 | mℓ |

# Appendix ii

# Enumeration data and statistical analyses

**Table 1** Presumptive bacterial indicator counts at all sampling points during October 2013.

| **Total heterotrophic bacteria (THB)** | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **P1** | | | **P2** | | | **P3** | | | **P4** | | | **P5** | | |
| | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^6$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^6$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^6$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^6$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^6$** |
| **10^4** | 83 | 1,66 | 1,66 | 71 | 1,42 | 1,42 | 87 | 1,74 | 1,74 | 89 | 1,78 | 1,78 | 92 | 1,84 | 1,84 |
| | 80 | 1,6 | 1,6 | 69 | 1,38 | 1,38 | 85 | 1,70 | 1,7 | 91 | 1,82 | 1,82 | 93 | 1,86 | 1,86 |
| **10^5** | 46 | 9,2 | 9,2 | 38 | 7,60 | 7,6 | 40 | 8,00 | 8 | 47 | 9,40 | 9,4 | 45 | 9,00 | 9 |
| | 47 | 9,4 | 9,4 | 34 | 6,80 | 6,8 | 40 | 8,00 | 8 | 46 | 9,20 | 9,2 | 49 | 9,80 | 9,8 |
| **10^6** | 19 | 3,8 | 38 | 17 | 3.40 | 34 | 20 | 4.00 | 40 | 25 | 5.00 | 50 | 32 | 6.40 | 64 |
| | 20 | 4 | 40 | 12 | 2.40 | 24 | 20 | 4.00 | 40 | 28 | 5.60 | 56 | 27 | 5.40 | 54 |
| **Average** | | | 9,30 | | | 7,20 | | | 8.00 | | | 9,30 | | | 9,40 |
| **StDev** | | | 0,14 | | | 0,57 | | | 0.00 | | | 0,14 | | | 0,57 |
| ***E.coli* (EC)** | | | | | | | | | | | | | | | |
| | **P1** | | | **P2** | | | **P3** | | | **P4** | | | **P5** | | |
| | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** |
| **10^2** | 15 | 3.0 | 3 | 11 | 2.2 | 2,2 | 14 | 2.8 | 2,8 | 19 | 3.8 | 3,8 | 14 | 2.8 | 2,8 |
| | 12 | 2.4 | 2,4 | 12 | 2.4 | 2,4 | 15 | 3.0 | 3 | 18 | 3.6 | 3,6 | 12 | 2.4 | 2,4 |
| **10^3** | 2 | 4.0 | 4 | 1 | 2.0 | 2 | 1 | 2.0 | 2 | 7 | 1.4 | 14 | 7 | 1.4 | 14 |
| | 1 | 2.0 | 2 | 2 | 4.0 | 4 | 3 | 6.0 | 6 | 5 | 1.0 | 10 | 3 | 6.0 | 6 |
| **Average** | | | 2,70 | | | 2,30 | | | 2,90 | | | 3,70 | | | 2,60 |
| **StDev** | | | 0,42 | | | 0,14 | | | 0,14 | | | 0,14 | | | 0,28 |
| **Total coliforms (TC)** | | | | | | | | | | | | | | | |
| | **P1** | | | **P2** | | | **P3** | | | **P4** | | | **P5** | | |
| | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** |
| **10^2** | 19 | 3.8 | 3,8 | 17 | 3.4 | 3,4 | 20 | 4.0 | 4 | 17 | 3.4 | 3,4 | 21 | 4,2 | 4,2 |
| | 19 | 3.8 | 3,8 | 18 | 3.6 | 3,6 | 17 | 3.4 | 3,4 | 19 | 3.8 | 3,8 | 20 | 4,0 | 4 |
| **10^3** | 7 | 1.4 | 14 | 9 | 1.8 | 18 | 9 | 1.8 | 18 | 7 | 1.4 | 14 | 12 | 2,4 | 24 |
| | 12 | 2.4 | 24 | 8 | 1.6 | 16 | 7 | 1.4 | 14 | 5 | 1.0 | 10 | 9 | 1,8 | 18 |
| **Average** | | | 3,80 | | | 3,50 | | | 3,70 | | | 3,60 | | | 4,10 |
| **StDev** | | | 0,00 | | | 0,14 | | | 0,42 | | | 0,28 | | | 0,14 |
| **Faecal coliforms (FC)** | | | | | | | | | | | | | | | |
| | **P1** | | | **P2** | | | **P3** | | | **P4** | | | **P5** | | |
| | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** |

| | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **10^1** | 110 | 2.20 | 2,20 | 113 | 2.26 | 2,26 | 78 | 1.56 | 1,56 | 42 | 8.40 | 0,84 | 84 | 1.68 | 1,68 |
| | 112 | 2.24 | 2,24 | 115 | 2.30 | 2,30 | 81 | 1.62 | 1,62 | 43 | 8.60 | 0,86 | 88 | 1.76 | 1,76 |
| **10^2** | 55 | 1.10 | 11.0 | 82 | 1.64 | 16,4 | 40 | 8.00 | 8.00 | 34 | 6.80 | 6,80 | 22 | 4.40 | 4,40 |
| | 61 | 1.22 | 12,2 | 87 | 1.74 | 17,4 | 40 | 8.00 | 8.00 | 31 | 6.20 | 6,20 | 24 | 4.80 | 4,80 |
| **10^3** | 19 | 3.80 | 38.0 | 5 | 1.00 | 10.0 | 21 | 4.20 | 42.0 | 18 | 3.60 | 36.0 | 3 | 6.00 | 6.00 |
| | 22 | 4.40 | 44.0 | 6 | 1.20 | 12.0 | 18 | 3.60 | 36.0 | 20 | 4.00 | 40.0 | 0 | 0.00 | 0.00 |
| **Average** | | | 2,22 | | | 2,28 | | | 1,59 | | | 0,85 | | | 1,72 |
| **StDev** | | | 0,03 | | | 0,03 | | | 0,04 | | | 0,01 | | | 0,06 |
| **Faecal streptococci (FS)** | | | | | | | | | | | | | | | |
| | **P1** | | | **P2** | | | **P3** | | | **P4** | | | **P5** | | |
| | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** |
| **10^1** | 62 | 1.24 | 1,24 | 100 | 2.00 | 2.00 | 46 | 9.20 | 0,92 | 55 | 1.10 | 1,10 | 29 | 5.80 | 0,58 |
| | 64 | 1.28 | 1,28 | 100 | 2.00 | 2.00 | 46 | 9.20 | 0,92 | 57 | 1.14 | 1,14 | 60 | 1.20 | 1,20 |
| **10^2** | 39 | 7.80 | 7,80 | 52 | 1.04 | 10,4 | 32 | 6.40 | 6,40 | 21 | 4.20 | 4,20 | 33 | 6.60 | 6,60 |
| | 24 | 4.80 | 4,80 | 30 | 6.00 | 6.00 | 29 | 5.80 | 5,80 | 20 | 4.00 | 4.00 | 31 | 6.20 | 6,20 |
| **10^3** | 5 | 1.00 | 10.0 | 2 | 4.00 | 4.00 | 17 | 3.40 | 34.0 | 7 | 1.40 | 14.0 | 10 | 2.00 | 20.0 |
| | 6 | 1.20 | 12.0 | 3 | 6.00 | 6.00 | 12 | 2.40 | 24.0 | 10 | 2.00 | 20.0 | 14 | 2.80 | 28.0 |
| **Average** | | | 1,26 | | | 2.00 | | | 6,10 | | | 4,10 | | | 6,40 |
| **StDev** | | | 0,03 | | | 0,00 | | | 0,42 | | | 0,14 | | | 0,28 |
| ***Vibrio* species (VIB spp.)** | | | | | | | | | | | | | | | |
| | **P1** | | | **P2** | | | **P3** | | | **P4** | | | **P5** | | |
| | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** |
| **10^1** | 128 | 2.56 | 2,56 | 304 | 6.08 | 6,08 | 69 | 1.38 | 1,38 | 154 | 3.08 | 3,08 | 98 | 1.96 | 1,96 |
| | 137 | 2.74 | 2,74 | 311 | 6.22 | 6,22 | 50 | 1.00 | 1.00 | 138 | 2.76 | 2,76 | 101 | 2.02 | 2,02 |
| **10^2** | 98 | 1.96 | 19,6 | 268 | 5.36 | 53,6 | 5 | 1.00 | 1.00 | 72 | 1.44 | 14,4 | 96 | 1.92 | 19,2 |
| | 114 | 2.28 | 22,8 | 252 | 5.04 | 50,4 | 14 | 2.80 | 2,80 | 78 | 1.56 | 15,6 | 87 | 1.74 | 17,4 |
| **10^3** | 52 | 1.04 | 104 | 41 | 8.20 | 82.0 | 1 | 2.00 | 2.00 | 1 | 2.00 | 2.00 | 22 | 4.40 | 44.0 |
| | 62 | 1.24 | 124 | 33 | 6.60 | 66.0 | 0 | 0.00 | 0.00 | 0 | 0.00 | 0.00 | 23 | 4.60 | 46.0 |
| **Average** | | | 2,65 | | | 6,15 | | | 2,40 | | | 2,92 | | | 1,99 |
| **StDev** | | | 0,13 | | | 0,10 | | | 0,57 | | | 0,23 | | | 0,04 |
| ***Salmonella* species (SAL spp.)** | | | | | | | | | | | | | | | |
| | **P1** | | | **P2** | | | **P3** | | | **P4** | | | **P5** | | |
| | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** |
| **10^1** | 80 | 1.60 | 1,60 | 41 | 8.20 | 0,82 | 41 | 8.20 | 0,82 | 52 | 1.04 | 1,04 | 69 | 1.38 | 1,38 |
| | 81 | 1.62 | 1,62 | 40 | 8.00 | 0,80 | 46 | 9.20 | 0,92 | 52 | 1.04 | 1,04 | 69 | 1.38 | 1,38 |

| | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **10^2** | 8 | 1.60 | 1,60 | 8 | 1.60 | 1,60 | 22 | 4.40 | 4,40 | 27 | 5.40 | 5,40 | 47 | 9.40 | 9,40 |
| | 6 | 1.20 | 1,20 | 10 | 2.00 | 2.00 | 29 | 5.80 | 5,80 | 21 | 4.20 | 4,20 | 40 | 8.00 | 8.00 |
| **10^3** | 0 | 0.00 | 0.00 | 2 | 4.00 | 4.00 | 14 | 2.80 | 28.0 | 9 | 1.80 | 18.0 | 32 | 6.40 | 64.0 |
| | 1 | 2.00 | 2.00 | 1 | 2.00 | 2.00 | 13 | 2.60 | 26.0 | 3 | 6.00 | 6.00 | 20 | 4.00 | 40.0 |
| **Average** | | | 1,61 | | | 2.00 | | | 0,87 | | | 1,04 | | | 1,38 |
| **StDev** | | | 0,01 | | | 0,00 | | | 0,07 | | | 0,00 | | | 0,00 |
| ***Shigella* species (SHIG spp.)** | | | | | | | | | | | | | | | |
| | **P1** | | | **P2** | | | **P3** | | | **P4** | | | **P5** | | |
| | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** |
| **10^1** | 92 | 1.84 | 1,84 | 55 | 1.10 | 1,10 | 37 | 7.40 | 0,74 | 40 | 8.00 | 0,80 | 59 | 1.18 | 1,18 |
| | 102 | 2.04 | 2,04 | 58 | 1.16 | 1,16 | 36 | 7.20 | 0,72 | 41 | 8.20 | 0,82 | 49 | 9.80 | 0,98 |
| **10^2** | 19 | 3.80 | 3,80 | 12 | 2.40 | 2,4 | 15 | 3.00 | 3.00 | 15 | 3.00 | 3.00 | 32 | 6.40 | 6,40 |
| | 18 | 3.60 | 3,60 | 17 | 3.40 | 3,4 | 17 | 3.40 | 3,40 | 14 | 2.80 | 2,80 | 36 | 7.20 | 7,20 |
| **10^3** | 5 | 1.00 | 10.0 | 2 | 4.00 | 4.00 | 4 | 8.00 | 8.00 | 7 | 1.40 | 14.0 | 11 | 2.20 | 22.0 |
| | 4 | 8.00 | 8.0 | 2 | 4.00 | 4.00 | 1 | 2.00 | 2.00 | 0 | 0.00 | 0.00 | 3 | 6.00 | 6.00 |
| **Average** | | | 1,94 | | | 1,13 | | | 0,73 | | | 0,81 | | | 1,08 |
| **StDev** | | | 0,14 | | | 0,04 | | | 0,01 | | | 0,01 | | | 0,14 |

**Table 2** Presumptive bacterial indicator counts at all sampling points during November 2013.

| **Total heterotrophic bacteria (THB)** | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **P1** | | | **P2** | | | **P3** | | | **P4** | | | **P5** | | |
| | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^6$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^6$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^6$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^6$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^6$** |
| **10^5** | 47 | 9.40 | 9,40 | 43 | 8.60 | 8,60 | 33 | 6.60 | 6,60 | 38 | 7.60 | 7,60 | 37 | 7.40 | 7,40 |
| | 45 | 9.00 | 9.00 | 42 | 8.40 | 8,40 | 31 | 6.20 | 6,20 | 37 | 7.40 | 7,40 | 40 | 8.00 | 8.00 |
| **10^6** | 29 | 5.80 | 58 | 20 | 4.00 | 40 | 17 | 3.40 | 34 | 23 | 4.60 | 46 | 21 | 4.20 | 42 |
| | 26 | 5.20 | 52 | 24 | 4.80 | 48 | 19 | 3.80 | 38 | 23 | 4.60 | 46 | 14 | 2.80 | 28 |
| **Average** | | | 9,20 | | | 8,50 | | | 6,40 | | | 7,50 | | | 7,70 |
| **StDev** | | | 0,28 | | | 0,14 | | | 0,28 | | | 0,14 | | | 0,42 |
| ***E.coli* (EC)** | | | | | | | | | | | | | | | |
| | **P1** | | | **P2** | | | **P3** | | | **P4** | | | **P5** | | |
| | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** |
| **10^2** | 20 | 4.00 | 4.00 | 21 | 4.20 | 4,20 | 14 | 2.80 | 2,80 | 19 | 3.80 | 3,80 | 14 | 2.80 | 2,80 |
| | 20 | 4.00 | 4.00 | 17 | 3.40 | 3,40 | 15 | 3.00 | 3.00 | 18 | 3.60 | 3,60 | 12 | 2.40 | 2,40 |
| **10^3** | 9 | 1.80 | 18.0 | 7 | 1.40 | 14.0 | 1 | 2.00 | 2.00 | 7 | 1.40 | 14.0 | 7 | 1.40 | 14.0 |

| | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 2 | 4.00 | 4.00 | 11 | 2.20 | 22.0 | 3 | 6.00 | 6.00 | 3 | 6.00 | 6.00 | 3 | 6.00 | 6.00 |
| **Average** | | | 4.00 | | | 3,80 | | | 2,90 | | | 3,70 | | | 2,60 |
| **StDev** | | | 0.00 | | | 0,57 | | | 0,14 | | | 0,14 | | | 0,28 |
| **Total coliforms (TC)** | | | | | | | | | | | | | | | |
| | **P1** | | | **P2** | | | **P3** | | | **P4** | | | **P5** | | |
| | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** |
| **10^2** | 21 | 4.20 | 4,20 | 17 | 3.40 | 3,40 | 22 | 4.40 | 4,40 | 11 | 2.20 | 2,20 | 19 | 3.80 | 3,80 |
| | 20 | 4.00 | 4.00 | 17 | 3.40 | 3,40 | 23 | 4.60 | 4,60 | 14 | 2.80 | 2,80 | 21 | 4.20 | 4,20 |
| **10^3** | 9 | 1.80 | 18.0 | 5 | 1.00 | 10.0 | 4 | 8.00 | 8.00 | 5 | 1.00 | 10.0 | 12 | 2.40 | 24.0 |
| | 8 | 1.60 | 16.0 | 3 | 6.00 | 6.00 | 3 | 6.00 | 6.00 | 1 | 2.00 | 2.00 | 7 | 1.40 | 14.0 |
| **Average** | | | 4,10 | | | 3,40 | | | 4,50 | | | 2,50 | | | 4.00 |
| **StDev** | | | 0,14 | | | 0.00 | | | 0,14 | | | 0,43 | | | 0,28 |
| **Faecal coliforms (FC)** | | | | | | | | | | | | | | | |
| | **P1** | | | **P2** | | | **P3** | | | **P4** | | | **P5** | | |
| | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** |
| **10^1** | 51 | 1.02 | 1,02 | 24 | 4.80 | 0,48 | 35 | 7.00 | 0,70 | 26 | 5.20 | 0,52 | 30 | 6.00 | 0,60 |
| | 50 | 1.00 | 1.00 | 22 | 4.40 | 0,44 | 42 | 8.40 | 0,84 | 24 | 4.80 | 0,48 | 32 | 6.40 | 0,64 |
| **10^2** | 27 | 5.40 | 5,40 | 16 | 3.20 | 3,20 | 19 | 3.80 | 3,8 | 17 | 3.40 | 3,4 | 15 | 3.00 | 3.00 |
| | 31 | 6.20 | 6,20 | 13 | 2.60 | 2,60 | 19 | 3.80 | 3,8 | 13 | 2.60 | 2,6 | 17 | 3.40 | 3,40 |
| **10^3** | 14 | 2.80 | 28.0 | 3 | 6.00 | 6.00 | 5 | 1.00 | 10.0 | 8 | 1.60 | 16.0 | 4 | 8.00 | 8.00 |
| | 11 | 2.20 | 22.0 | 3 | 6.00 | 6.00 | 0 | 0.00 | 0.00 | 3 | 6.00 | 6.00 | 7 | 1.40 | 14.0 |
| **Average** | | | 1,01 | | | 2,90 | | | 3,80 | | | 3.00 | | | 3,20 |
| **StDev** | | | 0,01 | | | 0,42 | | | 0.00 | | | 0,57 | | | 0,28 |
| **Faecal streptococci (FS)** | | | | | | | | | | | | | | | |
| | **P1** | | | **P2** | | | **P3** | | | **P4** | | | **P5** | | |
| | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** |
| **10^1** | 49 | 9.80 | 0,98 | 35 | 7.00 | 0,70 | 22 | 4.40 | 0,44 | 24 | 4.80 | 0,48 | 29 | 5.80 | 0,58 |
| | 49 | 9.80 | 0,98 | 30 | 6.00 | 0,60 | 20 | 4.00 | 0,40 | 25 | 5.00 | 0,50 | 23 | 4.60 | 0,46 |
| **10^2** | 32 | 6.40 | 6,40 | 17 | 3.40 | 3,40 | 13 | 2.60 | 2,60 | 14 | 2.80 | 2,80 | 14 | 2.80 | 2,80 |
| | 27 | 5.40 | 5,40 | 19 | 3.80 | 3,80 | 12 | 2.40 | 2,40 | 7 | 1.40 | 1,40 | 12 | 2.40 | 2,40 |
| **10^3** | 10 | 2.00 | 20.0 | 4 | 8.00 | 8.00 | 0 | 0.00 | 0.00 | 3 | 6.00 | 6.00 | 2 | 4.00 | 4.00 |
| | 12 | 2.40 | 24.0 | 1 | 2.00 | 2.00 | 1 | 2.00 | 2.00 | 2 | 4.00 | 4.00 | 1 | 2.00 | 2.00 |
| **Average** | | | 0,98 | | | 3,60 | | | 2,50 | | | 2,1 | | | 2,60 |
| **StDev** | | | 0.00 | | | 0,28 | | | 0,14 | | | 0,99 | | | 0,28 |
| ***Vibrio* species (VIB spp.)** | | | | | | | | | | | | | | | |

| | P1 | | | P2 | | | P3 | | | P4 | | | P5 | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Count | cfu/100mℓ | (cfu/100mℓ) x $10^3$ | Count | cfu/100mℓ | (cfu/100mℓ) x $10^3$ | Count | cfu/100mℓ | (cfu/100mℓ) x $10^3$ | Count | cfu/100mℓ | (cfu/100mℓ) x $10^3$ | Count | cfu/100mℓ | (cfu/100mℓ) x $10^3$ |
| **10^1** | 128 | 2.56 | 2,56 | 129 | 2.58 | 2,58 | 92 | 1.84 | 1,84 | 123 | 2.46 | 2,46 | 125 | 2.50 | 2,50 |
| | 127 | 2.50 | 2,54 | 128 | 2.56 | 2,56 | 95 | 1.90 | 1,90 | 122 | 2.44 | 2,44 | 126 | 2.52 | 2,52 |
| **10^2** | 72 | 1.44 | 14,4 | 19 | 3.80 | 3,80 | 88 | 1.76 | 17,6 | 28 | 5.60 | 5,60 | 45 | 9.00 | 9.00 |
| | 72 | 1.44 | 14,4 | 25 | 5.00 | 5.00 | 92 | 1.84 | 18,4 | 32 | 6.40 | 6,4 | 43 | 8.60 | 8,60 |
| **10^3** | 6 | 1.20 | 12.0 | 3 | 6.00 | 6.00 | 20 | 4.00 | 40.0 | 2 | 4.00 | 4.00 | 17 | 3.40 | 34.0 |
| | 4 | 8.00 | 8.0 | 1 | 2.00 | 2.00 | 24 | 4.80 | 48.0 | 4 | 8.00 | 8.00 | 14 | 2.80 | 28.0 |
| **Average** | | | 2,55 | | | 2,57 | | | 1,87 | | | 2,45 | | | 2,51 |
| **StDev** | | | 0,01 | | | 0,01 | | | 0,04 | | | 0,01 | | | 0,01 |
| ***Salmonella* species (SAL spp.)** | | | | | | | | | | | | | | | |
| | P1 | | | P2 | | | P3 | | | P4 | | | P5 | | |
| | Count | cfu/100mℓ | (cfu/100mℓ) x $10^3$ | Count | cfu/100mℓ | (cfu/100mℓ) x $10^3$ | Count | cfu/100mℓ | (cfu/100mℓ) x $10^3$ | Count | cfu/100mℓ | (cfu/100mℓ) x $10^3$ | Count | cfu/100mℓ | (cfu/100mℓ) x $10^3$ |
| **10^1** | 26 | 5.20 | 0,52 | 22 | 4.40 | 0,44 | 38 | 7.60 | 0,76 | 37 | 7.40 | 0,74 | 39 | 7.80 | 0,78 |
| | 27 | 5.40 | 0,54 | 23 | 4.60 | 0,46 | 43 | 8.60 | 0,86 | 35 | 7.00 | 0,70 | 37 | 7.40 | 0,74 |
| **10^2** | 10 | 2.00 | 2.00 | 14 | 2.80 | 2,80 | 5 | 1.00 | 1.00 | 12 | 2.40 | 2,40 | 19 | 3.80 | 3,80 |
| | 12 | 2.40 | 2,40 | 12 | 2.40 | 2,40 | 9 | 1.80 | 1,80 | 8 | 1.60 | 1,60 | 15 | 3.00 | 3.00 |
| **10^3** | 2 | 4.00 | 4.00 | 0 | 0.00 | 0.00 | 1 | 2.00 | 2.00 | 6 | 1.20 | 12.0 | 4 | 8.00 | 8.00 |
| | 1 | 2.00 | 2.00 | 1 | 2.00 | 2.00 | 1 | 2.00 | 2.00 | 3 | 6.00 | 6.00 | 0 | 0.00 | 0.00 |
| **Average** | | | 2,20 | | | 2,60 | | | 1,40 | | | 2.00 | | | 3,40 |
| **StDev** | | | 0,28 | | | 0,28 | | | 0,57 | | | 0,57 | | | 0,57 |
| ***Shigella* species (SHIG spp.)** | | | | | | | | | | | | | | | |
| | P1 | | | P2 | | | P3 | | | P4 | | | P5 | | |
| | Count | cfu/100mℓ | (cfu/100mℓ) x $10^3$ | Count | cfu/100mℓ | (cfu/100mℓ) x $10^3$ | Count | cfu/100mℓ | (cfu/100mℓ) x $10^3$ | Count | cfu/100mℓ | (cfu/100mℓ) x $10^3$ | Count | cfu/100mℓ | (cfu/100mℓ) x $10^3$ |
| **10^1** | 38 | 7.60 | 0,76 | 35 | 7.00 | 0,70 | 40 | 8.00 | 0,80 | 22 | 4.40 | 0,44 | 17 | 3.40 | 0,34 |
| | 31 | 6.20 | 0,62 | 32 | 6.40 | 0,64 | 41 | 8.20 | 0,82 | 21 | 4.20 | 0,42 | 14 | 2.80 | 0,28 |
| **10^2** | 19 | 3.80 | 3,80 | 14 | 2.80 | 2,80 | 28 | 5.60 | 5,6 | 7 | 1.40 | 1,40 | 6 | 1.20 | 1,20 |
| | 22 | 4.40 | 4,40 | 14 | 2.80 | 2,80 | 22 | 4.40 | 4,4 | 5 | 1.00 | 1.00 | 4 | 8.00 | 0,80 |
| **10^3** | 6 | 1.20 | 12.0 | 8 | 1.60 | 16.0 | 7 | 1.40 | 14.0 | 0 | 0.00 | 0.00 | 1 | 2.00 | 2.00 |
| | 8 | 1.60 | 16.0 | 0 | 0.00 | 0.00 | 3 | 6.00 | 6.00 | 0 | 0.00 | 0.00 | 0 | 0.00 | 0.00 |
| **Average** | | | 4,10 | | | 2,80 | | | 5.00 | | | 1,20 | | | 1.00 |
| **StDev** | | | 0,42 | | | 0.00 | | | 0,85 | | | 0,28 | | | 0,28 |

**Table 3** Presumptive bacterial indicator counts at all sampling points during December 2013.

| Total heterotrophic bacteria (THB) | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | P1 | | | P2 | | | P3 | | | P4 | | | P5 | | |
| | Count | cfu/100mℓ | (cfu/100mℓ) x $10^6$ | Count | cfu/100mℓ | (cfu/100mℓ) x $10^6$ | Count | cfu/100mℓ | (cfu/100mℓ) x $10^6$ | Count | cfu/100mℓ | (cfu/100mℓ) x $10^6$ | Count | cfu/100mℓ | (cfu/100mℓ) x $10^6$ |
| 10^5 | 50 | 1.00 | 10 | 47 | 9.40 | 9,4 | 49 | 9.80 | 9,8 | 40 | 8.00 | 8 | 44 | 8.80 | 8,8 |
| | 51 | 1.02 | 10,2 | 46 | 9.20 | 9,2 | 48 | 9.60 | 9,6 | 40 | 8.00 | 8 | 43 | 8.60 | 8,6 |
| 10^6 | 32 | 6.40 | 64 | 27 | 5.40 | 54 | 30 | 6.00 | 60 | 27 | 5.40 | 54 | 20 | 4.00 | 40 |
| | 32 | 6.40 | 64 | 29 | 5.80 | 58 | 29 | 5.80 | 58 | 24 | 4.80 | 48 | 21 | 4.20 | 42 |
| Average | | | 10,1 | | | 9,3 | | | 9,7 | | | 8 | | | 8,7 |
| StDev | | | 0,141 | | | 0,141 | | | 0,141 | | | 0 | | | 0,141 |
| *E.coli* (EC) | | | | | | | | | | | | | | | |
| | P1 | | | P2 | | | P3 | | | P4 | | | P5 | | |
| | Count | cfu/100mℓ | (cfu/100mℓ) x $10^3$ | Count | cfu/100mℓ | (cfu/100mℓ) x $10^3$ | Count | cfu/100mℓ | (cfu/100mℓ) x $10^3$ | Count | cfu/100mℓ | (cfu/100mℓ) x $10^3$ | Count | cfu/100mℓ | (cfu/100mℓ) x $10^3$ |
| 10^2 | 21 | 4.20 | 4,2 | 17 | 3.40 | 3,4 | 14 | 2.80 | 2,8 | 18 | 3.60 | 3,6 | 20 | 4.00 | 4 |
| | 22 | 4.40 | 4,4 | 15 | 3.00 | 3 | 11 | 2.20 | 2,2 | 20 | 4.00 | 4 | 20 | 4.00 | 4 |
| 10^3 | 10 | 2.00 | 20 | 9 | 1.80 | 18 | 4 | 8.00 | 8 | 7 | 1.40 | 14 | 11 | 2.20 | 22 |
| | 11 | 2.20 | 22 | 7 | 1.40 | 14 | 1 | 2.00 | 2 | 8 | 1.60 | 16 | 7 | 1.40 | 14 |
| Average | | | 4,3 | | | 3,2 | | | 2,5 | | | 3,8 | | | 4 |
| StDev | | | 0,141 | | | 0,283 | | | 0,424 | | | 0,283 | | | 0 |
| Total coliforms (TC) | | | | | | | | | | | | | | | |
| | P1 | | | P2 | | | P3 | | | P4 | | | P5 | | |
| | Count | cfu/100mℓ | (cfu/100mℓ) x $10^3$ | Count | cfu/100mℓ | (cfu/100mℓ) x $10^3$ | Count | cfu/100mℓ | (cfu/100mℓ) x $10^3$ | Count | cfu/100mℓ | (cfu/100mℓ) x $10^3$ | Count | cfu/100mℓ | (cfu/100mℓ) x $10^3$ |
| 10^2 | 27 | 5.40 | 5,4 | 21 | 4.20 | 4,2 | 17 | 3.40 | 3,4 | 22 | 4.40 | 4,4 | 25 | 5.00 | 5 |
| | 27 | 5.40 | 5,4 | 23 | 4.60 | 4,6 | 15 | 3.00 | 3 | 22 | 4.40 | 4,4 | 27 | 5.40 | 5,4 |
| 10^3 | 19 | 3.80 | 38 | 14 | 2.80 | 28 | 7 | 1.40 | 14 | 17 | 3.40 | 34 | 11 | 2.20 | 22 |
| | 14 | 2.80 | 28 | 13 | 2.60 | 26 | 7 | 1.40 | 14 | 16 | 3.20 | 32 | 9 | 1.80 | 18 |
| Average | | | 5,4 | | | 4,4 | | | 3,2 | | | 4,4 | | | 5,2 |
| StDev | | | 0 | | | 0,283 | | | 0,283 | | | 0 | | | 0,283 |
| Faecal coliforms (FC) | | | | | | | | | | | | | | | |
| | P1 | | | P2 | | | P3 | | | P4 | | | P5 | | |
| | Count | cfu/100mℓ | (cfu/100mℓ) x $10^3$ | Count | cfu/100mℓ | (cfu/100mℓ) x $10^3$ | Count | cfu/100mℓ | (cfu/100mℓ) x $10^3$ | Count | cfu/100mℓ | (cfu/100mℓ) x $10^3$ | Count | cfu/100mℓ | (cfu/100mℓ) x $10^3$ |
| 10^2 | 33 | 6.60 | 6,6 | 24 | 4.80 | 4,8 | 9 | 1.80 | 1,8 | 11 | 2.20 | 2,2 | 19 | 3.80 | 3,8 |
| | 33 | 6.60 | 6,6 | 25 | 5.00 | 5 | 10 | 2.00 | 2 | 6 | 1.20 | 1,2 | 14 | 2.80 | 2,8 |
| 10^3 | 14 | 2.80 | 28 | 7 | 1.40 | 14 | 0 | 0.00 | 0 | 0 | 0.00 | 0 | 0 | 0.00 | 0 |
| | 13 | 2.60 | 26 | 8 | 1.60 | 16 | 1 | 2.00 | 2 | 3 | 6.00 | 0,6 | 0 | 0.00 | 0 |

| | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Average** | | | 6,6 | | | 4,9 | | | 1,9 | | | 1,7 | | | 3,3 |
| **StDev** | | | 0 | | | 0,141 | | | 0,141 | | | 0,707 | | | 0,707 |
| **Faecal streptococci (FS)** | | | | | | | | | | | | | | | |
| | **P1** | | | **P2** | | | **P3** | | | **P4** | | | **P5** | | |
| | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** |
| **10^2** | 28 | 5.60 | 5,6 | 21 | 4.20 | 4,2 | 14 | 2.80 | 2,8 | 23 | 4.60 | 4,6 | 25 | 5.00 | 5 |
| | 27 | 5.40 | 5,4 | 20 | 4.00 | 4 | 13 | 2.60 | 2,6 | 24 | 4.80 | 4,8 | 25 | 5.00 | 5 |
| **10^3** | 11 | 2.20 | 22 | 7 | 1.40 | 14 | 3 | 6.00 | 6 | 14 | 2.80 | 28 | 6 | 1.20 | 12 |
| | 12 | 2.40 | 24 | 1 | 2.00 | 2 | 1 | 2.00 | 2 | 9 | 1.80 | 18 | 9 | 1.80 | 18 |
| **Average** | | | 5,5 | | | 4,1 | | | 2,7 | | | 4,7 | | | 5 |
| **StDev** | | | 0,141 | | | 0,141 | | | 0,141 | | | 0,141 | | | 0 |
| ***Vibrio* species (VIB spp.)** | | | | | | | | | | | | | | | |
| | **P1** | | | **P2** | | | **P3** | | | **P4** | | | **P5** | | |
| | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** |
| **10^1** | 92 | 1.84 | 1,84 | 62 | 1.24 | 1,24 | 51 | 1.02 | 1,02 | 61 | 1.22 | 1,22 | 42 | 8.40 | 0,84 |
| | 103 | 2.06 | 2,06 | 71 | 1.42 | 1,42 | 54 | 1.08 | 1,08 | 59 | 1.18 | 1,18 | 43 | 8.60 | 0,86 |
| **10^2** | 66 | 1.32 | 13,2 | 30 | 6.00 | 6 | 22 | 4.40 | 4,4 | 22 | 4.40 | 4,4 | 36 | 7.20 | 7,2 |
| | 62 | 1.24 | 12,4 | 30 | 6.00 | 6 | 23 | 4.60 | 4,6 | 26 | 5.20 | 5,2 | 32 | 6.40 | 6,4 |
| **10^3** | 23 | 4.60 | 46 | 17 | 3.40 | 34 | 11 | 2.20 | 22 | 13 | 2.60 | 26 | 16 | 3.20 | 32 |
| | 21 | 4.20 | 42 | 25 | 5.00 | 50 | 10 | 2.00 | 20 | 6 | 1.20 | 12 | 4 | 8.00 | 8 |
| **Average** | | | 1,95 | | | 6 | | | 4,5 | | | 4,8 | | | 6,8 |
| **StDev** | | | 0,156 | | | 0 | | | 0,141 | | | 0,566 | | | 0,566 |
| ***Salmonella* species (SAL spp.)** | | | | | | | | | | | | | | | |
| | **P1** | | | **P2** | | | **P3** | | | **P4** | | | **P5** | | |
| | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** |
| **10^2** | 14 | 2.80 | 2,8 | 10 | 2.00 | 2 | 14 | 2.80 | 2,8 | 14 | 2.80 | 2,8 | 6 | 1.20 | 1,2 |
| | 10 | 2.00 | 2 | 6 | 1.20 | 1,2 | 17 | 3.40 | 3,4 | 10 | 2.00 | 2 | 4 | 8.00 | 0,8 |
| **10^3** | 2 | 4.00 | 4 | 2 | 4.00 | 4 | 6 | 1.20 | 12 | 3 | 6.00 | 6 | 0 | 0.00 | 0 |
| | 1 | 2.00 | 2 | 0 | 0.00 | 0 | 6 | 1.20 | 12 | 1 | 2.00 | 2 | 3 | 6.00 | 6 |
| **Average** | | | 2,4 | | | 1,6 | | | 3,1 | | | 2,4 | | | 1 |
| **StDev** | | | 0,566 | | | 0,566 | | | 0,424 | | | 0,566 | | | 0,283 |
| ***Shigella* species (SHIG spp.)** | | | | | | | | | | | | | | | |
| | **P1** | | | **P2** | | | **P3** | | | **P4** | | | **P5** | | |
| | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** |

| | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **10^2** | 19 | 3.80 | 3,8 | 8 | 1.60 | 1,6 | 6 | 1.20 | 1,2 | 13 | 2.60 | 2,6 | 13 | 2.60 | 2,6 |
| | 18 | 3.60 | 3,6 | 9 | 1.80 | 1,8 | 3 | 6.00 | 0,6 | 18 | 3.60 | 3,6 | 14 | 2.80 | 2,8 |
| **10^3** | 4 | 8.00 | 8 | 0 | 0.00 | 0 | 2 | 4.00 | 4 | 3 | 6.00 | 6 | 2 | 4.00 | 4 |
| | 5 | 1.00 | 10 | 0 | 0.00 | 0 | 1 | 2.00 | 2 | 4 | 8.00 | 8 | 0 | 0.00 | 0 |
| **Average** | | | 3,7 | | | 1,7 | | | 0,9 | | | 3,1 | | | 2,7 |
| **StDev** | | | 0,141 | | | 0,141 | | | 0,424 | | | 0,707 | | | 0,141 |

**Table 4** Presumptive bacterial indicator counts at all sampling points during January 2014.

| **Total heterotrophic bacteria (THB)** | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **P1** | | | **P2** | | | **P3** | | | **P4** | | | **P5** | | |
| | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^6$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^6$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^6$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^6$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^6$** |
| **10^5** | 74 | 1.48 | 14,8 | 63 | 1.26 | 12,6 | 47 | 9.40 | 9,4 | 61 | 1.22 | 12,2 | 68 | 1.36 | 13,6 |
| | 75 | 1.50 | 15 | 60 | 1.20 | 12 | 49 | 9.80 | 9,8 | 63 | 1.26 | 12,6 | 64 | 1.28 | 12,8 |
| **10^6** | 22 | 4.40 | 44 | 31 | 6.20 | 62 | 21 | 4.20 | 42 | 27 | 5.40 | 54 | 19 | 3.80 | 38 |
| | 20 | 4.00 | 40 | 29 | 5.80 | 58 | 25 | 5.00 | 50 | 27 | 5.40 | 54 | 20 | 4.00 | 40 |
| **Average** | | | 14,9 | | | 12,3 | | | 9,6 | | | 12,4 | | | 13,2 |
| **StDev** | | | 0,141 | | | 0,424 | | | 0,283 | | | 0,283 | | | 0,566 |
| ***E.coli* (EC)** | | | | | | | | | | | | | | | |
| | **P1** | | | **P2** | | | **P3** | | | **P4** | | | **P5** | | |
| | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** |
| **10^2** | 29 | 5.80 | 5,8 | 25 | 5.00 | 5 | 31 | 6.20 | 6,2 | 27 | 5.40 | 5,4 | 29 | 5.80 | 5,8 |
| | 26 | 5.20 | 5,2 | 23 | 4.60 | 4,6 | 30 | 6.00 | 6 | 26 | 5.20 | 5,2 | 25 | 5.00 | 5 |
| **10^3** | 15 | 3.00 | 30 | 16 | 3.20 | 32 | 17 | 3.40 | 34 | 6 | 1.20 | 12 | 14 | 2.80 | 28 |
| | 13 | 2.60 | 26 | 16 | 3.20 | 32 | 12 | 2.40 | 24 | 4 | 8.00 | 8 | 14 | 2.80 | 28 |
| **Average** | | | 5,5 | | | 4,8 | | | 6,1 | | | 5,3 | | | 5,4 |
| **StDev** | | | 0,424 | | | 0,283 | | | 0,141 | | | 0,141 | | | 0,566 |
| **Total coliforms (TC)** | | | | | | | | | | | | | | | |
| | **P1** | | | **P2** | | | **P3** | | | **P4** | | | **P5** | | |
| | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** |
| **10^2** | 29 | 5.80 | 5,8 | 27 | 5.40 | 5,4 | 25 | 5.00 | 5 | 23 | 4.60 | 4,6 | 26 | 5.20 | 5,2 |
| | 30 | 6.00 | 6 | 28 | 5.60 | 5,6 | 25 | 5.00 | 5 | 20 | 4.00 | 4 | 24 | 4.80 | 4,8 |
| **10^3** | 12 | 2.40 | 24 | 18 | 3.60 | 36 | 3 | 6.00 | 6 | 12 | 2.40 | 24 | 10 | 2.00 | 20 |
| | 17 | 3.40 | 34 | 17 | 3.40 | 34 | 8 | 1.60 | 16 | 16 | 3.20 | 32 | 14 | 2.80 | 28 |
| **Average** | | | 5,9 | | | 5,5 | | | 5 | | | 4,3 | | | 5 |

| | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **StDev** | | | 0,141 | | | 0,141 | | | 0 | | | 0,424 | | | 0,283 |
| **Faecal coliforms (FC)** | | | | | | | | | | | | | | | |
| | **P1** | | | **P2** | | | **P3** | | | **P4** | | | **P5** | | |
| | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** |
| **10^2** | 29 | 5.80 | 5,8 | 26 | 5.20 | 5,2 | 19 | 3.80 | 3,8 | 23 | 4.60 | 4,6 | 19 | 3.80 | 3,8 |
| | 28 | 5.60 | 5,6 | 27 | 5.40 | 5,4 | 17 | 3.40 | 3,4 | 25 | 5.00 | 5 | 14 | 2.80 | 2,8 |
| **10^3** | 7 | 1.40 | 14 | 3 | 6.00 | 6 | 1 | 2.00 | 2 | 14 | 2.80 | 28 | 3 | 6.00 | 6 |
| | 5 | 1.00 | 10 | 3 | 6.00 | 6 | 0 | 0.00 | 0 | 9 | 1.80 | 18 | 1 | 2.00 | 2 |
| **Average** | | | 5,7 | | | 5,3 | | | 3,6 | | | 4,8 | | | 3,3 |
| **StDev** | | | 0,141 | | | 0,141 | | | 0,283 | | | 0,283 | | | 0,707 |
| **Faecal streptococci (FS)** | | | | | | | | | | | | | | | |
| | **P1** | | | **P2** | | | **P3** | | | **P4** | | | **P5** | | |
| | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** |
| **10^2** | 33 | 6.60 | 6,6 | 31 | 6.20 | 6,2 | 19 | 3.80 | 3,8 | 25 | 5.00 | 5 | 30 | 6.00 | 6 |
| | 31 | 6.20 | 6,2 | 29 | 5.80 | 5,8 | 21 | 4.20 | 4,2 | 27 | 5.40 | 5,4 | 29 | 5.80 | 5,8 |
| **10^3** | 19 | 3.80 | 38 | 17 | 3.40 | 34 | 6 | 1.20 | 12 | 12 | 2.40 | 24 | 16 | 3.20 | 32 |
| | 17 | 3.40 | 34 | 14 | 2.80 | 28 | 11 | 2.20 | 22 | 12 | 2.40 | 24 | 11 | 2.20 | 22 |
| **Average** | | | 6,4 | | | 6 | | | 4 | | | 5,2 | | | 5,9 |
| **StDev** | | | 0,283 | | | 0,283 | | | 0,283 | | | 0,283 | | | 0,141 |
| ***Vibrio* species (VIB spp.)** | | | | | | | | | | | | | | | |
| | **P1** | | | **P2** | | | **P3** | | | **P4** | | | **P5** | | |
| | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** |
| **10^1** | 132 | 2.64 | 2,64 | 79 | 1.58 | 1,58 | 89 | 1.78 | 1,78 | 39 | 7.80 | 0,78 | 47 | 9.40 | 0,94 |
| | 133 | 2.66 | 2,66 | 80 | 1.60 | 1,6 | 92 | 1.84 | 1,84 | 32 | 6.40 | 0,64 | 51 | 1.02 | 1,02 |
| **10^2** | 79 | 1.58 | 15,8 | 23 | 4.60 | 4,6 | 42 | 8.40 | 8,4 | 19 | 3.80 | 3,8 | 22 | 4.40 | 4,4 |
| | 72 | 1.44 | 14,4 | 24 | 4.80 | 4,8 | 48 | 9.60 | 9,6 | 17 | 3.40 | 3,4 | 28 | 5.60 | 5,6 |
| **10^3** | 11 | 2.20 | 22 | 17 | 3.40 | 34 | 9 | 1.80 | 18 | 5 | 1.00 | 10 | 7 | 1.40 | 14 |
| | 18 | 3.60 | 36 | 11 | 2.20 | 22 | 7 | 1.40 | 14 | 6 | 1.20 | 12 | 2 | 4.00 | 4 |
| **Average** | | | 2,65 | | | 4,7 | | | 1,81 | | | 3,6 | | | 5 |
| **StDev** | | | 0,014 | | | 0,141 | | | 0,042 | | | 0,283 | | | 0,849 |
| ***Salmonella* species (SAL spp.)** | | | | | | | | | | | | | | | |
| | **P1** | | | **P2** | | | **P3** | | | **P4** | | | **P5** | | |
| | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** |
| **10^2** | 19 | 3.80 | 3,8 | 24 | 4.80 | 4,8 | 6 | 1.20 | 1,2 | 14 | 2.80 | 2,8 | 11 | 2.20 | 2,2 |

| | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 21 | 4.20 | 4,2 | 26 | 5.20 | 5,2 | 4 | 8.00 | 0,8 | 15 | 3.00 | 3 | 6 | 1.20 | 1,2 |
| **10^3** | 14 | 2.80 | 28 | 12 | 2.40 | 24 | 0 | 0.00 | 0 | 1 | 2.00 | 2 | 2 | 4.00 | 4 |
| | 6 | 1.20 | 12 | 5 | 1.00 | 10 | 3 | 6.00 | 6 | 1 | 2.00 | 2 | 0 | 0.00 | 0 |
| **Average** | | | 4 | | | 5 | | | 1 | | | 2,9 | | | 1,7 |
| **StDev** | | | 0,283 | | | 0,283 | | | 0,283 | | | 0,141 | | | 0,707 |
| ***Shigella* species (SHIG spp.)** | | | | | | | | | | | | | | | |
| | **P1** | | | **P2** | | | **P3** | | | **P4** | | | **P5** | | |
| | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** |
| **10^2** | 31 | 6.20 | 6,2 | 19 | 3.80 | 3,8 | 14 | 2.80 | 2,8 | 14 | 2.80 | 2,8 | 16 | 3.20 | 3,2 |
| | 25 | 5.00 | 5 | 7 | 1.40 | 1,4 | 18 | 3.60 | 3,6 | 15 | 3.00 | 3 | 16 | 3.20 | 3,2 |
| **10^3** | 3 | 6.00 | 6 | 2 | 4.00 | 4 | 6 | 1.20 | 12 | 4 | 8.00 | 8 | 4 | 8.00 | 8 |
| | 2 | 4.00 | 4 | 2 | 4.00 | 4 | 1 | 2.00 | 2 | 2 | 4.00 | 4 | 3 | 6.00 | 6 |
| **Average** | | | 5,5 | | | 4 | | | 3,2 | | | 2,9 | | | 3,2 |
| **StDev** | | | 0,707 | | | 0 | | | 0,566 | | | 0,141 | | | 0 |

**Table 5** Presumptive bacterial indicator counts at all sampling points during February 2014.

| **Total heterotrophic bacteria (THB)** | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **P1** | | | **P2** | | | **P3** | | | **P4** | | | **P5** | | |
| | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^6$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^6$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^6$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^6$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^6$** |
| **10^5** | 58 | 1.16 | 11,6 | 49 | 9.80 | 9,8 | 37 | 7.40 | 7,4 | 47 | 9.40 | 9,4 | 51 | 1.02 | 10,2 |
| | 51 | 1.02 | 10,2 | 49 | 9.80 | 9,8 | 34 | 6.80 | 6,8 | 43 | 8.60 | 8,6 | 53 | 1.06 | 10,6 |
| **10^6** | 19 | 3.80 | 38 | 17 | 3.40 | 34 | 17 | 3.40 | 34 | 20 | 4.00 | 40 | 14 | 2.80 | 28 |
| | 11 | 2.20 | 22 | 15 | 3.00 | 30 | 19 | 3.80 | 38 | 20 | 4.00 | 40 | 14 | 2.80 | 28 |
| **Average** | | | 10,9 | | | 9,8 | | | 7,1 | | | 9 | | | 10,4 |
| **StDev** | | | 0,990 | | | 0 | | | 0,424 | | | 0,566 | | | 0,283 |
| ***E.coli* (EC)** | | | | | | | | | | | | | | | |
| | **P1** | | | **P2** | | | **P3** | | | **P4** | | | **P5** | | |
| | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** |
| **10^2** | 27 | 5.40 | 5,4 | 22 | 4.40 | 4,4 | 17 | 3.40 | 3,4 | 19 | 3.80 | 3,8 | 24 | 4.80 | 4,8 |
| | 25 | 5.00 | 5 | 19 | 3.80 | 3,8 | 15 | 3.00 | 3 | 19 | 3.80 | 3,8 | 26 | 5.20 | 5,2 |
| **10^3** | 13 | 2.60 | 26 | 10 | 2.00 | 20 | 7 | 1.40 | 14 | 6 | 1.20 | 12 | 14 | 2.80 | 28 |
| | 12 | 2.40 | 24 | 10 | 2.00 | 20 | 3 | 6.00 | 6 | 4 | 8.00 | 8 | 9 | 1.80 | 18 |
| **Average** | | | 5,2 | | | 4,1 | | | 3,2 | | | 3,8 | | | 5 |
| **StDev** | | | 0,283 | | | 0,424 | | | 0,283 | | | 0 | | | 0,283 |

| Total coliforms (TC) | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | P1 | | | P2 | | | P3 | | | P4 | | | P5 | | |
| | Count | cfu/100mℓ | (cfu/100mℓ) x $10^3$ | Count | cfu/100mℓ | (cfu/100mℓ) x $10^3$ | Count | cfu/100mℓ | (cfu/100mℓ) x $10^3$ | Count | cfu/100mℓ | (cfu/100mℓ) x $10^3$ | Count | cfu/100mℓ | (cfu/100mℓ) x $10^3$ |
| 10^2 | 28 | 5.60 | 5,6 | 26 | 5.20 | 5,2 | 23 | 4.60 | 4,6 | 23 | 4.60 | 4,6 | 22 | 4.40 | 4,4 |
| | 28 | 5.60 | 5,6 | 26 | 5.20 | 5,2 | 27 | 5.40 | 5,4 | 24 | 4.80 | 4,8 | 16 | 3.20 | 3,2 |
| 10^3 | 5 | 1.00 | 10 | 13 | 2.60 | 26 | 16 | 3.20 | 32 | 15 | 3.00 | 30 | 14 | 2.80 | 28 |
| | 11 | 2.20 | 22 | 15 | 3.00 | 30 | 14 | 2.80 | 28 | 11 | 2.20 | 22 | 10 | 2.00 | 20 |
| Average | | | 5,6 | | | 5,2 | | | 5 | | | 4,7 | | | 3,8 |
| StDev | | | 0 | | | 0 | | | 0,566 | | | 0,141 | | | 0,849 |
| **Faecal coliforms (FC)** | | | | | | | | | | | | | | | |
| | P1 | | | P2 | | | P3 | | | P4 | | | P5 | | |
| | Count | cfu/100mℓ | (cfu/100mℓ) x $10^3$ | Count | cfu/100mℓ | (cfu/100mℓ) x $10^3$ | Count | cfu/100mℓ | (cfu/100mℓ) x $10^3$ | Count | cfu/100mℓ | (cfu/100mℓ) x $10^3$ | Count | cfu/100mℓ | (cfu/100mℓ) x $10^3$ |
| 10^1 | 120 | 2.40 | 2,4 | 124 | 2.48 | 2,48 | 92 | 1.84 | 1,84 | 82 | 1.64 | 1,64 | 33 | 6.60 | 0,66 |
| | 121 | 2.42 | 2,42 | 123 | 2.46 | 2,46 | 89 | 1.78 | 1,78 | 74 | 1.48 | 1,48 | 33 | 6.60 | 0,66 |
| 10^2 | 86 | 1.72 | 17,2 | 101 | 2.02 | 20,2 | 26 | 5.20 | 5,2 | 15 | 3.00 | 3 | 25 | 5.00 | 5 |
| | 74 | 1.48 | 14,8 | 99 | 1.98 | 19,8 | 21 | 4.20 | 4,2 | 7 | 1.40 | 1,4 | 15 | 3.00 | 3 |
| 10^3 | 13 | 2.60 | 26 | 12 | 2.40 | 24 | 15 | 3.00 | 30 | 2 | 4.00 | 4 | 2 | 4.00 | 4 |
| | 12 | 2.40 | 24 | 15 | 3.00 | 30 | 1 | 2.00 | 2 | 1 | 2.00 | 2 | 2 | 4.00 | 4 |
| Average | | | 2,41 | | | 2,47 | | | 4,7 | | | 3,5 | | | 4,5 |
| StDev | | | 0,014 | | | 0,014 | | | 0,707 | | | 0,707 | | | 0,707 |
| **Faecal streptococci (FS)** | | | | | | | | | | | | | | | |
| | P1 | | | P2 | | | P3 | | | P4 | | | P5 | | |
| | Count | cfu/100mℓ | (cfu/100mℓ) x $10^3$ | Count | cfu/100mℓ | (cfu/100mℓ) x $10^3$ | Count | cfu/100mℓ | (cfu/100mℓ) x $10^3$ | Count | cfu/100mℓ | (cfu/100mℓ) x $10^3$ | Count | cfu/100mℓ | (cfu/100mℓ) x $10^3$ |
| 10^1 | 113 | 2.26 | 2,26 | 102 | 2.04 | 2,04 | 82 | 1.64 | 1,64 | 92 | 1.84 | 1,84 | 56 | 1.12 | 1,12 |
| | 113 | 2.26 | 2,26 | 99 | 1.98 | 1,98 | 61 | 1.22 | 1,22 | 79 | 1.58 | 1,58 | 48 | 9.60 | 0,96 |
| 10^2 | 66 | 1.32 | 13,2 | 13 | 2.60 | 2,6 | 27 | 5.40 | 5,4 | 31 | 6.20 | 6,2 | 14 | 2.80 | 2,8 |
| | 54 | 1.08 | 10,8 | 12 | 2.40 | 2,4 | 23 | 4.60 | 4,6 | 35 | 7.00 | 7 | 15 | 3.00 | 3 |
| 10^3 | 15 | 3.00 | 30 | 5 | 1.00 | 10 | 9 | 1.80 | 18 | 5 | 1.00 | 10 | 2 | 4.00 | 4 |
| | 14 | 2.80 | 28 | 1 | 2.00 | 2 | 2 | 4.00 | 4 | 10 | 2.00 | 20 | 3 | 6.00 | 6 |
| Average | | | 2,26 | | | 2,5 | | | 5 | | | 6,6 | | | 2,9 |
| StDev | | | 0 | | | 0,141 | | | 0,566 | | | 0,566 | | | 0,141 |
| ***Vibrio* species (VIB spp.)** | | | | | | | | | | | | | | | |
| | P1 | | | P2 | | | P3 | | | P4 | | | P5 | | |
| | Count | cfu/100mℓ | (cfu/100mℓ) x $10^3$ | Count | cfu/100mℓ | (cfu/100mℓ) x $10^3$ | Count | cfu/100mℓ | (cfu/100mℓ) x $10^3$ | Count | cfu/100mℓ | (cfu/100mℓ) x $10^3$ | Count | cfu/100mℓ | (cfu/100mℓ) x $10^3$ |

| | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **10^1** | 156 | 3.12 | 3,12 | 111 | 2.22 | 2,22 | 91 | 1.82 | 1,82 | 75 | 1.50 | 1,5 | 80 | 1.60 | 1,6 |
| | 162 | 3.24 | 3,24 | 92 | 1.84 | 1,84 | 63 | 1.26 | 1,26 | 74 | 1.48 | 1,48 | 80 | 1.60 | 1,6 |
| **10^2** | 87 | 1.74 | 17,4 | 49 | 9.80 | 9,8 | 32 | 6.40 | 6,4 | 54 | 1.08 | 10,8 | 31 | 6.20 | 6,2 |
| | 80 | 1.60 | 16 | 45 | 9.00 | 9 | 40 | 8.00 | 8 | 56 | 1.12 | 11,2 | 25 | 5.00 | 5 |
| **10^3** | 37 | 7.40 | 74 | 22 | 4.40 | 44 | 12 | 2.40 | 24 | 9 | 1.80 | 18 | 13 | 2.60 | 26 |
| | 30 | 6.00 | 60 | 27 | 5.40 | 54 | 13 | 2.60 | 26 | 10 | 2.00 | 20 | 7 | 1.40 | 14 |
| **Average** | | | 3,18 | | | 2,03 | | | 7,2 | | | 1,49 | | | 5,6 |
| **StDev** | | | 0,085 | | | 0,269 | | | 1,131 | | | 0,014 | | | 0,849 |
| ***Salmonella* species (SAL spp.)** | | | | | | | | | | | | | | | |
| | **P1** | | | **P2** | | | **P3** | | | **P4** | | | **P5** | | |
| | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** |
| **10^2** | 11 | 2.20 | 2,2 | 16 | 3.20 | 3,2 | 16 | 3.20 | 3,2 | 9 | 1.80 | 1,8 | 16 | 3.20 | 3,2 |
| | 14 | 2.80 | 2,8 | 8 | 1.60 | 1,6 | 15 | 3.00 | 3 | 5 | 1.00 | 1 | 15 | 3.00 | 3 |
| **10^3** | 7 | 1.40 | 14 | 3 | 6.00 | 6 | 2 | 4.00 | 4 | 1 | 2.00 | 2 | 2 | 4.00 | 4 |
| | 3 | 6.00 | 6 | 2 | 4.00 | 4 | 2 | 4.00 | 4 | 0 | 0.00 | 0 | 0 | 0.00 | 0 |
| **Average** | | | 2,5 | | | 2,4 | | | 4 | | | 1,9 | | | 3,1 |
| **StDev** | | | 0,424 | | | 1,131 | | | 0 | | | 0,141 | | | 0,141 |
| ***Shigella* species (SHIG spp.)** | | | | | | | | | | | | | | | |
| | **P1** | | | **P2** | | | **P3** | | | **P4** | | | **P5** | | |
| | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** |
| **10^2** | 16 | 3.20 | 3,2 | 5 | 1.00 | 1 | 8 | 1.60 | 1,6 | 14 | 2.80 | 2,8 | 10 | 2.00 | 2 |
| | 10 | 2.00 | 2 | 11 | 2.20 | 2,2 | 3 | 6.00 | 0,6 | 16 | 3.20 | 3,2 | 13 | 2.60 | 2,6 |
| **10^3** | 5 | 1.00 | 10 | 3 | 6.00 | 6 | 2 | 4.00 | 4 | 2 | 4.00 | 4 | 7 | 1.40 | 14 |
| | 2 | 4.00 | 4 | 1 | 2.00 | 2 | 1 | 2.00 | 2 | 2 | 4.00 | 4 | 0 | 0.00 | 0 |
| **Average** | | | 3,6 | | | 2,1 | | | 3 | | | 4 | | | 2,3 |
| **StDev** | | | 0,566 | | | 0,141 | | | 1,414 | | | 0 | | | 0,424 |

**Table 6** Presumptive bacterial indicator counts at all sampling points during March 2014.

| **Total heterotrophic bacteria (THB)** | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **P1** | | | **P2** | | | **P3** | | | **P4** | | | **P5** | | |
| | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^6$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^6$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^6$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^6$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^6$** |
| **10^5** | 43 | 8.60 | 8,6 | 46 | 9.20 | 9,2 | 40 | 8.00 | 8 | 47 | 9.40 | 9,4 | 45 | 9.00 | 9 |
| | 40 | 800 | 8 | 43 | 8.60 | 8,6 | 40 | 8.00 | 8 | 46 | 9.20 | 9,2 | 49 | 9.80 | 9,8 |
| **10^6** | 19 | 380 | 38 | 24 | 4.8 | 48 | 12 | 2.4 | 24 | 17 | 3.4 | 34 | 24 | 4.8 | 48 |
| | 10 | 200 | 20 | 20 | 4.0 | 40 | 13 | 2.6 | 26 | 16 | 3.2 | 32 | 24 | 4.8 | 48 |

| | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Average** | | | 8,3 | | | 8,9 | | | 8 | | | 9,3 | | | 9,4 |
| **StDev** | | | 0,424 | | | 0,424 | | | 0 | | | 0,141 | | | 0,566 |
| ***E.coli* (EC)** | | | | | | | | | | | | | | | |
| | **P1** | | | **P2** | | | **P3** | | | **P4** | | | **P5** | | |
| | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** |
| **10^2** | 23 | 4.60 | 4,6 | 21 | 4.20 | 4,2 | 15 | 3.00 | 3 | 21 | 4.20 | 4,2 | 21 | 4.20 | 4,2 |
| | 22 | 4.40 | 4,4 | 19 | 3.80 | 3,8 | 16 | 3.20 | 3,2 | 21 | 4.20 | 4,2 | 22 | 4.40 | 4,4 |
| **10^3** | 15 | 3.00 | 30 | 13 | 2.60 | 26 | 7 | 1.40 | 14 | 10 | 2.00 | 20 | 10 | 2.00 | 20 |
| | 12 | 2.40 | 24 | 11 | 2.20 | 22 | 4 | 8.00 | 8 | 8 | 1.60 | 16 | 8 | 1.60 | 16 |
| **Average** | | | 4,5 | | | 4 | | | 3,1 | | | 4,2 | | | 4,3 |
| **StDev** | | | 0,141 | | | 0,283 | | | 0,141 | | | 0 | | | 0,141 |
| **Total coliforms (TC)** | | | | | | | | | | | | | | | |
| | **P1** | | | **P2** | | | **P3** | | | **P4** | | | **P5** | | |
| | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** |
| **10^2** | 22 | 4.40 | 4,4 | 26 | 5.20 | 5,2 | 20 | 4.00 | 4 | 16 | 3.20 | 3,2 | 23 | 4.60 | 4,6 |
| | 24 | 4.80 | 4,8 | 23 | 4.60 | 4,6 | 20 | 4.00 | 4 | 13 | 2.60 | 2,6 | 21 | 4.20 | 4,2 |
| **10^3** | 10 | 2.00 | 20 | 9 | 1.80 | 18 | 16 | 3.20 | 32 | 5 | 1.00 | 10 | 6 | 1.20 | 12 |
| | 12 | 2.40 | 24 | 10 | 2.00 | 20 | 14 | 2.80 | 28 | 7 | 1.40 | 14 | 5 | 1.00 | 10 |
| **Average** | | | 4,6 | | | 4,9 | | | 4 | | | 2,9 | | | 4,4 |
| **StDev** | | | 0,283 | | | 0,424 | | | 0 | | | 0,424 | | | 0,283 |
| **Faecal coliforms (FC)** | | | | | | | | | | | | | | | |
| | **P1** | | | **P2** | | | **P3** | | | **P4** | | | **P5** | | |
| | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** |
| **10^2** | 31 | 6.200 | 6 | 23 | 4600 | 4,6 | 10 | 2000 | 2 | 14 | 2800 | 2,8 | 17 | 3400 | 3,4 |
| | 35 | 7.000 | 7 | 21 | 4200 | 4,2 | 6 | 1200 | 1,2 | 19 | 3800 | 3,8 | 13 | 2600 | 2,6 |
| **10^3** | 14 | 2.8000 | 28 | 5 | 10000 | 10 | 2 | 4000 | 4 | 3 | 6000 | 6 | 1 | 2000 | 2 |
| | 7 | 1.4000 | 14 | 3 | 6000 | 6 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| **Average** | | | 6,5 | | | 4,4 | | | 1,6 | | | 3,3 | | | 3 |
| **StDev** | | | 0,707 | | | 0,283 | | | 0,566 | | | 0,707 | | | 0,566 |
| **Faecal streptococci (FS)** | | | | | | | | | | | | | | | |
| | **P1** | | | **P2** | | | **P3** | | | **P4** | | | **P5** | | |
| | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** |
| **10^2** | 10 | 2.00 | 2 | 33 | 6.60 | 6,6 | 10 | 2.00 | 2 | 15 | 3.00 | 3 | 14 | 2.80 | 2,8 |
| | 11 | 2.20 | 2,2 | 29 | 5.80 | 5,8 | 6 | 1.20 | 1,2 | 15 | 3.00 | 3 | 8 | 1.60 | 1,6 |

| | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **10^3** | 1 | 2.00 | 2 | 11 | 2.20 | 22 | 2 | 4.00 | 4 | 2 | 4.00 | 4 | 5 | 1.00 | 10 |
| | 0 | 0.00 | 0 | 4 | 8.00 | 8 | 1 | 2.00 | 2 | 2 | 4.00 | 4 | 1 | 2.00 | 2 |
| **Average** | | | 2,1 | | | 6,2 | | | 1,6 | | | 4 | | | 2,4 |
| **StDev** | | | 0,141 | | | 0,566 | | | 0,566 | | | 0 | | | 0,566 |
| ***Vibrio* species (VIB spp.)** | | | | | | | | | | | | | | | |
| | **P1** | | | **P2** | | | **P3** | | | **P4** | | | **P5** | | |
| | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** |
| **10^2** | 19 | 3.80 | 3,8 | 12 | 2.40 | 2,4 | 3 | 6.00 | 0,6 | 6 | 1.20 | 1,2 | 26 | 5.20 | 5,2 |
| | 20 | 4.00 | 4 | 10 | 2.00 | 2 | 2 | 4.00 | 0,4 | 8 | 1.60 | 1,6 | 21 | 4.20 | 4,2 |
| **10^3** | 5 | 1.00 | 10 | 3 | 6.00 | 6 | 0 | 0.00 | 0 | 2 | 4.00 | 4 | 11 | 2.20 | 22 |
| | 6 | 1.20 | 12 | 1 | 2.00 | 2 | 0 | 0.00 | 0 | 3 | 6.00 | 6 | 10 | 2.00 | 20 |
| **Average** | | | 3,9 | | | 2,2 | | | 0,5 | | | 1,4 | | | 4,7 |
| **StDev** | | | 0,141 | | | 0,283 | | | 0,141 | | | 0,283 | | | 0,707 |
| ***Salmonella* species (SAL spp.)** | | | | | | | | | | | | | | | |
| | **P1** | | | **P2** | | | **P3** | | | **P4** | | | **P5** | | |
| | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** |
| **10^1** | 42 | 8.40 | 0,84 | 31 | 6.20 | 0,62 | 14 | 2.80 | 0,28 | 37 | 7.40 | 0,74 | 41 | 8.20 | 0,82 |
| | 43 | 8.60 | 0,86 | 29 | 5.80 | 0,58 | 19 | 3.80 | 0,38 | 39 | 7.80 | 0,78 | 36 | 7.20 | 0,72 |
| **10^2** | 16 | 3.20 | 3,2 | 9 | 1.80 | 1,8 | 6 | 1.20 | 1,2 | 22 | 4.40 | 4,4 | 19 | 3.80 | 3,8 |
| | 11 | 2.20 | 2,2 | 5 | 1.00 | 1 | 1 | 2.00 | 0,2 | 21 | 4.20 | 4,2 | 14 | 2.80 | 2,8 |
| **10^3** | 2 | 4.00 | 4 | 2 | 4.00 | 4 | 0 | 0.00 | 0 | 3 | 6.00 | 6 | 2 | 4.00 | 4 |
| | 1 | 2.00 | 2 | 0 | 0.00 | 0 | 0 | 0.00 | 0 | 1 | 2.00 | 2 | 1 | 2.00 | 2 |
| **Average** | | | 2,7 | | | 1,4 | | | 0,33 | | | 4,3 | | | 3,3 |
| **StDev** | | | 0,707 | | | 0,566 | | | 0,071 | | | 0,141 | | | 0,707 |
| ***Shigella* species (SHIG spp.)** | | | | | | | | | | | | | | | |
| | **P1** | | | **P2** | | | **P3** | | | **P4** | | | **P5** | | |
| | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** |
| **10^2** | 16 | 3.20 | 3,2 | 17 | 3.40 | 3,4 | 17 | 3.40 | 3,4 | 15 | 3.00 | 3 | 8 | 1.60 | 1,6 |
| | 6 | 1.20 | 1,2 | 13 | 2.60 | 2,6 | 18 | 3.60 | 3,6 | 8 | 1.60 | 1,6 | 5 | 1.00 | 1 |
| **10^3** | 1 | 2.00 | 2 | 5 | 1.00 | 10 | 2 | 4.00 | 4 | 4 | 8.00 | 8 | 2 | 4.00 | 4 |
| | 3 | 6.00 | 6 | 1 | 2.00 | 2 | 2 | 4.00 | 4 | 2 | 4.00 | 4 | 1 | 2.00 | 2 |
| **Average** | | | 2,6 | | | 3 | | | 3,5 | | | 3,5 | | | 1,3 |
| **StDev** | | | 0,849 | | | 0,566 | | | 0,141 | | | 0,707 | | | 0,424 |

**Table 7** Presumptive bacterial indicator counts at all sampling points during April 2014.

| **Total heterotrophic bacteria (THB)** | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **P1** | | | **P2** | | | **P3** | | | **P4** | | | **P5** | | |
| | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^6$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^6$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^6$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^6$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^6$** |
| **10^5** | 38 | 7.60 | 7,6 | 35 | 7.00 | 7 | 33 | 6.60 | 6,6 | 36 | 7.20 | 7,2 | 33 | 6.60 | 6,6 |
| | 37 | 7.40 | 7,4 | 35 | 7.00 | 7 | 30 | 6.00 | 6 | 38 | 7.60 | 7,6 | 34 | 6.80 | 6,8 |
| **10^6** | 17 | 3.40 | 34 | 21 | 4.20 | 42 | 15 | 3.00 | 30 | 19 | 3.80 | 38 | 21 | 4.20 | 42 |
| | 17 | 3.40 | 34 | 20 | 4.00 | 40 | 14 | 2.80 | 28 | 19 | 3.80 | 38 | 21 | 4.20 | 42 |
| **Average** | | | 7,5 | | | 7 | | | 6,3 | | | 7,4 | | | 6,7 |
| **StDev** | | | 0,141 | | | 0 | | | 0,424 | | | 0,283 | | | 0,141 |
| ***E.coli* (EC)** | | | | | | | | | | | | | | | |
| | **P1** | | | **P2** | | | **P3** | | | **P4** | | | **P5** | | |
| | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** |
| **10^2** | 23 | 4.60 | 4,6 | 22 | 4.40 | 4,4 | 19 | 3.80 | 3,8 | 19 | 3.80 | 3,8 | 12 | 2.40 | 2,4 |
| | 20 | 4.00 | 4 | 23 | 4.60 | 4,6 | 20 | 4.00 | 4 | 14 | 2.80 | 2,8 | 13 | 2.60 | 2,6 |
| **10^3** | 14 | 2.80 | 28 | 14 | 2.80 | 28 | 7 | 1.40 | 14 | 3 | 6.00 | 6 | 8 | 1.60 | 16 |
| | 7 | 1.40 | 14 | 14 | 2.80 | 28 | 5 | 1.00 | 10 | 4 | 8.00 | 8 | 1 | 2.00 | 2 |
| **Average** | | | 4,3 | | | 4,5 | | | 3,9 | | | 3,3 | | | 2,5 |
| **StDev** | | | 0,424 | | | 0,141 | | | 0,141 | | | 0,707 | | | 0,141 |
| **Total coliforms (TC)** | | | | | | | | | | | | | | | |
| | **P1** | | | **P2** | | | **P3** | | | **P4** | | | **P5** | | |
| | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** |
| **10^2** | 20 | 4.00 | 4 | 19 | 3.80 | 3,8 | 21 | 4.20 | 4,2 | 22 | 4.40 | 4,4 | 17 | 3.40 | 3,4 |
| | 21 | 4.20 | 4,2 | 14 | 2.80 | 2,8 | 21 | 4.20 | 4,2 | 23 | 4.60 | 4,6 | 16 | 3.20 | 3,2 |
| **10^3** | 11 | 2.20 | 22 | 7 | 1.40 | 14 | 14 | 2.80 | 28 | 14 | 2.80 | 28 | 3 | 6.00 | 6 |
| | 11 | 2.20 | 22 | 2 | 4.00 | 4 | 12 | 2.40 | 24 | 11 | 2.20 | 22 | 2 | 4.00 | 4 |
| **Average** | | | 4,1 | | | 3,3 | | | 4,2 | | | 4,5 | | | 3,3 |
| **StDev** | | | 0,141 | | | 0,707 | | | 0 | | | 0,141 | | | 0,141 |
| **Faecal coliforms (FC)** | | | | | | | | | | | | | | | |
| | **P1** | | | **P2** | | | **P3** | | | **P4** | | | **P5** | | |
| | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** |
| **10^2** | 69 | 1.38 | 1,38 | 72 | 1.44 | 1,44 | 54 | 1.08 | 1,08 | 47 | 9.40 | 0,94 | 32 | 6.40 | 0,64 |
| | 72 | 1.44 | 1,44 | 71 | 1.42 | 1,42 | 56 | 1.12 | 1,12 | 49 | 9.80 | 0,98 | 37 | 7.40 | 0,74 |

| | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **10^3** | 47 | 9.40 | 9,4 | 44 | 8.80 | 8,8 | 13 | 2.60 | 2,6 | 16 | 3.20 | 3,2 | 14 | 2.80 | 2,8 |
| | 42 | 8.40 | 8,4 | 43 | 8.60 | 8,6 | 11 | 2.20 | 2,2 | 19 | 3.80 | 3,8 | 18 | 3.60 | 3,6 |
| **Average** | | | 1,41 | | | 1,43 | | | 2,4 | | | 3,5 | | | 3,2 |
| **StDev** | | | 0,042 | | | 0,014 | | | 0,283 | | | 0,424 | | | 0,566 |
| **Faecal streptococci (FS)** | | | | | | | | | | | | | | | |
| | **P1** | | | **P2** | | | **P3** | | | **P4** | | | **P5** | | |
| | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** |
| **10^2** | 37 | 7.40 | 7,4 | 24 | 4.80 | 4,8 | 37 | 7.40 | 7,4 | 24 | 4.80 | 4,8 | 23 | 4.60 | 4,6 |
| | 38 | 7.60 | 7,6 | 24 | 4.80 | 4,8 | 34 | 6.80 | 6,8 | 21 | 4.20 | 4,2 | 27 | 5.40 | 5,4 |
| **10^3** | 21 | 4.20 | 42 | 13 | 2.60 | 26 | 11 | 2.20 | 22 | 7 | 1.40 | 14 | 14 | 2.80 | 28 |
| | 20 | 4.00 | 40 | 11 | 2.20 | 22 | 6 | 1.20 | 12 | 0 | 0.00 | 0 | 8 | 1.60 | 16 |
| **Average** | | | 7,5 | | | 4,8 | | | 7,1 | | | 4,5 | | | 5 |
| **StDev** | | | 0,141 | | | 0 | | | 0,424 | | | 0,424 | | | 0,566 |
| ***Vibrio* species (VIB spp.)** | | | | | | | | | | | | | | | |
| | **P1** | | | **P2** | | | **P3** | | | **P4** | | | **P5** | | |
| | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** |
| **10^1** | 123 | 246 | 2,46 | 119 | 2.38 | 2,38 | 78 | 1.56 | 1,56 | 92 | 1.84 | 1,84 | 87 | 1.74 | 1,74 |
| | 120 | 240 | 2,4 | 114 | 2.28 | 2,28 | 72 | 1.44 | 1,44 | 98 | 1.96 | 1,96 | 84 | 1.68 | 1,68 |
| **10^2** | 47 | 940 | 9,4 | 36 | 7.20 | 7,2 | 26 | 5.20 | 5,2 | 50 | 1.00 | 10 | 31 | 6.20 | 6,2 |
| | 40 | 8.00 | 8 | 32 | 6.40 | 6,4 | 21 | 4.20 | 4,2 | 53 | 1.06 | 10,6 | 30 | 6.00 | 6 |
| **10^3** | 13 | 2.60 | 26 | 11 | 2.20 | 22 | 14 | 2.80 | 28 | 23 | 4.60 | 46 | 11 | 2.20 | 22 |
| | 13 | 2.60 | 26 | 19 | 3.80 | 38 | 8 | 1.60 | 16 | 20 | 4.00 | 40 | 3 | 6.00 | 6 |
| **Average** | | | 2,43 | | | 2,33 | | | 4,7 | | | 1,9 | | | 6,1 |
| **StDev** | | | 0,042 | | | 0,071 | | | 0,707 | | | 0,085 | | | 0,141 |
| ***Salmonella* species (SAL spp.)** | | | | | | | | | | | | | | | |
| | **P1** | | | **P2** | | | **P3** | | | **P4** | | | **P5** | | |
| | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** |
| **10^2** | 23 | 4.60 | 4,6 | 32 | 6.40 | 6,4 | 14 | 2.80 | 2,8 | 23 | 4.60 | 4,6 | 22 | 4.40 | 4,4 |
| | 21 | 4.20 | 4,2 | 33 | 6.60 | 6,6 | 11 | 2.20 | 2,2 | 20 | 4.00 | 4 | 19 | 3.80 | 3,8 |
| **10^3** | 7 | 1.40 | 14 | 17 | 3.40 | 34 | 3 | 6.00 | 6 | 7 | 1.40 | 14 | 3 | 6.00 | 6 |
| | 10 | 2.00 | 20 | 18 | 3.60 | 36 | 4 | 8.00 | 8 | 11 | 2.20 | 22 | 4 | 8.00 | 8 |
| **Average** | | | 4,4 | | | 6,5 | | | 2,5 | | | 4,3 | | | 4,1 |
| **StDev** | | | 0,283 | | | 0,141 | | | 0,424 | | | 0,424 | | | 0,424 |
| ***Shigella* species (SHIG spp.)** | | | | | | | | | | | | | | | |
| | **P1** | | | **P2** | | | **P3** | | | **P4** | | | **P5** | | |

| | Count | cfu/100mℓ | (cfu/100mℓ) x $10^3$ | Count | cfu/100mℓ | (cfu/100mℓ) x $10^3$ | Count | cfu/100mℓ | (cfu/100mℓ) x $10^3$ | Count | cfu/100mℓ | (cfu/100mℓ) x $10^3$ | Count | cfu/100mℓ | (cfu/100mℓ) x $10^3$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **10^1** | 61 | 1.22 | 1,22 | 55 | 1.10 | 1,1 | 40 | 8.00 | 0,8 | 46 | 9.20 | 0,92 | 51 | 1.02 | 1,02 |
| | 66 | 1.32 | 1,32 | 57 | 1.14 | 1,14 | 40 | 8.00 | 0,8 | 47 | 9.40 | 0,92 | 53 | 1.06 | 1,06 |
| **10^2** | 48 | 9.60 | 9,6 | 32 | 6.40 | 6,4 | 28 | 5.60 | 5,6 | 13 | 2.60 | 2,5 | 22 | 4.40 | 4,4 |
| | 49 | 9.80 | 9,8 | 39 | 7.80 | 7,8 | 25 | 5.00 | 5 | 15 | 3.00 | 3 | 19 | 3.80 | 3,8 |
| **10^3** | 23 | 4.60 | 46 | 17 | 3.40 | 34 | 12 | 2.40 | 24 | 8 | 1.60 | 16 | 10 | 2.00 | 20 |
| | 21 | 4.20 | 42 | 21 | 4.20 | 42 | 7 | 1.40 | 14 | 10 | 2.00 | 20 | 7 | 1.40 | 14 |
| **Average** | | | 1,27 | | | 1,12 | | | 5,3 | | | 2,75 | | | 4,1 |
| **StDev** | | | 0,071 | | | 0,028 | | | 0,424 | | | 0,354 | | | 0,424 |

**Table 8** Presumptive bacterial indicator counts at all sampling points during May 2014.

| **Total heterotrophic bacteria (THB)** | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **P1** | | | **P2** | | | **P3** | | | **P4** | | | **P5** | | |
| | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^6$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^6$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^6$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^6$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^6$** |
| **10^5** | 33 | 6.60 | 6,6 | 29 | 5.80 | 5,8 | 31 | 6.20 | 6,2 | 34 | 6.80 | 6,8 | 38 | 7.60 | 7,6 |
| | 33 | 6.60 | 6,6 | 30 | 6.00 | 6 | 30 | 6.00 | 6 | 34 | 6.80 | 6,8 | 37 | 7.40 | 7,4 |
| **10^6** | 19 | 3.80 | 38 | 12 | 2.40 | 24 | 20 | 4.00 | 40 | 17 | 3.40 | 34 | 20 | 4.00 | 40 |
| | 20 | 4.00 | 40 | 11 | 2.20 | 22 | 20 | 4.00 | 40 | 16 | 3.20 | 32 | 21 | 4.20 | 42 |
| **Average** | | | 6,6 | | | 5,9 | | | 6,1 | | | 6,8 | | | 7,5 |
| **StDev** | | | 0 | | | 0,141 | | | 0,141 | | | 0 | | | 0,141 |
| ***E.coli* (EC)** | | | | | | | | | | | | | | | |
| | **P1** | | | **P2** | | | **P3** | | | **P4** | | | **P5** | | |
| | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** |
| **10^1** | 192 | 3.84 | 3,84 | 208 | 4.16 | 4,16 | 129 | 2.58 | 2,58 | 176 | 3.52 | 3,52 | 229 | 4.58 | 4,58 |
| | 224 | 4.48 | 4,48 | 220 | 4.40 | 4,4 | 144 | 2.88 | 2,88 | 182 | 3.64 | 3,64 | 201 | 4.02 | 4,02 |
| **10^2** | 88 | 1.76 | 17,6 | 123 | 2.46 | 24,6 | 69 | 1.38 | 13,8 | 89 | 1.78 | 17,8 | 119 | 2.38 | 23,8 |
| | 86 | 1.72 | 17,2 | 142 | 2.84 | 28,4 | 69 | 1.38 | 13,8 | 61 | 1.22 | 12,2 | 87 | 1.74 | 17,4 |
| **10^3** | 1 | 2.00 | 2 | 2 | 4.00 | 4 | 31 | 6.20 | 62 | 44 | 8.80 | 88 | 53 | 1.06 | 106 |
| | 4 | 8.00 | 8 | 3 | 6.00 | 6 | 38 | 7.60 | 76 | 45 | 9.00 | 90 | 58 | 1.16 | 116 |
| **Average** | | | 4,16 | | | 4,28 | | | 2,73 | | | 3,58 | | | 4,3 |
| **StDev** | | | 0,453 | | | 0,170 | | | 0,212 | | | 0,085 | | | 0,396 |
| **Total coliforms (TC)** | | | | | | | | | | | | | | | |
| | **P1** | | | **P2** | | | **P3** | | | **P4** | | | **P5** | | |
| | **Count** | **cfu/100mℓ** | **(cfu/100mℓ)** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ)** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ)** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ)** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ)** |

| | | | x $10^3$ | | | x $10^3$ | | | x $10^3$ | | | x $10^3$ | | | x $10^3$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **10^2** | 17 | 3.40 | 3,4 | 15 | 3.00 | 3 | 13 | 2.60 | 2,6 | 19 | 3.80 | 3,4 | 21 | 4.20 | 4,2 |
| | 17 | 3.40 | 3,4 | 14 | 2.80 | 2,8 | 12 | 2.40 | 2,4 | 20 | 4.00 | 4 | 21 | 4.20 | 4,2 |
| **10^3** | 4 | 8.00 | 8 | 4 | 8.00 | 8 | 0 | 0.00 | 0 | 1 | 2.00 | 2 | 6 | 1.20 | 12 |
| | 3 | 6.00 | 6 | 1 | 2.00 | 2 | 0 | 0.00 | 0 | 2 | 4.00 | 4 | 4 | 8.00 | 8 |
| **Average** | | | 3,4 | | | 2,4 | | | 2,5 | | | 3,7 | | | 4,2 |
| **StDev** | | | 0 | | | 0,566 | | | 0,141 | | | 0,424 | | | 0 |
| **Faecal coliforms (FC)** | | | | | | | | | | | | | | | |
| | **P1** | | | **P2** | | | **P3** | | | **P4** | | | **P5** | | |
| | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** |
| **10^1** | 61 | 1.22 | 1,22 | 57 | 1.14 | 1,14 | 43 | 8.60 | 0,86 | 53 | 1.06 | 1,06 | 57 | 1.14 | 1,14 |
| | 60 | 1.20 | 1,2 | 54 | 1.08 | 1,08 | 47 | 9.40 | 0,94 | 53 | 1.06 | 1,06 | 61 | 1.22 | 1,22 |
| **10^2** | 15 | 3.00 | 3 | 18 | 3.60 | 3,6 | 10 | 2.00 | 2 | 12 | 2.40 | 2,4 | 10 | 2.00 | 2 |
| | 18 | 3.60 | 3,6 | 17 | 3.40 | 3,4 | 11 | 2.20 | 2,2 | 9 | 1.80 | 1,8 | 5 | 1.00 | 1 |
| **10^3** | 7 | 1.40 | 14 | 11 | 2.20 | 22 | 1 | 2.00 | 2 | 1 | 2.00 | 2 | 0 | 0.00 | 0 |
| | 3 | 6.00 | 6 | 6 | 1.20 | 12 | 3 | 6.00 | 6 | 0 | 0.00 | 0 | 0 | 0.00 | 0 |
| **Average** | | | 1,21 | | | 1,11 | | | 2 | | | 2,2 | | | 1,18 |
| **StDev** | | | 0,014 | | | 0,042 | | | 0 | | | 0,283 | | | 0,057 |
| **Faecal streptococci (FS)** | | | | | | | | | | | | | | | |
| | **P1** | | | **P2** | | | **P3** | | | **P4** | | | **P5** | | |
| | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** |
| **10^1** | 152 | 3.04 | 3,04 | 44 | 8.80 | 0,88 | 47 | 9.4 | 0,94 | 66 | 1.32 | 1,32 | 116 | 2.32 | 2,32 |
| | 178 | 3.56 | 3,56 | 45 | 9.00 | 0,9 | 67 | 1.34 | 1,34 | 72 | 1.44 | 1,44 | 85 | 1.70 | 1,7 |
| **10^2** | 37 | 7.40 | 7,4 | 22 | 4.40 | 4,4 | 15 | 3.00 | 3 | 33 | 6.60 | 6,6 | 10 | 2.00 | 2 |
| | 35 | 7.00 | 7 | 26 | 5.20 | 5,2 | 16 | 3.20 | 3,2 | 38 | 7.60 | 7,6 | 18 | 3.60 | 3,6 |
| **10^3** | 1 | 2.0 | 2 | 0 | 0.00 | 0 | 11 | 2.20 | 22 | 12 | 2.40 | 24 | 3 | 6.00 | 6 |
| | 5 | 1.00 | 10 | 0 | 0.00 | 0 | 10 | 2.00 | 20 | 17 | 3.40 | 34 | 3 | 6.00 | 6 |
| **Average** | | | 3,3 | | | 4,8 | | | 3,1 | | | 1,38 | | | 1,85 |
| **StDev** | | | 0,368 | | | 0,566 | | | 0,141 | | | 0,085 | | | 0,212 |
| ***Vibrio* species (VIB spp.)** | | | | | | | | | | | | | | | |
| | **P1** | | | **P2** | | | **P3** | | | **P4** | | | **P5** | | |
| | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** |
| **10^1** | 280 | 5.60 | 5,6 | 180 | 3.60 | 3,6 | 247 | 4.94 | 4,94 | 99 | 1.98 | 1,98 | 192 | 3.84 | 3,84 |
| | 266 | 5.32 | 5,32 | 144 | 2.88 | 2,88 | 199 | 3.98 | 3,98 | 97 | 1.94 | 1,94 | 127 | 2.54 | 2,54 |
| **10^2** | 32 | 6.40 | 6,4 | 43 | 8.60 | 8,6 | 176 | 3.52 | 35,2 | 31 | 6.20 | 6,2 | 86 | 1.72 | 17,2 |

| | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 33 | 6.60 | 6,6 | 49 | 9.80 | 9,8 | 162 | 3.24 | 32,4 | 34 | 6.80 | 6,8 | 61 | 1.22 | 12,2 |
| **10^3** | 1 | 2.00 | 2 | 7 | 1.40 | 14 | 17 | 3.40 | 34 | 17 | 3.40 | 34 | 24 | 4.80 | 48 |
| | 2 | 4.00 | 4 | 5 | 1.00 | 10 | 18 | 3.60 | 36 | 18 | 3.60 | 36 | 27 | 5.40 | 54 |
| **Average** | | | 5,46 | | | 3,24 | | | 4,46 | | | 1,96 | | | 3,19 |
| **StDev** | | | 0,198 | | | 0,509 | | | 0,679 | | | 0,028 | | | 0,919 |
| ***Salmonella* species (SAL spp.)** | | | | | | | | | | | | | | | |
| | **P1** | | | **P2** | | | **P3** | | | **P4** | | | **P5** | | |
| | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** |
| **10^2** | 16 | 3.20 | 3,2 | 12 | 2.40 | 2,4 | 7 | 1.40 | 1,4 | 10 | 2.00 | 2 | 15 | 3.00 | 3 |
| | 14 | 2.80 | 2,8 | 16 | 3.20 | 3,2 | 6 | 1.20 | 1,2 | 11 | 2.20 | 2,2 | 12 | 2.40 | 2,4 |
| **10^3** | 1 | 2.00 | 2 | 3 | 6.00 | 6 | 4 | 8.00 | 8 | 7 | 1.40 | 14 | 2 | 4.00 | 4 |
| | 7 | 1.40 | 14 | 6 | 1.20 | 12 | 3 | 6.00 | 6 | 3 | 6.00 | 6 | 0 | 0.00 | 0 |
| **Average** | | | 3 | | | 2,8 | | | 1,3 | | | 2,1 | | | 2,7 |
| **StDev** | | | 0,283 | | | 0,566 | | | 0,141 | | | 0,141 | | | 0,424 |
| ***Shigella* species (SHIG spp.)** | | | | | | | | | | | | | | | |
| | **P1** | | | **P2** | | | **P3** | | | **P4** | | | **P5** | | |
| | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** |
| **10^1** | 49 | 9.80 | 0,98 | 144 | 2.88 | 2,88 | 32 | 6.40 | 0,64 | 35 | 7.00 | 0,7 | 45 | 9.00 | 0,9 |
| | 45 | 9.00 | 0,9 | 138 | 2.76 | 2,76 | 38 | 7.60 | 0,76 | 34 | 6.80 | 0,68 | 48 | 9.60 | 0,96 |
| **10^2** | 15 | 3.00 | 3 | 50 | 1.00 | 10 | 24 | 4.80 | 4,8 | 23 | 4.60 | 4,6 | 23 | 4.60 | 4,6 |
| | 17 | 3.40 | 3,4 | 51 | 1.02 | 10,2 | 22 | 4.40 | 4,4 | 22 | 4.40 | 4,4 | 24 | 4.80 | 4,8 |
| **10^3** | 1 | 2.00 | 2 | 8 | 1.60 | 16 | 10 | 2.00 | 20 | 10 | 2.00 | 20 | 1 | 2.00 | 2 |
| | 3 | 6.00 | 6 | 7 | 1.40 | 14 | 16 | 3.20 | 32 | 12 | 2.40 | 24 | 0 | 0.00 | 0 |
| **Average** | | | 3,2 | | | 2,82 | | | 4,6 | | | 4,5 | | | 4,7 |
| **StDev** | | | 0,283 | | | 0,085 | | | 0,283 | | | 0,141 | | | 0,141 |

**Table 9** Presumptive bacterial indicator counts at all sampling points during June 2014.

| **Total heterotrophic bacteria (THB)** | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **P1** | | | **P2** | | | **P3** | | | **P4** | | | **P5** | | |
| | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^6$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^6$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^6$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^6$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^6$** |
| **10^5** | 22 | 4.40 | 4,4 | 29 | 5.80 | 5,8 | 19 | 3.80 | 3,8 | 25 | 5.00 | 5 | 23 | 4.60 | 4,6 |
| | 21 | 4.20 | 4,2 | 28 | 5.60 | 5,6 | 15 | 3.00 | 3 | 24 | 4.80 | 4,8 | 22 | 4.40 | 4,4 |
| **10^6** | 5 | 1.00 | 10 | 17 | 3.40 | 34 | 7 | 1.40 | 14 | 12 | 2.40 | 24 | 14 | 2.80 | 28 |
| | 6 | 1.20 | 12 | 17 | 3.40 | 34 | 9 | 1.80 | 18 | 13 | 2.60 | 26 | 18 | 3.60 | 36 |

| | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Average** | | | 4,3 | | | 5,7 | | | 3,4 | | | 4,9 | | | 4,5 |
| **StDev** | | | 0,141 | | | 0,141 | | | 0,566 | | | 0,141 | | | 0,141 |
| ***E.coli* (EC)** | | | | | | | | | | | | | | | |
| | **P1** | | | **P2** | | | **P3** | | | **P4** | | | **P5** | | |
| | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** |
| **10^1** | 66 | 1.32 | 1,32 | 75 | 1.50 | 1,5 | 24 | 4.80 | 0,48 | 35 | 7.00 | 0,7 | 42 | 8.40 | 0,84 |
| | 98 | 1.96 | 1,96 | 44 | 8.80 | 0,88 | 26 | 5.20 | 0,52 | 32 | 6.40 | 0,64 | 40 | 8.00 | 0,8 |
| **10^2** | 36 | 7.20 | 7,2 | 20 | 4.00 | 4 | 14 | 2.80 | 2,8 | 14 | 2.80 | 2,8 | 20 | 4.00 | 4 |
| | 37 | 7.40 | 7,4 | 39 | 7.80 | 7,8 | 13 | 2.60 | 2,6 | 18 | 3.60 | 3,6 | 21 | 4.20 | 4,2 |
| **10^3** | 8 | 1.60 | 16 | 11 | 2.20 | 22 | 0 | 0.00 | 0 | 7 | 1.40 | 14 | 4 | 8.00 | 8 |
| | 2 | 4.00 | 4 | 17 | 3.40 | 34 | 2 | 4.00 | 4 | 8 | 1.60 | 16 | 5 | 1.00 | 10 |
| **Average** | | | 1,64 | | | 1,19 | | | 2,7 | | | 3,2 | | | 4,1 |
| **StDev** | | | 0,453 | | | 0,438 | | | 0,141 | | | 0,566 | | | 0,141 |
| **Total coliforms (TC)** | | | | | | | | | | | | | | | |
| | **P1** | | | **P2** | | | **P3** | | | **P4** | | | **P5** | | |
| | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** |
| **10^1** | 123 | 2.46 | 2,46 | 67 | 1.34 | 1,34 | 54 | 1.08 | 1,08 | 98 | 1.96 | 1,96 | 100 | 2.00 | 2 |
| | 130 | 2.60 | 2,6 | 72 | 1.44 | 1,44 | 52 | 1.04 | 1,04 | 87 | 1.74 | 1,74 | 103 | 2.06 | 2,06 |
| **10^2** | 20 | 4.00 | 4 | 15 | 3.00 | 3 | 11 | 2.20 | 2,2 | 18 | 3.60 | 3,6 | 57 | 1.14 | 11,4 |
| | 15 | 3.00 | 3 | 11 | 2.20 | 2,2 | 13 | 2.60 | 2,6 | 12 | 2.40 | 2,4 | 59 | 1.18 | 11,8 |
| **10^3** | 2 | 4.00 | 4 | 2 | 4.00 | 4 | 3 | 6.00 | 6 | 6 | 1.20 | 12 | 21 | 4.20 | 42 |
| | 2 | 4.00 | 4 | 8 | 1.60 | 16 | 5 | 1.00 | 10 | 4 | 8.00 | 8 | 20 | 4.00 | 40 |
| **Average** | | | 2,53 | | | 1,39 | | | 1,06 | | | 1,85 | | | 2,03 |
| **StDev** | | | 0,099 | | | 0,071 | | | 0,028 | | | 0,156 | | | 0,042 |
| **Faecal coliforms (FC)** | | | | | | | | | | | | | | | |
| | **P1** | | | **P2** | | | **P3** | | | **P4** | | | **P5** | | |
| | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** |
| **10^1** | 80 | 1.60 | 1,6 | 92 | 1.84 | 1,84 | 70 | 1.40 | 1,4 | 61 | 1.22 | 1,22 | 85 | 1.70 | 1,7 |
| | 92 | 1.84 | 1,84 | 98 | 1.96 | 1,96 | 69 | 1.38 | 1,38 | 61 | 1.22 | 1,22 | 90 | 1.80 | 1,8 |
| **10^2** | 15 | 3.00 | 3 | 37 | 7.40 | 7,4 | 33 | 6.60 | 6,6 | 42 | 8.40 | 8,4 | 51 | 1.02 | 10,2 |
| | 13 | 2.60 | 2,6 | 30 | 6.00 | 6 | 31 | 6.20 | 6,2 | 43 | 8.60 | 8,6 | 50 | 1.00 | 10 |
| **10^3** | 4 | 8.00 | 8 | 14 | 2.80 | 28 | 12 | 2.40 | 24 | 9 | 1.80 | 18 | 14 | 2.80 | 28 |
| | 4 | 8.00 | 8 | 11 | 2.20 | 22 | 8 | 1.60 | 16 | 3 | 6.00 | 6 | 17 | 3.40 | 34 |
| **Average** | | | 2,8 | | | 1,9 | | | 1,39 | | | 1,22 | | | 1,75 |
| **StDev** | | | 0,283 | | | 0,085 | | | 0,014 | | | 0 | | | 0,071 |

| Faecal streptococci (FS) | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | P1 | | | P2 | | | P3 | | | P4 | | | P5 | | |
| | Count | cfu/100mℓ | (cfu/100mℓ) x $10^3$ | Count | cfu/100mℓ | (cfu/100mℓ) x $10^3$ | Count | cfu/100mℓ | (cfu/100mℓ) x $10^3$ | Count | cfu/100mℓ | (cfu/100mℓ) x $10^3$ | Count | cfu/100mℓ | (cfu/100mℓ) x $10^3$ |
| 10^1 | 182 | 3.64 | 3,64 | 84 | 1.68 | 1,68 | 113 | 2.26 | 2,26 | 127 | 2.54 | 2,54 | 100 | 2.00 | 2 |
| | 187 | 3.74 | 3,74 | 110 | 2.20 | 2,2 | 104 | 2.08 | 2,08 | 128 | 2.56 | 2,56 | 103 | 2.06 | 2,06 |
| 10^2 | 59 | 1.18 | 11,8 | 67 | 1.34 | 13,4 | 26 | 5.20 | 5,2 | 51 | 1.02 | 10,2 | 47 | 9.40 | 9,4 |
| | 52 | 1.04 | 10,4 | 62 | 1.24 | 12,4 | 23 | 4.60 | 4,6 | 49 | 9.80 | 9,8 | 43 | 8.60 | 8,6 |
| 10^3 | 15 | 3.00 | 30 | 9 | 1.80 | 18 | 8 | 1.60 | 16 | 13 | 2.60 | 26 | 26 | 5.20 | 52 |
| | 15 | 3.00 | 30 | 10 | 2.00 | 20 | 3 | 6.00 | 6 | 12 | 2.40 | 24 | 28 | 5.60 | 56 |
| Average | | | 3,69 | | | 1,94 | | | 2,17 | | | 2,55 | | | 2,03 |
| StDev | | | 0,071 | | | 0,368 | | | 0,127 | | | 0,014 | | | 0,042 |
| *Vibrio* species (VIB spp.) | | | | | | | | | | | | | | | |
| | P1 | | | P2 | | | P3 | | | P4 | | | P5 | | |
| | Count | cfu/100mℓ | (cfu/100mℓ) x $10^3$ | Count | cfu/100mℓ | (cfu/100mℓ) x $10^3$ | Count | cfu/100mℓ | (cfu/100mℓ) x $10^3$ | Count | cfu/100mℓ | (cfu/100mℓ) x $10^3$ | Count | cfu/100mℓ | (cfu/100mℓ) x $10^3$ |
| 10^1 | 95 | 1.90 | 1,9 | 101 | 2.02 | 2,02 | 72 | 1.44 | 1,44 | 119 | 2.38 | 2,38 | 142 | 2.84 | 2,84 |
| | 99 | 1.98 | 1,98 | 100 | 2.00 | 2 | 70 | 1.40 | 1,4 | 99 | 1.98 | 1,98 | 143 | 2.86 | 2,86 |
| 10^2 | 45 | 9.00 | 9 | 58 | 1.16 | 11,6 | 40 | 8.00 | 8 | 52 | 1.04 | 10,4 | 66 | 1.32 | 13,2 |
| | 39 | 7.80 | 7,8 | 52 | 1.04 | 10,4 | 39 | 7.80 | 7,8 | 52 | 1.04 | 10,4 | 71 | 1.42 | 14,2 |
| 10^3 | 22 | 4.40 | 44 | 32 | 6.40 | 64 | 14 | 2.80 | 28 | 13 | 2.60 | 26 | 23 | 4.60 | 46 |
| | 27 | 5.40 | 54 | 33 | 6.60 | 66 | 8 | 1.60 | 16 | 13 | 2.60 | 26 | 18 | 3.60 | 36 |
| Average | | | 1,94 | | | 2,01 | | | 1,42 | | | 2,18 | | | 2,85 |
| StDev | | | 0,057 | | | 0,014 | | | 0,028 | | | 0,283 | | | 0,014 |
| *Salmonella* species (SAL spp.) | | | | | | | | | | | | | | | |
| | P1 | | | P2 | | | P3 | | | P4 | | | P5 | | |
| | Count | cfu/100mℓ | (cfu/100mℓ) x $10^3$ | Count | cfu/100mℓ | (cfu/100mℓ) x $10^3$ | Count | cfu/100mℓ | (cfu/100mℓ) x $10^3$ | Count | cfu/100mℓ | (cfu/100mℓ) x $10^3$ | Count | cfu/100mℓ | (cfu/100mℓ) x $10^3$ |
| 10^1 | 39 | 7.80 | 0,78 | 66 | 1.32 | 1,32 | 23 | 4.60 | 0,46 | 33 | 6.60 | 0,66 | 44 | 8.80 | 0,88 |
| | 32 | 6.40 | 0,64 | 69 | 1.38 | 1,38 | 22 | 4.40 | 0,44 | 30 | 6.00 | 0,6 | 40 | 8.00 | 0,8 |
| 10^2 | 17 | 3.40 | 3,4 | 42 | 8.40 | 8,4 | 13 | 2.60 | 2,6 | 15 | 3.00 | 3 | 17 | 3.40 | 3,4 |
| | 19 | 3.80 | 3,8 | 43 | 8.60 | 8,6 | 12 | 2.40 | 2,4 | 15 | 3.00 | 3 | 19 | 3.80 | 3,8 |
| 10^3 | 4 | 8.00 | 8 | 14 | 2.80 | 28 | 1 | 2.00 | 2 | 3 | 6.00 | 6 | 2 | 4.00 | 4 |
| | 3 | 6.00 | 6 | 17 | 3.40 | 34 | 0 | 0.00 | 0 | 1 | 2.00 | 2 | 2 | 4.00 | 4 |
| Average | | | 3,6 | | | 1,35 | | | 2,5 | | | 3 | | | 3,6 |
| StDev | | | 0,283 | | | 0,042 | | | 0,141 | | | 0 | | | 0,283 |
| *Shigella* species (SHIG spp.) | | | | | | | | | | | | | | | |
| | P1 | | | P2 | | | P3 | | | P4 | | | P5 | | |

| | Count | cfu/100mℓ | (cfu/100mℓ) x $10^3$ | Count | cfu/100mℓ | (cfu/100mℓ) x $10^3$ | Count | cfu/100mℓ | (cfu/100mℓ) x $10^3$ | Count | cfu/100mℓ | (cfu/100mℓ) x $10^3$ | Count | cfu/100mℓ | (cfu/100mℓ) x $10^3$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **10^1** | 60 | 1.20 | 1,2 | 50 | 1.00 | 1 | 38 | 7.60 | 0,76 | 22 | 4.40 | 0,44 | 30 | 6.00 | 0,6 |
| | 61 | 1.22 | 1,22 | 51 | 1.02 | 1,02 | 32 | 6.40 | 0,64 | 21 | 4.20 | 0,42 | 22 | 4.40 | 0,44 |
| **10^2** | 20 | 4.00 | 4 | 14 | 2.80 | 2,8 | 15 | 3.00 | 3 | 14 | 2.80 | 2,8 | 23 | 4.60 | 4,6 |
| | 23 | 4.60 | 4,6 | 7 | 1.40 | 1,4 | 1 | 2.00 | 0,2 | 10 | 2.00 | 2 | 18 | 3.60 | 3,6 |
| **10^3** | 10 | 2.00 | 20 | 3 | 6.00 | 6 | 0 | 0.00 | 0 | 7 | 1.40 | 14 | 3 | 6.00 | 6 |
| | 9 | 1.80 | 18 | 0 | 0.00 | 0 | 0 | 0.00 | 0 | 1 | 2.00 | 2 | 0 | 0.00 | 0 |
| **Average** | | | 1,21 | | | 2,1 | | | 0,7 | | | 2,4 | | | 0,52 |
| **StDev** | | | 0,014 | | | 0,990 | | | 0,085 | | | 0,566 | | | 0,113 |

**Table 10** Presumptive bacterial indicator counts at all sampling points during July 2014.

| **Total heterotrophic bacteria (THB)** | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **P1** | | | **P2** | | | **P3** | | | **P4** | | | **P5** | | |
| | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^6$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^6$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^6$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^6$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^6$** |
| **10^5** | 14 | 2.80 | 2,8 | 11 | 2.20 | 2,2 | 7 | 1.40 | 1,4 | 10 | 2.00 | 2 | 17 | 3.40 | 3,4 |
| | 15 | 3.00 | 3 | 10 | 2.00 | 2 | 6 | 1.20 | 1,2 | 10 | 2.00 | 2 | 19 | 3.80 | 3,8 |
| **10^6** | 7 | 1.40 | 14 | 1 | 2.00 | 2.00 | 0 | 0.00 | 0.00 | 1 | 2.00 | 2.00 | 3 | 6.00 | 6.00 |
| | 7 | 1.40 | 14 | 0 | 0.00 | 0.00 | 0 | 0.00 | 0.00 | 1 | 2.00 | 2.00 | 4 | 8.00 | 8.00 |
| **Average** | | | 2,9 | | | 2,1 | | | 1,3 | | | 2 | | | 3,6 |
| **StDev** | | | 0,141 | | | 0,141 | | | 0,141 | | | 0 | | | 0,283 |
| ***E.coli* (EC)** | | | | | | | | | | | | | | | |
| | **P1** | | | **P2** | | | **P3** | | | **P4** | | | **P5** | | |
| | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** |
| **10^1** | 172 | 3.44 | 3,44 | 97 | 1.94 | 1,94 | 74 | 1.48 | 1,48 | 77 | 1.54 | 1,54 | 54 | 1.08 | 1,08 |
| | 175 | 3.50 | 3,5 | 92 | 1.84 | 1,84 | 78 | 1.56 | 1,56 | 71 | 1.42 | 1,42 | 41 | 8.20 | 0,82 |
| **10^2** | 33 | 6.60 | 6,6 | 19 | 3.80 | 3,8 | 32 | 6.40 | 6,4 | 48 | 9.60 | 9,6 | 27 | 5.40 | 5,4 |
| | 33 | 6.60 | 6,6 | 15 | 3.00 | 3 | 39 | 7.80 | 7,8 | 49 | 9.80 | 9,8 | 22 | 4.40 | 4,4 |
| **10^3** | 7 | 1.40 | 14 | 2 | 4.00 | 4 | 15 | 3.00 | 30 | 9 | 1.80 | 18 | 13 | 2.60 | 26 |
| | 2 | 4.00 | 4 | 1 | 2.00 | 2 | 10 | 2.00 | 20 | 8 | 1.60 | 16 | 15 | 3.00 | 30 |
| **Average** | | | 3,47 | | | 3,4 | | | 1,52 | | | 1,48 | | | 0,95 |
| **StDev** | | | 0,042 | | | 0,566 | | | 0,057 | | | 0,085 | | | 0,184 |
| **Total coliforms (TC)** | | | | | | | | | | | | | | | |
| | **P1** | | | **P2** | | | **P3** | | | **P4** | | | **P5** | | |
| | **Count** | **cfu/100mℓ** | **(cfu/100mℓ)** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ)** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ)** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ)** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ)** |

| | | | x $10^3$ | | | x $10^3$ | | | x $10^3$ | | | x $10^3$ | | | x $10^3$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **10^1** | 52 | 1.04 | 1,04 | 72 | 1.44 | 1,44 | 82 | 1.64 | 1,64 | 53 | 1.06 | 1,06 | 48 | 9.60 | 0,96 |
| | 61 | 1.22 | 1,22 | 66 | 1.32 | 1,32 | 84 | 1.68 | 1,68 | 62 | 1.24 | 1,24 | 49 | 9.80 | 0,98 |
| **10^2** | 35 | 7.00 | 7 | 8 | 1.60 | 1,6 | 13 | 2.60 | 2,6 | 20 | 4.00 | 4 | 28 | 5.60 | 5,6 |
| | 33 | 6.60 | 6,6 | 8 | 1.60 | 1,6 | 15 | 3.00 | 3 | 21 | 4.20 | 4,2 | 23 | 4.60 | 4,6 |
| **10^3** | 1 | 2000 | 2 | 2 | 4000 | 4 | 9 | 18000 | 18 | 2 | 4000 | 4 | 18 | 36000 | 36 |
| | 2 | 4000 | 4 | 1 | 2000 | 2 | 4 | 8000 | 8 | 8 | 16000 | 16 | 14 | 28000 | 28 |
| **Average** | | | 1,13 | | | 1,6 | | | 2,8 | | | 1,15 | | | 0,97 |
| **StDev** | | | 0,127 | | | 0 | | | 0,283 | | | 0,127 | | | 0,014 |
| **Faecal coliforms (FC)** | | | | | | | | | | | | | | | |
| | **P1** | | | **P2** | | | **P3** | | | **P4** | | | **P5** | | |
| | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** |
| **10^1** | 96 | 1.92 | 1,92 | 82 | 1.64 | 1,64 | 68 | 1.36 | 1,36 | 108 | 2.16 | 2,16 | 86 | 1.72 | 1,72 |
| | 96 | 1.92 | 1,92 | 80 | 1.60 | 1,6 | 62 | 1.24 | 1,24 | 112 | 2.24 | 2,24 | 90 | 1.80 | 1,8 |
| **10^2** | 34 | 6.80 | 6,8 | 56 | 1.12 | 11,2 | 46 | 9.20 | 9,2 | 38 | 7.60 | 7,6 | 49 | 9.80 | 9,8 |
| | 35 | 7.00 | 7 | 52 | 1.04 | 10,4 | 42 | 8.40 | 8,4 | 32 | 6.40 | 6,4 | 45 | 9.00 | 9 |
| **10^3** | 10 | 2.00 | 20 | 7 | 1.40 | 14 | 25 | 5.00 | 50 | 10 | 2.00 | 20 | 14 | 2.80 | 28 |
| | 14 | 2.80 | 28 | 9 | 1.80 | 18 | 25 | 5.00 | 50 | 13 | 2.60 | 26 | 12 | 2.40 | 24 |
| **Average** | | | 1,92 | | | 1,62 | | | 1,3 | | | 2,2 | | | 1,76 |
| **StDev** | | | 0 | | | 0,028 | | | 0,085 | | | 0,057 | | | 0,057 |
| **Faecal streptococci (FS)** | | | | | | | | | | | | | | | |
| | **P1** | | | **P2** | | | **P3** | | | **P4** | | | **P5** | | |
| | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** |
| **10^1** | 64 | 1.28 | 1,28 | 118 | 2.36 | 2,36 | 31 | 6.20 | 0,62 | 95 | 1.90 | 1,9 | 40 | 8.00 | 0,8 |
| | 87 | 1.74 | 1,74 | 170 | 3.40 | 3,4 | 38 | 7.60 | 0,76 | 98 | 1.96 | 1,96 | 65 | 1.30 | 1,3 |
| **10^2** | 34 | 6.80 | 6,8 | 22 | 4.40 | 4,4 | 14 | 2.80 | 2,8 | 15 | 3.00 | 3 | 11 | 2.20 | 2,2 |
| | 33 | 6.60 | 6,6 | 28 | 5.60 | 5,6 | 13 | 2.60 | 2,6 | 14 | 2.80 | 2,8 | 14 | 2.80 | 2,8 |
| **10^3** | 8 | 1.60 | 16 | 7 | 1.40 | 14 | 1 | 2.00 | 2 | 4 | 8.00 | 8 | 0 | 0.00 | 0 |
| | 3 | 6.00 | 6 | 6 | 1.20 | 12 | 0 | 0.00 | 0 | 4 | 8.00 | 8 | 1 | 2.00 | 2 |
| **Average** | | | 1,51 | | | 2,88 | | | 2,7 | | | 2,9 | | | 2,5 |
| **StDev** | | | 0,325 | | | 0,735 | | | 0,141 | | | 0,141 | | | 0,424 |
| ***Vibrio* species (VIB spp.)** | | | | | | | | | | | | | | | |
| | **P1** | | | **P2** | | | **P3** | | | **P4** | | | **P5** | | |
| | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** |
| **10^1** | 64 | 1.28 | 1,28 | 30 | 6.00 | 0,6 | 84 | 1.68 | 1,68 | 44 | 8.80 | 0,88 | 92 | 1.84 | 1,84 |

| | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 60 | 1.200 | 1,2 | 31 | 6.20 | 0,62 | 63 | 1.26 | 1,26 | 44 | 8.80 | 0,88 | 93 | 1.86 | 1,86 |
| **10^2** | 32 | 6.40 | 6,4 | 13 | 2.60 | 2,6 | 44 | 8.80 | 8,8 | 25 | 5.00 | 5 | 25 | 5.00 | 5 |
| | 31 | 6.20 | 6,2 | 18 | 3.60 | 3,6 | 51 | 1.02 | 10,2 | 28 | 5.60 | 5,6 | 27 | 5.40 | 5,4 |
| **10^3** | 0 | 0.00 | 0 | 1 | 2.00 | 2 | 22 | 4.40 | 44 | 10 | 2.00 | 20 | 15 | 3.00 | 30 |
| | 3 | 6.00 | 6 | 0 | 0.00 | 0 | 27 | 5.40 | 54 | 2 | 4.00 | 4 | 4 | 8.00 | 8 |
| **Average** | | | 1,24 | | | 2,3 | | | 1,47 | | | 0,88 | | | 1,85 |
| **StDev** | | | 0,057 | | | 0,424 | | | 0,297 | | | 0 | | | 0,014 |
| ***Salmonella* species (SAL spp.)** | | | | | | | | | | | | | | | |
| | **P1** | | | **P2** | | | **P3** | | | **P4** | | | **P5** | | |
| | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** |
| **10^1** | 42 | 8.40 | 0,84 | 39 | 7.80 | 0,78 | 39 | 7.80 | 0,78 | 54 | 1.08 | 1,08 | 23 | 4.60 | 0,46 |
| | 40 | 8.00 | 0,8 | 48 | 9.60 | 0,96 | 47 | 9.40 | 0,94 | 62 | 1.24 | 1,24 | 26 | 5.20 | 0,52 |
| **10^2** | 22 | 4.40 | 4,4 | 24 | 4.80 | 4,8 | 22 | 4.40 | 4,4 | 32 | 6.40 | 6,4 | 10 | 2.00 | 2 |
| | 24 | 4.80 | 4,8 | 25 | 5.00 | 5 | 22 | 4.40 | 4,4 | 31 | 6.20 | 6,2 | 15 | 3.00 | 3 |
| **10^3** | 7 | 1.40 | 14 | 0 | 0.00 | 0 | 12 | 2.40 | 24 | 2 | 4.00 | 4 | 0 | 0.00 | 0 |
| | 3 | 6.00 | 6 | 1 | 2.00 | 2 | 30 | 6.00 | 60 | 16 | 3.20 | 32 | 10 | 2.00 | 20 |
| **Average** | | | 0,82 | | | 0,87 | | | 0,86 | | | 1,16 | | | 0,49 |
| **StDev** | | | 0,028 | | | 0,127 | | | 0,113 | | | 0,113 | | | 0,042 |
| ***Shigella* species (SHIG spp.)** | | | | | | | | | | | | | | | |
| | **P1** | | | **P2** | | | **P3** | | | **P4** | | | **P5** | | |
| | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** |
| **10^1** | 50 | 1.00 | 1 | 42 | 8.40 | 0,84 | 48 | 9.60 | 0,96 | 32 | 6.40 | 0,64 | 48 | 9.60 | 0,96 |
| | 50 | 1.00 | 1 | 40 | 8.00 | 0,8 | 49 | 9.80 | 0,98 | 37 | 7.40 | 0,74 | 47 | 9.40 | 0,94 |
| **10^2** | 18 | 3.60 | 3,6 | 22 | 4.40 | 4,4 | 19 | 3.80 | 3,8 | 20 | 4.00 | 4 | 32 | 6.40 | 6,4 |
| | 17 | 3.40 | 3,4 | 22 | 4.40 | 4,4 | 20 | 4.00 | 4 | 22 | 4.40 | 4,4 | 30 | 6.00 | 6 |
| **10^3** | 2 | 4.00 | 4 | 0 | 0.00 | 0 | 12 | 2.40 | 24 | 0 | 0.00 | 0 | 7 | 1.40 | 14 |
| | 0 | 0.00 | 0 | 0 | 0.00 | 0 | 1 | 2.00 | 2 | 0 | 0.00 | 0 | 12 | 2.40 | 24 |
| **Average** | | | 1 | | | 0,82 | | | 0,97 | | | 0,69 | | | 0,95 |
| **StDev** | | | 0 | | | 0,028 | | | 0,014 | | | 0,071 | | | 0,014 |

**Table 11** Presumptive bacterial indicator counts at all sampling points during August 2014.

| **Total heterotrophic bacteria (THB)** | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **P1** | | | **P2** | | | **P3** | | | **P4** | | | **P5** | | |
| | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^6$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^6$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^6$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^6$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^6$** |

| | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **10^5** | 19 | 3.80 | 3,8 | 14 | 2.80 | 2,8 | 9 | 1.80 | 1,8 | 13 | 2.60 | 2,6 | 16 | 3.20 | 3,2 |
| | 19 | 3.80 | 3,8 | 15 | 3.00 | 3 | 7 | 1.40 | 1,4 | 10 | 2.00 | 2 | 19 | 3.80 | 3,8 |
| **10^6** | 3 | 6.00 | 6.00 | 2 | 4.00 | 4.00 | 0 | 0.00 | 0.00 | 0 | 0.00 | 0.00 | 2 | 4.00 | 4.00 |
| | 4 | 8.00 | 8.00 | 1 | 2.00 | 2.00 | 1 | 2.00 | 2.00 | 0 | 0.00 | 0.00 | 2 | 4.00 | 4.00 |
| **Average** | | | 3,8 | | | 2,9 | | | 1,6 | | | 2,3 | | | 3,5 |
| **StDev** | | | 0 | | | 0,141 | | | 0,283 | | | 0,424 | | | 0,424 |
| ***E.coli* (EC)** | | | | | | | | | | | | | | | |
| | **P1** | | | **P2** | | | **P3** | | | **P4** | | | **P5** | | |
| | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** |
| **10^1** | 99 | 1.98 | 1,98 | 77 | 1.54 | 1,54 | 61 | 1.22 | 1,22 | 40 | 8.00 | 0,8 | 51 | 1.02 | 1,02 |
| | 101 | 2.02 | 2,02 | 72 | 1.44 | 1,44 | 60 | 1.20 | 1,2 | 47 | 9.40 | 0,94 | 59 | 1.18 | 1,18 |
| **10^2** | 50 | 1.00 | 10 | 35 | 7.00 | 7 | 47 | 9.40 | 9,4 | 28 | 5.60 | 5,6 | 33 | 6.60 | 6,6 |
| | 51 | 1.02 | 10,2 | 40 | 8.00 | 8 | 40 | 8.00 | 8 | 23 | 4.60 | 4,6 | 35 | 7.00 | 7 |
| **10^3** | 23 | 4.60 | 46 | 19 | 3.80 | 38 | 23 | 4.60 | 46 | 14 | 2.80 | 28 | 19 | 3.80 | 38 |
| | 20 | 4.00 | 40 | 14 | 2.80 | 28 | 16 | 3.20 | 32 | 9 | 1.80 | 18 | 14 | 2.80 | 28 |
| **Average** | | | 2 | | | 1,49 | | | 1,21 | | | 0,87 | | | 1,1 |
| **StDev** | | | 0,028 | | | 0,071 | | | 0,014 | | | 0,099 | | | 0,113 |
| **Total coliforms (TC)** | | | | | | | | | | | | | | | |
| | **P1** | | | **P2** | | | **P3** | | | **P4** | | | **P5** | | |
| | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** |
| **10^1** | 87 | 1.74 | 1,74 | 50 | 1.00 | 1 | 32 | 6.40 | 0,64 | 49 | 9.80 | 0,98 | 57 | 1.14 | 1,14 |
| | 88 | 1.76 | 1,76 | 57 | 1.14 | 1,14 | 31 | 6.20 | 0,62 | 42 | 8.40 | 0,84 | 60 | 1.20 | 1,2 |
| **10^2** | 17 | 3.40 | 3,4 | 22 | 4.40 | 4,4 | 19 | 3.80 | 3,8 | 13 | 2.60 | 2,6 | 33 | 6.60 | 6,6 |
| | 16 | 3.20 | 3,2 | 23 | 4.60 | 4,6 | 21 | 4.20 | 4,2 | 11 | 2.20 | 2,2 | 33 | 6.60 | 6,6 |
| **10^3** | 4 | 8.00 | 8 | 4 | 8.00 | 8 | 4 | 8.00 | 8 | 3 | 6.00 | 6 | 11 | 2.20 | 22 |
| | 4 | 8.00 | 8 | 9 | 1.80 | 18 | 0 | 0.00 | 0 | 0 | 0.00 | 0 | 18 | 3.60 | 36 |
| **Average** | | | 1,75 | | | 1,07 | | | 0,63 | | | 0,91 | | | 1,17 |
| **StDev** | | | 0,014 | | | 0,099 | | | 0,014 | | | 0,099 | | | 0,042 |
| **Faecal coliforms (FC)** | | | | | | | | | | | | | | | |
| | **P1** | | | **P2** | | | **P3** | | | **P4** | | | **P5** | | |
| | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** |
| **10^1** | 45 | 9.0 | 0,9 | 43 | 8.60 | 0,86 | 32 | 6.40 | 0,64 | 40 | 8.00 | 0,8 | 49 | 9.80 | 0,98 |
| | 65 | 1.30 | 1,3 | 48 | 9.60 | 0,96 | 37 | 7.40 | 0,74 | 41 | 8.20 | 0,82 | 42 | 8.40 | 0,84 |
| **10^2** | 16 | 3.20 | 3,2 | 13 | 2.60 | 2,6 | 26 | 5.20 | 5,2 | 17 | 3.40 | 3,4 | 27 | 5.40 | 5,4 |
| | 21 | 4.20 | 4,2 | 11 | 2.20 | 2,2 | 19 | 3.80 | 3,8 | 13 | 2.60 | 2,6 | 22 | 4.40 | 4,4 |

| | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **10^3** | 2 | 4.00 | 4 | 7 | 1.40 | 14 | 4 | 8.00 | 8 | 5 | 1.00 | 10 | 5 | 1.00 | 10 |
| | 2 | 4.00 | 4 | 3 | 6.00 | 6 | 5 | 1.00 | 10 | 3 | 6.00 | 6 | 0 | 0.00 | 0 |
| **Average** | | | 1,1 | | | 0,91 | | | 0,69 | | | 0,81 | | | 0,91 |
| **StDev** | | | 0,283 | | | 0,071 | | | 0,071 | | | 0,014 | | | 0,099 |
| **Faecal streptococci (FS)** | | | | | | | | | | | | | | | |
| | **P1** | | | **P2** | | | **P3** | | | **P4** | | | **P5** | | |
| | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** |
| **10^1** | 40 | 8.00 | 0,8 | 52 | 1.04 | 1,04 | 30 | 6.00 | 0,6 | 37 | 7.40 | 0,74 | 42 | 8.40 | 0,84 |
| | 41 | 8.20 | 0,82 | 47 | 9.40 | 0,94 | 22 | 4.40 | 0,44 | 18 | 3.60 | 0,36 | 43 | 8.60 | 0,86 |
| **10^2** | 17 | 3.40 | 3,4 | 26 | 5.20 | 5,2 | 9 | 1.80 | 1,8 | 20 | 4.00 | 4 | 11 | 2.20 | 2,2 |
| | 14 | 2.80 | 2,8 | 23 | 4.60 | 4,6 | 6 | 1.20 | 1,2 | 21 | 4.20 | 4,2 | 10 | 2.00 | 2 |
| **10^3** | 6 | 1.20 | 12 | 8 | 1.60 | 16 | 0 | 0.00 | 0 | 1 | 2.00 | 2 | 3 | 6.00 | 6 |
| | 1 | 2.00 | 2 | 9 | 1.80 | 18 | 2 | 4.00 | 4 | 1 | 2.00 | 2 | 2 | 4.00 | 4 |
| **Average** | | | 0,81 | | | 0,99 | | | 0,52 | | | 0,55 | | | 0,85 |
| **StDev** | | | 0,014 | | | 0,071 | | | 0,113 | | | 0,269 | | | 0,014 |
| ***Vibrio* species (VIB spp.)** | | | | | | | | | | | | | | | |
| | **P1** | | | **P2** | | | **P3** | | | **P4** | | | **P5** | | |
| | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** |
| **10^1** | 115 | 2.30 | 2,3 | 88 | 1.76 | 1,76 | 96 | 1.92 | 1,92 | 88 | 1.76 | 1,76 | 110 | 2.20 | 2,2 |
| | 103 | 2.06 | 2,06 | 96 | 1.92 | 1,92 | 94 | 1.88 | 1,88 | 74 | 1.48 | 1,48 | 112 | 2.24 | 2,24 |
| **10^2** | 23 | 4.60 | 4,6 | 45 | 9.00 | 9 | 21 | 4.20 | 4,2 | 17 | 3.40 | 3,4 | 63 | 1.26 | 12,6 |
| | 36 | 7.20 | 7,2 | 41 | 8.20 | 8,2 | 22 | 4.40 | 4,4 | 12 | 2.40 | 2,4 | 68 | 1.36 | 13,6 |
| **10^3** | 11 | 2.20 | 22 | 19 | 3.80 | 38 | 6 | 1.20 | 12 | 4 | 8.00 | 8 | 23 | 4.60 | 46 |
| | 6 | 1.20 | 12 | 14 | 2.80 | 28 | 7 | 1.40 | 14 | 9 | 1.80 | 18 | 24 | 4.80 | 48 |
| **Average** | | | 2,18 | | | 1,84 | | | 1,9 | | | 1,62 | | | 2,22 |
| **StDev** | | | 0,170 | | | 0,113 | | | 0,028 | | | 0,198 | | | 0,028 |
| ***Salmonella* species (SAL spp.)** | | | | | | | | | | | | | | | |
| | **P1** | | | **P2** | | | **P3** | | | **P4** | | | **P5** | | |
| | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** |
| **10^1** | 37 | 7.40 | 0,74 | 21 | 4.20 | 0,42 | 20 | 4.00 | 0,4 | 15 | 3.00 | 0,3 | 12 | 2.40 | 0,24 |
| | 31 | 6.20 | 0,62 | 29 | 5.80 | 0,58 | 21 | 4.20 | 0,42 | 12 | 2.40 | 0,24 | 18 | 3.60 | 0,36 |
| **10^2** | 15 | 3.00 | 3 | 19 | 3.80 | 3,8 | 13 | 2.60 | 2,6 | 4 | 8.00 | 0,8 | 0 | 0.00 | 0 |
| | 7 | 1.40 | 1,4 | 16 | 3.20 | 3,2 | 11 | 2.20 | 2,2 | 7 | 1.40 | 1,4 | 6 | 1.20 | 1,2 |
| **10^3** | 0 | 0.00 | 0 | 2 | 4.00 | 4 | 0 | 0.00 | 0 | 1 | 2.00 | 2 | 0 | 0.00 | 0 |
| | 0 | 0.00 | 0 | 0 | 0.00 | 0 | 0 | 0.00 | 0 | 0 | 0.00 | 0 | 0 | 0.00 | 0 |

| | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Average** | | | 0,68 | | | 0,5 | | | 0,41 | | | 0,27 | | | 0,3 |
| **StDev** | | | 0,085 | | | 0,113 | | | 0,014 | | | 0,042 | | | 0,085 |
| ***Shigella* species (SHIG spp.)** | | | | | | | | | | | | | | | |
| | **P1** | | | **P2** | | | **P3** | | | **P4** | | | **P5** | | |
| | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** |
| **10^1** | 46 | 9.20 | 0,92 | 66 | 1.32 | 1,32 | 51 | 1.02 | 1,02 | 42 | 8.40 | 0,84 | 21 | 4.20 | 0,42 |
| | 54 | 1.08 | 1,08 | 36 | 7.20 | 0,72 | 43 | 8.60 | 0,86 | 40 | 8.00 | 0,8 | 22 | 4.40 | 0,44 |
| **10^2** | 20 | 4.00 | 4 | 40 | 8.00 | 8 | 21 | 4.20 | 4,2 | 17 | 3.40 | 3,4 | 16 | 3.20 | 3,2 |
| | 20 | 4.00 | 4 | 32 | 6.40 | 6,4 | 18 | 3.60 | 3,6 | 19 | 3.80 | 3,8 | 14 | 2.80 | 2,8 |
| **10^3** | 9 | 1.80 | 18 | 10 | 2.00 | 20 | 13 | 2.60 | 26 | 5 | 1.00 | 10 | 2 | 4.00 | 4 |
| | 1 | 2.00 | 2 | 6 | 1.20 | 12 | 9 | 1.80 | 18 | 2 | 4.00 | 4 | 1 | 2.00 | 2 |
| **Average** | | | 1 | | | 1,02 | | | 0,94 | | | 0,82 | | | 0,43 |
| **StDev** | | | 0,113 | | | 0,424 | | | 0,113 | | | 0,028 | | | 0,014 |

**Table 12** Presumptive bacterial indicator counts at all sampling points during September 2014.

| **Total heterotrophic bacteria (THB)** | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **P1** | | | **P2** | | | **P3** | | | **P4** | | | **P5** | | |
| | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^6$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^6$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^6$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^6$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^6$** |
| **10^5** | 29 | 5.80 | 5,8 | 28 | 5.60 | 5,6 | 19 | 3.80 | 3,8 | 24 | 4.80 | 4,8 | 19 | 3.80 | 3,8 |
| | 29 | 5.80 | 5,8 | 26 | 5.20 | 5,2 | 17 | 3.40 | 3,4 | 23 | 4.60 | 4,6 | 21 | 4.20 | 4,2 |
| **10^6** | 7 | 1.40 | 14 | 11 | 2.00 | 20 | 9 | 1.80 | 18 | 13 | 2.60 | 26 | 12 | 2.40 | 24 |
| | 7 | 1.40 | 14 | 10 | 2.00 | 20 | 8 | 1.60 | 16 | 13 | 2.60 | 26 | 11 | 2.20 | 22 |
| **Average** | | | 5,8 | | | 5,4 | | | 3,6 | | | 4,7 | | | 4 |
| **StDev** | | | 0 | | | 0,283 | | | 0,283 | | | 0,141 | | | 0,283 |
| ***E.coli* (EC)** | | | | | | | | | | | | | | | |
| | **P1** | | | **P2** | | | **P3** | | | **P4** | | | **P5** | | |
| | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** |
| **10^1** | 72 | 1.44 | 1,44 | 83 | 1.66 | 1,66 | 88 | 1.76 | 1,76 | 47 | 9.40 | 0,94 | 51 | 1.02 | 1,02 |
| | 79 | 1.58 | 1,58 | 94 | 1.88 | 1,88 | 92 | 1.84 | 1,84 | 49 | 9.80 | 0,98 | 56 | 1.12 | 1,12 |
| **10^2** | 41 | 8.20 | 8,2 | 14 | 2.80 | 2,8 | 33 | 6.60 | 6,6 | 24 | 4.80 | 4,8 | 30 | 6.00 | 6 |
| | 35 | 7.00 | 7 | 13 | 2.60 | 2,6 | 21 | 4.20 | 4,2 | 22 | 4.40 | 4,4 | 31 | 6.20 | 6,2 |
| **10^3** | 7 | 1.40 | 14 | 5 | 1.00 | 10 | 8 | 1.60 | 16 | 7 | 1.40 | 14 | 6 | 1.20 | 12 |
| | 2 | 4.00 | 4 | 2 | 4.00 | 4 | 10 | 2.00 | 20 | 5 | 1.00 | 10 | 1 | 2.00 | 2 |
| **Average** | | | 1,51 | | | 2,7 | | | 1,8 | | | 0,96 | | | 1,07 |

| | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **StDev** | | | 0,099 | | | 0,141 | | | 0,057 | | | 0,028 | | | 0,071 |
| **Total coliforms (TC)** | | | | | | | | | | | | | | | |
| | **P1** | | | **P2** | | | **P3** | | | **P4** | | | **P5** | | |
| | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** |
| **10^1** | 78 | 1.56 | 1,56 | 53 | 1.06 | 1,06 | 44 | 8.80 | 0,88 | 49 | 9.80 | 0,98 | 26 | 5.20 | 0,52 |
| | 83 | 1.66 | 1,66 | 59 | 1.18 | 1,18 | 43 | 8.60 | 0,86 | 32 | 6.40 | 0,64 | 19 | 3.80 | 0,38 |
| **10^2** | 37 | 7.40 | 7,4 | 15 | 3.00 | 3 | 17 | 3.40 | 3,4 | 22 | 4.40 | 4,4 | 10 | 2.00 | 2 |
| | 34 | 6.80 | 6,8 | 19 | 3.80 | 3,8 | 13 | 2.60 | 2,6 | 16 | 3.20 | 3,2 | 10 | 2.00 | 2 |
| **10^3** | 10 | 2.00 | 20 | 7 | 1.40 | 14 | 0 | 0.00 | 0 | 4 | 8.00 | 8 | 6 | 1.20 | 12 |
| | 11 | 2.20 | 22 | 0 | 0.00 | 0 | 5 | 1.00 | 10 | 6 | 1.20 | 12 | 1 | 2.00 | 2 |
| **Average** | | | 1,61 | | | 1,12 | | | 0,87 | | | 0,81 | | | 2 |
| **StDev** | | | 0,071 | | | 0,085 | | | 0,014 | | | 0,240 | | | 0,099 |
| **Faecal coliforms (FC)** | | | | | | | | | | | | | | | |
| | **P1** | | | **P2** | | | **P3** | | | **P4** | | | **P5** | | |
| | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** |
| **10^1** | 50 | 1.00 | 1 | 42 | 8.40 | 0,84 | 19 | 3.80 | 0,38 | 32 | 6.40 | 0,64 | 41 | 8.20 | 0,82 |
| | 69 | 1.38 | 1,38 | 37 | 7.40 | 0,74 | 17 | 3.40 | 0,34 | 34 | 6.80 | 0,68 | 40 | 8.00 | 0,8 |
| **10^2** | 12 | 2.40 | 2,4 | 28 | 5.60 | 5,6 | 2 | 4.00 | 0,4 | 16 | 3.20 | 3,2 | 22 | 4.40 | 4,4 |
| | 11 | 2.20 | 2,2 | 23 | 4.60 | 4,6 | 3 | 6.00 | 0,6 | 8 | 1.60 | 1,6 | 19 | 3.80 | 3,8 |
| **10^3** | 5 | 1.00 | 10 | 9 | 1.80 | 18 | 0 | 0.00 | 0 | 2 | 4.00 | 4 | 10 | 2.00 | 20 |
| | 4 | 8.00 | 8 | 2 | 4.00 | 4 | 1 | 2.00 | 2 | 1 | 2.00 | 2 | 7 | 1.40 | 14 |
| **Average** | | | 2,3 | | | 0,79 | | | 0,36 | | | 1,8 | | | 0,81 |
| **StDev** | | | 0,141 | | | 0,071 | | | 0,028 | | | 0,283 | | | 0,014 |
| **Faecal streptococci (FS)** | | | | | | | | | | | | | | | |
| | **P1** | | | **P2** | | | **P3** | | | **P4** | | | **P5** | | |
| | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** |
| **10^2** | 9 | 1.80 | 1,8 | 9 | 1.80 | 1,8 | 3 | 6.00 | 0,6 | 5 | 1.00 | 1 | 5 | 1.00 | 1 |
| | 13 | 2.60 | 2,6 | 14 | 2.80 | 2,8 | 1 | 2.00 | 0,2 | 1 | 2.00 | 0,2 | 5 | 1.00 | 1 |
| **10^3** | 1 | 2.00 | 2 | 2 | 4.00 | 4 | 0 | 0.00 | 0 | 0 | 0.00 | 0 | 0 | 0.00 | 0 |
| | 2 | 4.00 | 4 | 1 | 2.00 | 2 | 3 | 6.00 | 6 | 0 | 0.00 | 0 | 0 | 0.00 | 0 |
| **Average** | | | 2,2 | | | 2,3 | | | 0,4 | | | 0,6 | | | 1 |
| **StDev** | | | 0,566 | | | 0,707 | | | 0,283 | | | 0,566 | | | 0 |
| ***Vibrio* species (VIB spp.)** | | | | | | | | | | | | | | | |
| | **P1** | | | **P2** | | | **P3** | | | **P4** | | | **P5** | | |
| | **Count** | **cfu/100mℓ** | **(cfu/100mℓ)** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ)** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ)** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ)** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ)** |

| | | | x $10^3$ | | | x $10^3$ | | | x $10^3$ | | | x $10^3$ | | | x $10^3$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **10^1** | 123 | 2.46 | 2,46 | 130 | 2.60 | 2,6 | 49 | 9.80 | 0,98 | 108 | 2.16 | 2,16 | 55 | 1.10 | 1,1 |
| | 120 | 2.40 | 2,4 | 134 | 2.68 | 2,68 | 42 | 8.40 | 0,84 | 112 | 2.24 | 2,24 | 59 | 1.18 | 1,18 |
| **10^2** | 12 | 2.40 | 2,4 | 68 | 1.36 | 13,6 | 19 | 3.80 | 3,8 | 27 | 5.40 | 5,4 | 14 | 2.80 | 2,8 |
| | 23 | 4.60 | 4,6 | 79 | 1.58 | 15,8 | 19 | 3.80 | 3,8 | 67 | 1.34 | 13,4 | 17 | 3.40 | 3,4 |
| **10^3** | 0 | 0.00 | 0 | 18 | 3.60 | 36 | 11 | 2.20 | 22 | 16 | 3.20 | 32 | 6 | 1.20 | 12 |
| | 2 | 4.00 | 4 | 20 | 4.00 | 40 | 6 | 1.20 | 12 | 12 | 2.40 | 24 | 1 | 2.00 | 2 |
| **Average** | | | 2,43 | | | 2,64 | | | 3,8 | | | 2,2 | | | 3,1 |
| **StDev** | | | 0,042 | | | 0,057 | | | 0,099 | | | 0,057 | | | 0,057 |
| ***Salmonella* species (SAL spp.)** | | | | | | | | | | | | | | | |
| | **P1** | | | **P2** | | | **P3** | | | **P4** | | | **P5** | | |
| | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** |
| **10^2** | 20 | 4.00 | 4 | 6 | 1.20 | 1,2 | 9 | 1.80 | 1,8 | 12 | 2.40 | 2,4 | 7 | 1.40 | 1,4 |
| | 12 | 2.40 | 2,4 | 2 | 4.00 | 0,4 | 5 | 1.00 | 1 | 12 | 2.40 | 2,4 | 9 | 1.80 | 1,8 |
| **10^3** | 6 | 1.20 | 12 | 1 | 2.00 | 2 | 1 | 2.00 | 2 | 3 | 6.00 | 6 | 0 | 0.00 | 0 |
| | 2 | 4.00 | 4 | 0 | 0.00 | 0 | 0 | 0.00 | 0 | 1 | 2.00 | 2 | 0 | 0.00 | 0 |
| **Average** | | | 3,2 | | | 1,6 | | | 1,9 | | | 2,4 | | | 1,6 |
| **StDev** | | | 1,131 | | | 0,566 | | | 0,141 | | | 0 | | | 0,283 |
| ***Shigella* species (SHIG spp.)** | | | | | | | | | | | | | | | |
| | **P1** | | | **P2** | | | **P3** | | | **P4** | | | **P5** | | |
| | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** | **Count** | **cfu/100mℓ** | **(cfu/100mℓ) x $10^3$** |
| **10^2** | 14 | 2.80 | 2,8 | 18 | 3.60 | 3,2 | 13 | 2.60 | 2,6 | 18 | 3.60 | 3,6 | 5 | 1.00 | 1 |
| | 12 | 2.40 | 2,4 | 10 | 2.00 | 2 | 15 | 3.00 | 3 | 15 | 3.00 | 3 | 1 | 2.00 | 0,2 |
| **10^3** | 0 | 0.00 | 0 | 4 | 8.00 | 8 | 7 | 1.40 | 14 | 2 | 4.00 | 4 | 1 | 2.00 | 2 |
| | 1 | 2.00 | 2 | 2 | 4.00 | 4 | 7 | 1.40 | 14 | 3 | 6.00 | 6 | 0 | 0.00 | 0 |
| **Average** | | | 2,6 | | | 2,6 | | | 2,8 | | | 3,3 | | | 0,6 |
| **StDev** | | | 0,283 | | | 0,849 | | | 0,283 | | | 0,424 | | | 0,566 |

**Table 13 Somatic coliphage counts for all sampling points and months (October 2013 – September 2014).**

| **October_2013** | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **P1** | | | **P2** | | | **P3** | | | **P4** | | | **P5** | | |
| | **Count** | **pfu/mℓ** | **(pfu/mℓ) x $10^2$** | **Count** | **pfu/mℓ** | **(pfu/mℓ) x $10^2$** | **Count** | **pfu/mℓ** | **(pfu/mℓ) x $10^2$** | **Count** | **pfu/mℓ** | **(pfu/mℓ) x $10^2$** | **Count** | **pfu/mℓ** | **(pfu/mℓ) x $10^2$** |
| **10^2** | 48 | 4.8 | 48 | 43 | 4.30 | 43 | 34 | 3.40 | 34 | 44 | 4.40 | 44 | 51 | 5.10 | 51 |
| | 49 | 4.9 | 49 | 42 | 4.20 | 42 | 32 | 3.20 | 32 | 44 | 4.40 | 44 | 51 | 5.10 | 51 |

| | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **10^3** | 24 | 2.40 | 240 | 20 | 2.00 | 200 | 15 | 1.50 | 150 | 23 | 2.30 | 230 | 25 | 2.50 | 250 |
| | 21 | 2.10 | 210 | 20 | 2.00 | 200 | 19 | 1.90 | 190 | 24 | 2.40 | 240 | 29 | 2.90 | 290 |
| **Average** | | | 225 | | | 200 | | | 170 | | | 235 | | | 270 |
| **StDev** | | | 21,2 | | | 0 | | | 28,3 | | | 7,1 | | | 28,3 |
| **November_2013** | | | | | | | | | | | | | | | |
| | **P1** | | | **P2** | | | **P3** | | | **P4** | | | **P5** | | |
| | **Count** | **pfu/mℓ** | **(pfu/mℓ) x $10^2$** | **Count** | **pfu/mℓ** | **(pfu/mℓ) x $10^2$** | **Count** | **pfu/mℓ** | **(pfu/mℓ) x $10^2$** | **Count** | **pfu/mℓ** | **(pfu/mℓ) x $10^2$** | **Count** | **pfu/mℓ** | **(pfu/mℓ) x $10^2$** |
| **10^2** | 83 | 8.3 | 83 | 80 | 8.00 | 80 | 92 | 9.20 | 92 | 87 | 8.70 | 87 | 99 | 9.90 | 99 |
| | 85 | 8.50 | 85 | 80 | 8.00 | 80 | 98 | 9.80 | 98 | 89 | 8.90 | 89 | 99 | 9.90 | 99 |
| **10^3** | 46 | 4.60 | 460 | 40 | 4.00 | 400 | 37 | 3.70 | 370 | 49 | 4.90 | 490 | 51 | 5.10 | 510 |
| | 48 | 4.80 | 480 | 41 | 4.10 | 410 | 34 | 3.40 | 340 | 47 | 4.70 | 470 | 55 | 5.50 | 550 |
| **Average** | | | 470 | | | 405 | | | 355 | | | 480 | | | 530 |
| **StDev** | | | 14,1 | | | 7,1 | | | 21,2 | | | 14,1 | | | 28,3 |
| **December_2013** | | | | | | | | | | | | | | | |
| | **P1** | | | **P2** | | | **P3** | | | **P4** | | | **P5** | | |
| | **Count** | **pfu/mℓ** | **(pfu/mℓ) x $10^2$** | **Count** | **pfu/mℓ** | **(pfu/mℓ) x $10^2$** | **Count** | **pfu/mℓ** | **(pfu/mℓ) x $10^2$** | **Count** | **pfu/mℓ** | **(pfu/mℓ) x $10^2$** | **Count** | **pfu/mℓ** | **(pfu/mℓ) x $10^2$** |
| **10^2** | 151 | 1.51 | 151 | 112 | 1.12 | 112 | 112 | 1.12 | 112 | 133 | 1.33 | 133 | 142 | 1.42 | 142 |
| | 147 | 1.47 | 147 | 120 | 1.20 | 120 | 101 | 1.01 | 101 | 132 | 1.32 | 132 | 143 | 1.43 | 143 |
| **10^3** | 67 | 6.70 | 670 | 62 | 6.20 | 620 | 57 | 5.70 | 570 | 59 | 5.90 | 590 | 64 | 6.40 | 640 |
| | 64 | 6.40 | 640 | 62 | 6.20 | 620 | 54 | 5.40 | 540 | 61 | 6.10 | 610 | 63 | 6.30 | 630 |
| **Average** | | | 655 | | | 620 | | | 555 | | | 600 | | | 635 |
| **StDev** | | | 21,21 | | | 0 | | | 21,21 | | | 14,14 | | | 7,07 |
| **January_2014** | | | | | | | | | | | | | | | |
| | **P1** | | | **P2** | | | **P3** | | | **P4** | | | **P5** | | |
| | **Count** | **pfu/mℓ** | **(pfu/mℓ) x $10^2$** | **Count** | **pfu/mℓ** | **(pfu/mℓ) x $10^2$** | **Count** | **pfu/mℓ** | **(pfu/mℓ) x $10^2$** | **Count** | **pfu/mℓ** | **(pfu/mℓ) x $10^2$** | **Count** | **pfu/mℓ** | **(pfu/mℓ) x $10^2$** |
| **10^2** | 187 | 1.87 | 187 | 210 | 2.10 | 210 | 156 | 1.56 | 156 | 174 | 1.74 | 174 | 189 | 1.89 | 189 |
| | 174 | 1.74 | 174 | 212 | 2.12 | 212 | 156 | 1.56 | 156 | 171 | 1.71 | 171 | 180 | 1.80 | 180 |
| **10^3** | 79 | 7.90 | 790 | 67 | 6.70 | 670 | 54 | 5.40 | 540 | 61 | 6.10 | 610 | 72 | 7.20 | 720 |
| | 74 | 7.40 | 740 | 64 | 6.40 | 640 | 53 | 5.30 | 530 | 65 | 6.50 | 650 | 73 | 7.30 | 730 |
| **Average** | | | 765 | | | 655 | | | 535 | | | 630 | | | 725 |
| **StDev** | | | 35,36 | | | 21,21 | | | 7,07 | | | 28,28 | | | 7,07 |
| **February_2014** | | | | | | | | | | | | | | | |
| | **P1** | | | **P2** | | | **P3** | | | **P4** | | | **P5** | | |
| | **Count** | **pfu/mℓ** | **(pfu/mℓ) x $10^2$** | **Count** | **pfu/mℓ** | **(pfu/mℓ) x $10^2$** | **Count** | **pfu/mℓ** | **(pfu/mℓ) x $10^2$** | **Count** | **pfu/mℓ** | **(pfu/mℓ) x $10^2$** | **Count** | **pfu/mℓ** | **(pfu/mℓ) x $10^2$** |

| | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **10^2** | 143 | 1.43 | 143 | 138 | 1.38 | 138 | 92 | 9.20 | 92 | 187 | 1.87 | 187 | 122 | 1.22 | 122 |
| | 143 | 1.43 | 143 | 133 | 1.33 | 133 | 93 | 9.30 | 93 | 183 | 1.83 | 183 | 120 | 1.20 | 120 |
| **10^3** | 70 | 7.00 | 700 | 63 | 6.30 | 630 | 50 | 5.00 | 500 | 58 | 5.80 | 580 | 65 | 6.50 | 650 |
| | 69 | 6.90 | 690 | 62 | 6.20 | 620 | 50 | 5.00 | 500 | 54 | 5.40 | 540 | 66 | 6.60 | 660 |
| **Average** | | | 695 | | | 625 | | | 500 | | | 560 | | | 655 |
| **StDev** | | | 7,07 | | | 7,07 | | | 0 | | | 28,28 | | | 7,07 |
| **March_2014** | | | | | | | | | | | | | | | |
| | **P1** | | | **P2** | | | **P3** | | | **P4** | | | **P5** | | |
| | **Count** | **pfu/mℓ** | **(pfu/mℓ) x $10^2$** | **Count** | **pfu/mℓ** | **(pfu/mℓ) x $10^2$** | **Count** | **pfu/mℓ** | **(pfu/mℓ) x $10^2$** | **Count** | **pfu/mℓ** | **(pfu/mℓ) x $10^2$** | **Count** | **pfu/mℓ** | **(pfu/mℓ) x $10^2$** |
| **10^2** | 123 | 1.23 | 123 | 120 | 1.20 | 120 | 77 | 7.70 | 77 | 130 | 1.30 | 130 | 136 | 1.36 | 136 |
| | 121 | 1.21 | 121 | 123 | 1.23 | 123 | 79 | 7.90 | 79 | 127 | 1.27 | 127 | 138 | 1.38 | 138 |
| **10^3** | 60 | 6.00 | 600 | 59 | 5.90 | 590 | 48 | 4.80 | 480 | 53 | 5.30 | 530 | 57 | 5.70 | 570 |
| | 63 | 6.30 | 630 | 57 | 5.70 | 570 | 46 | 4.60 | 460 | 51 | 5.10 | 510 | 58 | 5.80 | 580 |
| **Average** | | | 615 | | | 580 | | | 470 | | | 520 | | | 575 |
| **StDev** | | | 21,21 | | | 14,14 | | | 14,14 | | | 14,14 | | | 7,07 |
| **April_2014** | | | | | | | | | | | | | | | |
| | **P1** | | | **P2** | | | **P3** | | | **P4** | | | **P5** | | |
| | **Count** | **pfu/mℓ** | **(pfu/mℓ) x $10^2$** | **Count** | **pfu/mℓ** | **(pfu/mℓ) x $10^2$** | **Count** | **pfu/mℓ** | **(pfu/mℓ) x $10^2$** | **Count** | **pfu/mℓ** | **(pfu/mℓ) x $10^2$** | **Count** | **pfu/mℓ** | **(pfu/mℓ) x $10^2$** |
| **10^2** | 113 | 1.13 | 113 | 123 | 1.23 | 123 | 116 | 1.16 | 116 | 127 | 1.27 | 127 | 130 | 1.30 | 130 |
| | 110 | 1.10 | 110 | 120 | 1.20 | 120 | 112 | 1.12 | 112 | 123 | 1.23 | 123 | 125 | 1.25 | 125 |
| **10^3** | 49 | 4.90 | 490 | 51 | 5.10 | 510 | 40 | 4.00 | 400 | 46 | 4.60 | 460 | 49 | 4.90 | 490 |
| | 45 | 4.50 | 450 | 52 | 5.20 | 520 | 41 | 4.10 | 410 | 43 | 4.30 | 430 | 48 | 4.80 | 480 |
| **Average** | | | 470 | | | 515 | | | 405 | | | 445 | | | 485 |
| **StDev** | | | 28,28 | | | 7,07 | | | 7,07 | | | 21,21 | | | 7,07 |
| **May_2014** | | | | | | | | | | | | | | | |
| | **P1** | | | **P2** | | | **P3** | | | **P4** | | | **P5** | | |
| | **Count** | **pfu/mℓ** | **(pfu/mℓ) x $10^2$** | **Count** | **pfu/mℓ** | **(pfu/mℓ) x $10^2$** | **Count** | **pfu/mℓ** | **(pfu/mℓ) x $10^2$** | **Count** | **pfu/mℓ** | **(pfu/mℓ) x $10^2$** | **Count** | **pfu/mℓ** | **(pfu/mℓ) x $10^2$** |
| **10^2** | 86 | 8.60 | 86 | 67 | 6.70 | 67 | 42 | 4.20 | 42 | 74 | 7.40 | 74 | 82 | 8.20 | 82 |
| | 87 | 8.70 | 87 | 69 | 6.90 | 69 | 40 | 4.00 | 40 | 79 | 7.90 | 79 | 83 | 8.30 | 83 |
| **10^3** | 38 | 3.80 | 380 | 36 | 3.60 | 360 | 27 | 2.70 | 270 | 33 | 3.30 | 330 | 36 | 3.60 | 360 |
| | 39 | 3.90 | 390 | 36 | 3.60 | 360 | 29 | 2.90 | 290 | 34 | 3.40 | 340 | 35 | 3.50 | 350 |
| **Average** | | | 385 | | | 360 | | | 280 | | | 335 | | | 355 |
| **StDev** | | | 7,07 | | | 0 | | | 14,14 | | | 7,07 | | | 7,07 |
| **June_2014** | | | | | | | | | | | | | | | |
| | **P1** | | | **P2** | | | **P3** | | | **P4** | | | **P5** | | |

| | Count | pfu/mℓ | (pfu/mℓ) x $10^2$ | Count | pfu/mℓ | (pfu/mℓ) x $10^2$ | Count | pfu/mℓ | (pfu/mℓ) x $10^2$ | Count | pfu/mℓ | (pfu/mℓ) x $10^2$ | Count | pfu/mℓ | (pfu/mℓ) x $10^2$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **10^2** | 76 | 7.60 | 76 | 65 | 6.50 | 65 | 65 | 6.50 | 65 | 69 | 6.90 | 69 | 63 | 6.30 | 63 |
| | 52 | 5.20 | 52 | 68 | 6.80 | 68 | 61 | 6.10 | 61 | 51 | 5.10 | 51 | 58 | 5.80 | 58 |
| **10^3** | 25 | 2.50 | 250 | 29 | 2.90 | 290 | 21 | 2.10 | 210 | 23 | 2.30 | 230 | 31 | 3.10 | 310 |
| | 26 | 2.60 | 260 | 30 | 3.00 | 300 | 22 | 2.20 | 220 | 27 | 2.70 | 270 | 33 | 3.30 | 330 |
| **Average** | | | 255 | | | 295 | | | 215 | | | 250 | | | 320 |
| **StDev** | | | 7,1 | | | 7,1 | | | 7,1 | | | 28,3 | | | 14,1 |
| **July_2014** | | | | | | | | | | | | | | | |
| | **P1** | | | **P2** | | | **P3** | | | **P4** | | | **P5** | | |
| | **Count** | **pfu/mℓ** | **(pfu/mℓ) x $10^2$** | **Count** | **pfu/mℓ** | **(pfu/mℓ) x $10^2$** | **Count** | **pfu/mℓ** | **(pfu/mℓ) x $10^2$** | **Count** | **pfu/mℓ** | **(pfu/mℓ) x $10^2$** | **Count** | **pfu/mℓ** | **(pfu/mℓ) x $10^2$** |
| **10^1** | 108 | 1.08 | 10,8 | 110 | 1.10 | 11 | 92 | 9.20 | 9,2 | 134 | 1.34 | 13,4 | 82 | 8.20 | 8,2 |
| | 114 | 1.14 | 11,4 | 107 | 1.07 | 10,7 | 93 | 9.30 | 9,3 | 133 | 1.30 | 13,3 | 83 | 8.30 | 8,3 |
| **10^2** | 70 | 7.00 | 70 | 65 | 6.50 | 65 | 52 | 5.20 | 52 | 61 | 6.10 | 61 | 68 | 6.80 | 68 |
| | 69 | 6.90 | 69 | 69 | 6.90 | 69 | 53 | 5.30 | 53 | 64 | 6.40 | 64 | 69 | 6.90 | 69 |
| **Average** | | | 69,5 | | | 67 | | | 52,5 | | | 62,5 | | | 68,5 |
| **StDev** | | | 0,71 | | | 2,83 | | | 0,71 | | | 2,12 | | | 0,71 |
| **August_2014** | | | | | | | | | | | | | | | |
| | **P1** | | | **P2** | | | **P3** | | | **P4** | | | **P5** | | |
| | **Count** | **pfu/mℓ** | **(pfu/mℓ) x $10^2$** | **Count** | **pfu/mℓ** | **(pfu/mℓ) x $10^2$** | **Count** | **pfu/mℓ** | **(pfu/mℓ) x $10^2$** | **Count** | **pfu/mℓ** | **(pfu/mℓ) x $10^2$** | **Count** | **pfu/mℓ** | **(pfu/mℓ) x $10^2$** |
| **10^1** | 88 | 8.80 | 8,8 | 71 | 7.10 | 7,1 | 55 | 5.50 | 5,5 | 69 | 6.9 | 6,9 | 62 | 6.20 | 6,2 |
| | 88 | 8.80 | 8,8 | 73 | 7.30 | 7,3 | 56 | 5.60 | 5,6 | 69 | 6.9 | 6,9 | 63 | 6.30 | 6,3 |
| **10^2** | 43 | 4.30 | 43 | 39 | 3.90 | 39 | 25 | 2.50 | 25 | 33 | 3.30 | 33 | 35 | 3.50 | 35 |
| | 40 | 4.00 | 40 | 37 | 3.70 | 37 | 24 | 2.40 | 24 | 36 | 3.60 | 36 | 35 | 3.50 | 35 |
| **Average** | | | 41,5 | | | 38 | | | 24,5 | | | 34,5 | | | 35 |
| **StDev** | | | 2,12 | | | 1,41 | | | 0,71 | | | 2,12 | | | 0 |
| **September_2014** | | | | | | | | | | | | | | | |
| | **P1** | | | **P2** | | | **P3** | | | **P4** | | | **P5** | | |
| | **Count** | **pfu/mℓ** | **(pfu/mℓ) x $10^2$** | **Count** | **pfu/mℓ** | **(pfu/mℓ) x $10^2$** | **Count** | **pfu/mℓ** | **(pfu/mℓ) x $10^2$** | **Count** | **pfu/mℓ** | **(pfu/mℓ) x $10^2$** | **Count** | **pfu/mℓ** | **(pfu/mℓ) x $10^2$** |
| **10^1** | 103 | 1.03 | 103 | 123 | 1.23 | 123 | 98 | 9.80 | 98 | 110 | 1.10 | 110 | 132 | 1.32 | 132 |
| | 117 | 1.17 | 117 | 123 | 1.23 | 123 | 94 | 9.40 | 94 | 112 | 1.12 | 112 | 140 | 1.40 | 140 |
| **10^2** | 61 | 6.10 | 61 | 65 | 6.50 | 65 | 54 | 5.40 | 54 | 59 | 5.90 | 59 | 67 | 6.70 | 67 |
| | 62 | 6.20 | 62 | 66 | 6.60 | 66 | 53 | 5.30 | 53 | 63 | 6.30 | 63 | 64 | 6.40 | 64 |
| **Average** | | | 61,5 | | | 65,5 | | | 53,5 | | | 61 | | | 65,5 |
| **StDev** | | | 0,7 | | | 0,7 | | | 0,7 | | | 2,8 | | | 2,1 |

**Table 14 $F^{+}$RNA coliphage counts for all sampling points and months (October 2013 – September 2014).**

| October_2013 | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | P1 | | | P2 | | | P3 | | | P4 | | | P5 | | |
| | Count | pfu/mℓ | (pfu/mℓ) x $10^2$ | Count | pfu/mℓ | (pfu/mℓ) x $10^2$ | Count | pfu/mℓ | (pfu/mℓ) x $10^2$ | Count | pfu/mℓ | (pfu/mℓ) x $10^2$ | Count | pfu/mℓ | (pfu/mℓ) x $10^2$ |
| 10^2 | 42 | 4.20 | 42 | 98 | 9.90 | 99 | 57 | 5.70 | 57 | 61 | 6.10 | 61 | 87 | 8.70 | 87 |
| | 48 | 4.80 | 48 | 99 | 9.90 | 99 | 54 | 5.40 | 54 | 63 | 6.30 | 63 | 89 | 8.90 | 89 |
| 10^3 | 14 | 1.40 | 140 | 12 | 1.20 | 120 | 8 | 8.00 | 80 | 10 | 1.00 | 100 | 16 | 1.60 | 160 |
| | 15 | 1.50 | 150 | 11 | 1.10 | 110 | 9 | 9.00 | 90 | 10 | 1.00 | 100 | 17 | 1.70 | 170 |
| Average | | | 145 | | | 115 | | | 85 | | | 100 | | | 165 |
| StDev | | | 7,1 | | | 7,1 | | | 7,1 | | | 0 | | | 7,1 |
| **November_2013** | | | | | | | | | | | | | | | |
| | P1 | | | P2 | | | P3 | | | P4 | | | P5 | | |
| | Count | pfu/mℓ | (pfu/mℓ) x $10^2$ | Count | pfu/mℓ | (pfu/mℓ) x $10^2$ | Count | pfu/mℓ | (pfu/mℓ) x $10^2$ | Count | pfu/mℓ | (pfu/mℓ) x $10^2$ | Count | pfu/mℓ | (pfu/mℓ) x $10^2$ |
| 10^2 | 143 | 1.43 | 143 | 121 | 1.21 | 121 | 87 | 8.70 | 87 | 73 | 7.30 | 73 | 110 | 1.10 | 110 |
| | 143 | 1.43 | 143 | 121 | 1.21 | 121 | 89 | 8.90 | 89 | 79 | 7.90 | 79 | 110 | 1.10 | 110 |
| 10^3 | 41 | 4.10 | 410 | 35 | 3.50 | 350 | 30 | 3.00 | 300 | 33 | 3.30 | 330 | 38 | 3.80 | 380 |
| | 39 | 3.90 | 390 | 35 | 3.50 | 350 | 32 | 3.20 | 320 | 31 | 3.10 | 310 | 34 | 3.40 | 340 |
| Average | | | 400 | | | 350 | | | 310 | | | 320 | | | 360 |
| StDev | | | 14,1 | | | 0 | | | 14,1 | | | 14,1 | | | 28,3 |
| **December_2013** | | | | | | | | | | | | | | | |
| | P1 | | | P2 | | | P3 | | | P4 | | | P5 | | |
| | Count | pfu/mℓ | (pfu/mℓ) x $10^2$ | Count | pfu/mℓ | (pfu/mℓ) x $10^2$ | Count | pfu/mℓ | (pfu/mℓ) x $10^2$ | Count | pfu/mℓ | (pfu/mℓ) x $10^2$ | Count | pfu/mℓ | (pfu/mℓ) x $10^2$ |
| 10^2 | 113 | 1.13 | 113 | 131 | 1.31 | 131 | 97 | 9.70 | 97 | 101 | 1.01 | 101 | 117 | 1.17 | 117 |
| | 120 | 1.20 | 120 | 128 | 1.28 | 128 | 92 | 9.20 | 92 | 94 | 9.40 | 94 | 119 | 1.19 | 119 |
| 10^3 | 50 | 5.00 | 500 | 53 | 5.30 | 530 | 42 | 4.20 | 420 | 46 | 4.60 | 460 | 52 | 5.20 | 520 |
| | 50 | 5.00 | 500 | 57 | 5.70 | 570 | 43 | 4.30 | 430 | 45 | 4.50 | 450 | 51 | 5.10 | 510 |
| Average | | | 500 | | | 550 | | | 425 | | | 455 | | | 515 |
| StDev | | | 0 | | | 28,28 | | | 7,07 | | | 7,07 | | | 7,07 |
| **January_2014** | | | | | | | | | | | | | | | |
| | P1 | | | P2 | | | P3 | | | P4 | | | P5 | | |
| | Count | pfu/mℓ | (pfu/mℓ) x $10^2$ | Count | pfu/mℓ | (pfu/mℓ) x $10^2$ | Count | pfu/mℓ | (pfu/mℓ) x $10^2$ | Count | pfu/mℓ | (pfu/mℓ) x $10^2$ | Count | pfu/mℓ | (pfu/mℓ) x $10^2$ |
| 10^2 | 148 | 1.48 | 148 | 148 | 1.38 | 138 | 115 | 1.15 | 115 | 132 | 1.32 | 132 | 158 | 1.58 | 158 |

| | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 149 | 1.48 | 149 | 132 | 1.38 | 132 | 115 | 1.15 | 115 | 132 | 1.32 | 132 | 160 | 1.60 | 160 |
| **10^3** | 59 | 5.90 | 590 | 54 | 5.40 | 540 | 49 | 4.90 | 490 | 53 | 5.30 | 530 | 57 | 5.70 | 570 |
| | 58 | 5.80 | 580 | 56 | 5.60 | 560 | 51 | 5.10 | 510 | 53 | 5.30 | 530 | 58 | 5.80 | 580 |
| **Average** | | | 585 | | | 550 | | | 500 | | | 530 | | | 575 |
| **StDev** | | | 7,07 | | | 14,14 | | | 14,14 | | | 0 | | | 7,07 |
| **February_2014** | | | | | | | | | | | | | | | |
| | **P1** | | | **P2** | | | **P3** | | | **P4** | | | **P5** | | |
| | **Count** | **pfu/mℓ** | **(pfu/mℓ) x $10^2$** | **Count** | **pfu/mℓ** | **(pfu/mℓ) x $10^2$** | **Count** | **pfu/mℓ** | **(pfu/mℓ) x $10^2$** | **Count** | **pfu/mℓ** | **(pfu/mℓ) x $10^2$** | **Count** | **pfu/mℓ** | **(pfu/mℓ) x $10^2$** |
| **10^2** | 100 | 1.00 | 100 | 87 | 8.70 | 87 | 61 | 6.10 | 61 | 82 | 8.20 | 82 | 97 | 9.70 | 97 |
| | 97 | 9.70 | 97 | 84 | 8.40 | 84 | 65 | 6.50 | 65 | 82 | 8.20 | 82 | 94 | 9.40 | 94 |
| **10^3** | 55 | 5.50 | 550 | 51 | 5.10 | 510 | 46 | 4.60 | 460 | 49 | 4.90 | 490 | 54 | 5.40 | 540 |
| | 56 | 5.60 | 560 | 53 | 5.30 | 530 | 42 | 4.20 | 420 | 47 | 4.70 | 470 | 54 | 5.40 | 540 |
| **Average** | | | 555 | | | 520 | | | 440 | | | 480 | | | 540 |
| **StDev** | | | 7,1 | | | 14,1 | | | 28,3 | | | 14,1 | | | 0 |
| **March_2014** | | | | | | | | | | | | | | | |
| | **P1** | | | **P2** | | | **P3** | | | **P4** | | | **P5** | | |
| | **Count** | **pfu/mℓ** | **(pfu/mℓ) x $10^2$** | **Count** | **pfu/mℓ** | **(pfu/mℓ) x $10^2$** | **Count** | **pfu/mℓ** | **(pfu/mℓ) x $10^2$** | **Count** | **pfu/mℓ** | **(pfu/mℓ) x $10^2$** | **Count** | **pfu/mℓ** | **(pfu/mℓ) x $10^2$** |
| **10^2** | 86 | 8.60 | 86 | 80 | 8.00 | 80 | 74 | 7.40 | 74 | 65 | 6.50 | 65 | 92 | 9.20 | 92 |
| | 86 | 8.60 | 86 | 81 | 8.10 | 81 | 68 | 6.80 | 68 | 65 | 6.50 | 65 | 61 | 6.10 | 61 |
| **10^3** | 46 | 4.60 | 460 | 40 | 4.00 | 400 | 35 | 3.50 | 350 | 39 | 3.90 | 390 | 48 | 4.80 | 480 |
| | 47 | 4.70 | 470 | 41 | 4.10 | 410 | 38 | 3.80 | 380 | 36 | 3.60 | 360 | 49 | 4.90 | 490 |
| **Average** | | | 465 | | | 405 | | | 365 | | | 375 | | | 485 |
| **StDev** | | | 7,1 | | | 7,1 | | | 21,2 | | | 21,2 | | | 7,1 |
| **April_2014** | | | | | | | | | | | | | | | |
| | **P1** | | | **P2** | | | **P3** | | | **P4** | | | **P5** | | |
| | **Count** | **pfu/mℓ** | **(pfu/mℓ) x $10^2$** | **Count** | **pfu/mℓ** | **(pfu/mℓ) x $10^2$** | **Count** | **pfu/mℓ** | **(pfu/mℓ) x $10^2$** | **Count** | **pfu/mℓ** | **(pfu/mℓ) x $10^2$** | **Count** | **pfu/mℓ** | **(pfu/mℓ) x $10^2$** |
| **10^2** | 72 | 7.20 | 72 | 58 | 5.80 | 58 | 41 | 4.10 | 41 | 47 | 4.70 | 47 | 60 | 6.00 | 60 |
| | 64 | 6.40 | 64 | 53 | 5.30 | 53 | 45 | 4.50 | 45 | 44 | 4.40 | 44 | 60 | 6.00 | 60 |
| **10^3** | 30 | 3.00 | 300 | 27 | 2.70 | 270 | 21 | 2.10 | 210 | 25 | 2.50 | 250 | 31 | 3.10 | 310 |
| | 30 | 3.00 | 300 | 28 | 2.80 | 280 | 23 | 2.30 | 230 | 25 | 2.50 | 250 | 33 | 3.30 | 330 |
| **Average** | | | 300 | | | 275 | | | 220 | | | 250 | | | 320 |
| **StDev** | | | 0 | | | 7,07 | | | 14,14 | | | 0 | | | 14,14 |
| **May_2014** | | | | | | | | | | | | | | | |
| | **P1** | | | **P2** | | | **P3** | | | **P4** | | | **P5** | | |
| | **Count** | **pfu/mℓ** | **(pfu/mℓ) x** | **Count** | **pfu/mℓ** | **(pfu/mℓ) x** | **Count** | **pfu/mℓ** | **(pfu/mℓ) x** | **Count** | **pfu/mℓ** | **(pfu/mℓ) x** | **Count** | **pfu/mℓ** | **(pfu/mℓ) x** |

| | | | $10^2$ | | | $10^2$ | | | $10^2$ | | | $10^2$ | | | $10^2$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **10^2** | 58 | 5.50 | 58 | 43 | 4.30 | 43 | 32 | 3.20 | 32 | 38 | 3.80 | 38 | 53 | 5.30 | 53 |
| | 58 | 5.80 | 58 | 41 | 4.10 | 41 | 32 | 3.20 | 32 | 39 | 3.90 | 39 | 39 | 3.90 | 39 |
| **10^3** | 23 | 2.30 | 230 | 24 | 2.40 | 240 | 14 | 1.40 | 140 | 19 | 1.90 | 190 | 24 | 2.40 | 240 |
| | 22 | 2.20 | 220 | 23 | 2.30 | 230 | 14 | 1.40 | 140 | 21 | 2.10 | 210 | 22 | 2.20 | 220 |
| **Average** | | | 225 | | | 235 | | | 140 | | | 200 | | | 230 |
| **StDev** | | | 7,07 | | | 7,07 | | | 0 | | | 14,14 | | | 14,14 |
| **June_2014** | | | | | | | | | | | | | | | |
| | **P1** | | | **P2** | | | **P3** | | | **P4** | | | **P5** | | |
| | **Count** | **pfu/mℓ** | **(pfu/mℓ) x $10^2$** | **Count** | **pfu/mℓ** | **(pfu/mℓ) x $10^2$** | **Count** | **pfu/mℓ** | **(pfu/mℓ) x $10^2$** | **Count** | **pfu/mℓ** | **(pfu/mℓ) x $10^2$** | **Count** | **pfu/mℓ** | **(pfu/mℓ) x $10^2$** |
| **10^2** | 38 | 3.80 | 38 | 28 | 2.80 | 28 | 17 | 1.70 | 17 | 25 | 2.51 | 25 | 23 | 2.30 | 23 |
| | 31 | 3.51 | 31 | 25 | 2.50 | 25 | 19 | 1.90 | 19 | 21 | 2.10 | 21 | 24 | 2.40 | 24 |
| **10^3** | 15 | 1.50 | 150 | 17 | 1.70 | 170 | 10 | 1.00 | 100 | 16 | 1.60 | 160 | 19 | 1.90 | 190 |
| | 17 | 1.70 | 170 | 18 | 1.80 | 180 | 11 | 1.10 | 110 | 16 | 1.60 | 160 | 21 | 2.10 | 210 |
| **Average** | | | 160 | | | 175 | | | 105 | | | 160 | | | 200 |
| **StDev** | | | 14,1 | | | 7,1 | | | 7,1 | | | 0 | | | 14,1 |
| **July_2014** | | | | | | | | | | | | | | | |
| | **P1** | | | **P2** | | | **P3** | | | **P4** | | | **P5** | | |
| | **Count** | **pfu/mℓ** | **(pfu/mℓ) x $10^2$** | **Count** | **pfu/mℓ** | **(pfu/mℓ) x $10^2$** | **Count** | **pfu/mℓ** | **(pfu/mℓ) x $10^2$** | **Count** | **pfu/mℓ** | **(pfu/mℓ) x $10^2$** | **Count** | **pfu/mℓ** | **(pfu/mℓ) x $10^2$** |
| **10^2** | 38 | 3.80 | 38 | 31 | 3.10 | 31 | 22 | 2.20 | 22 | 24 | 2.40 | 24 | 39 | 3.90 | 39 |
| | 37 | 3.70 | 37 | 34 | 3.40 | 34 | 28 | 2.80 | 28 | 26 | 2.60 | 26 | 39 | 3.90 | 39 |
| **Average** | | | 37,5 | | | 32,5 | | | 25 | | | 25 | | | 39 |
| **StDev** | | | 0,7 | | | 2,1 | | | 4,2 | | | 1,4 | | | 0 |
| **August_2014** | | | | | | | | | | | | | | | |
| | **P1** | | | **P2** | | | **P3** | | | **P4** | | | **P5** | | |
| | **Count** | **pfu/mℓ** | **(pfu/mℓ) x $10^2$** | **Count** | **pfu/mℓ** | **(pfu/mℓ) x $10^2$** | **Count** | **pfu/mℓ** | **(pfu/mℓ) x $10^2$** | **Count** | **pfu/mℓ** | **(pfu/mℓ) x $10^2$** | **Count** | **pfu/mℓ** | **(pfu/mℓ) x $10^2$** |
| **10^2** | 17 | 1.70 | 17 | 14 | 1.40 | 14 | 9 | 9.00 | 9 | 12 | 1.20 | 12 | 19 | 1.90 | 19 |
| | 16 | 1.60 | 16 | 14 | 1.40 | 14 | 11 | 1.10 | 11 | 14 | 1.40 | 14 | 21 | 2.10 | 21 |
| **Average** | | | 16,5 | | | 14 | | | 10 | | | 13 | | | 20 |
| **StDev** | | | 0,71 | | | 0 | | | 1,41 | | | 1,41 | | | 1,41 |
| **September_2014** | | | | | | | | | | | | | | | |
| | **P1** | | | **P2** | | | **P3** | | | **P4** | | | **P5** | | |
| | **Count** | **pfu/mℓ** | **(pfu/mℓ) x $10^2$** | **Count** | **pfu/mℓ** | **(pfu/mℓ) x $10^2$** | **Count** | **pfu/mℓ** | **(pfu/mℓ) x $10^2$** | **Count** | **pfu/mℓ** | **(pfu/mℓ) x $10^2$** | **Count** | **pfu/mℓ** | **(pfu/mℓ) x $10^2$** |
| **10^2** | 30 | 3.00 | 30 | 31 | 3.10 | 31 | 24 | 2.40 | 24 | 32 | 3.20 | 32 | 33 | 3.30 | 33 |

| | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 33 | 3.30 | 33 | 31 | 3.10 | 31 | 26 | 2.60 | 26 | 36 | 3.60 | 36 | 35 | 3.50 | 35 |
| **Average** | | | 31,5 | | | 31 | | | 25 | | | 34 | | | 34 |
| **StDev** | | | 2,12 | | | 0 | | | 1,41 | | | 2,83 | | | 1,41 |

**Table 15 Direct virus-like particle (VLP) counts from April 201 – September 2014.**

| **April_2014** | | | | | | **May_2014** | | | | | | **June_2014** | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **VLP counts** | **VLP/mℓ** | | | | | **VLP counts** | **VLP/mℓ** | | | | | **VLP counts** | **VLP/mℓ** | | | | |
| | **P1** | **P2** | **P3** | **P4** | **P5** | | **P1** | **P2** | **P3** | **P4** | **P5** | | **P1** | **P2** | **P3** | **P4** | **P5** |
| **a** | 1059 | 991 | 720 | 801 | 1011 | **a** | 856 | 973 | 713 | 802 | 630 | **a** | 552 | 524 | 413 | 492 | 530 |
| **b** | 997 | 872 | 832 | 899 | 983 | **b** | 793 | 942 | 766 | 839 | 613 | **b** | 601 | 486 | 446 | 549 | 493 |
| **c** | 1039 | 1013 | 749 | 947 | 1027 | **c** | 901 | 860 | 601 | 771 | 729 | **c** | 583 | 440 | 504 | 461 | 556 |
| **d** | 1109 | 1022 | 761 | 959 | 835 | **d** | 920 | 801 | 818 | 711 | 694 | **d** | 599 | 500 | 489 | 473 | 532 |
| **Average** | 1051 | 974,5 | 765,5 | 901,5 | 964 | **Average** | 867,5 | 894 | 724,5 | 780,75 | 666,5 | **Average** | 583,75 | 487,5 | 463 | 493,75 | 527,75 |
| **StDev** | 46,5 | 69,6 | 47,6 | 71,8 | 87,9 | **StDev** | 56,5 | 78,2 | 92,8 | 54,2 | 54,3 | **StDev** | 22,6 | 35,3 | 41,4 | 39,0 | 26,0 |
| **July_2014** | | | | | | **August_2014** | | | | | | **September_2014** | | | | | |
| **VLP counts** | **VLP/mℓ** | | | | | **VLP counts** | **VLP/mℓ** | | | | | **VLP counts** | **VLP/mℓ** | | | | |
| | **P1** | **P2** | **P3** | **P4** | **P5** | | **P1** | **P2** | **P3** | **P4** | **P5** | | **P1** | **P2** | **P3** | **P4** | **P5** |
| **a** | 274 | 313 | 183 | 143 | 260 | **a** | 135 | 116 | 147 | 164 | 232 | **a** | 349 | 277 | 333 | 242 | 310 |
| **b** | 231 | 349 | 258 | 220 | 513 | **b** | 186 | 99 | 121 | 86 | 173 | **b** | 273 | 293 | 261 | 184 | 273 |
| **c** | 288 | 261 | 160 | 189 | 304 | **c** | 233 | 134 | 173 | 222 | 131 | **c** | 313 | 238 | 220 | 230 | 214 |
| **d** | 300 | 241 | 232 | 241 | 287 | **d** | 179 | 155 | 191 | 197 | 187 | **d** | 362 | 303 | 139 | 216 | 209 |
| **Average** | 273,25 | 291 | 208,25 | 198,25 | 341 | **Average** | 183,25 | 126 | 158 | 167,25 | 180,75 | **Average** | 324,25 | 277,75 | 238,25 | 218 | 251,5 |
| **StDev** | 30,1 | 49,2 | 44,7 | 42,6 | 116,1 | **StDev** | 40,1 | 24,0 | 30,6 | 59,1 | 41,6 | **StDev** | 40,0 | 28,6 | 81,0 | 25,0 | 48,6 |

**Table 16 Average faecal coliform to faecal streptococci (FC/FS) ratio for all sampling points.**

| | | P1 | P2 | P3 | P4 | P5 | Average cfu/100mℓ | Average FC/FS ratio |
|---|---|---|---|---|---|---|---|---|
| October | FC | 2,22 | 2,28 | 1,59 | 0,85 | 1,72 | 1,732 | 0,44 |
| | FS | 1,26 | 2 | 6,1 | 4,1 | 6,4 | 3,972 | |
| November | FC | 1,01 | 2,9 | 3,8 | 3 | 3,2 | 2,782 | 1,18 |
| | FS | 0,98 | 3,6 | 2,5 | 2,1 | 2,6 | 2,356 | |
| December | FC | 6,6 | 4,9 | 1,9 | 1,7 | 3,3 | 3,68 | 0,84 |
| | FS | 5,5 | 4,1 | 2,7 | 4,7 | 5 | 4,4 | |
| January | FC | 5,7 | 5,3 | 3,6 | 4,8 | 3,3 | 4,54 | 0,83 |
| | FS | 6,4 | 6 | 4 | 5,2 | 5,9 | 5,5 | |
| February | FC | 2,41 | 2,47 | 4,7 | 3,5 | 4,5 | 3,516 | 0,91 |
| | FS | 2,26 | 2,5 | 5 | 6,6 | 2,9 | 3,852 | |
| March | FC | 6,5 | 4,4 | 1,6 | 3,3 | 3 | 3,76 | 1,15 |
| | FS | 2,1 | 6,2 | 1,6 | 4 | 2,4 | 3,26 | |
| April | FC | 1,41 | 1,43 | 2,4 | 3,5 | 3,2 | 2,388 | 0,41 |
| | FS | 7,5 | 4,8 | 7,1 | 4,5 | 5 | 5,78 | |
| May | FC | 1,21 | 1,11 | 2 | 2,2 | 1,18 | 1,54 | 0,53 |
| | FS | 3,3 | 4,8 | 3,1 | 1,38 | 1,85 | 2,886 | |
| June | FC | 2,8 | 1,9 | 1,39 | 1,22 | 1,75 | 1,812 | 0,73 |
| | FS | 3,69 | 1,94 | 2,17 | 2,55 | 2,03 | 2,476 | |
| July | FC | 1,92 | 1,62 | 1,3 | 2,2 | 1,76 | 1,76 | 0,70 |
| | FS | 1,51 | 2,88 | 2,7 | 2,9 | 2,5 | 2,498 | |
| August | FC | 1,1 | 0,91 | 0,69 | 0,81 | 0,91 | 0,884 | 1,19 |
| | FS | 0,81 | 0,99 | 0,52 | 0,55 | 0,85 | 0,744 | |
| September | FC | 2,3 | 0,79 | 0,36 | 1,8 | 0,81 | 1,212 | 0,93 |
| | FS | 2,2 | 2,3 | 0,4 | 0,6 | 1 | 1,3 | |

**Descriptive Statistics**

| | Mean | Std. Deviation | N |
|---|---|---|---|
| Month | 6.500 | 3.4812 | 60 |
| Point | 3.000 | 1.4261 | 60 |
| THB | 7.032 | 3.0218 | 60 |
| EC | 3.2235 | 1.32399 | 60 |
| TC | 3.2442 | 1.51548 | 60 |
| FC | 2.4672 | 1.48576 | 60 |

**Correlations**

| | | Month | Point | THB | EC | TC | FC |
|---|---|---|---|---|---|---|---|
| Month | Pearson Correlation | 1 | .000 | -.750** | -.532** | -.749** | -.468** |
| | Sig. (2-tailed) | | 1.000 | .000 | .000 | .000 | .000 |
| | N | 60 | 60 | 60 | 60 | 60 | 60 |
| Point | Pearson Correlation | .000 | 1 | -.045 | -.113 | -.078 | -.114 |
| | Sig. (2-tailed) | 1.000 | | .734 | .390 | .553 | .387 |
| | N | 60 | 60 | 60 | 60 | 60 | 60 |
| THB | Pearson Correlation | -.750** | -.045 | 1 | .753** | .843** | .627** |
| | Sig. (2-tailed) | .000 | .734 | | .000 | .000 | .000 |
| | N | 60 | 60 | 60 | 60 | 60 | 60 |
| EC | Pearson Correlation | -.532** | -.113 | .753** | 1 | .745** | .514** |
| | Sig. (2-tailed) | .000 | .390 | .000 | | .000 | .000 |
| | N | 60 | 60 | 60 | 60 | 60 | 60 |
| TC | Pearson Correlation | -.749** | -.078 | .843** | .745** | 1 | .662** |
| | Sig. (2-tailed) | .000 | .553 | .000 | .000 | | .000 |
| | N | 60 | 60 | 60 | 60 | 60 | 60 |
| FC | Pearson Correlation | -.468** | -.114 | .627** | .514** | .662** | 1 |
| | Sig. (2-tailed) | .000 | .387 | .000 | .000 | .000 | |
| | N | 60 | 60 | 60 | 60 | 60 | 60 |

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

**Descriptive Statistics**

| | Mean | Std. Deviation | N |
|---|---|---|---|
| Month | 6.500 | 3.4812 | 60 |
| Point | 3.000 | 1.4261 | 60 |
| FS | 3.2437 | 1.88688 | 60 |
| VIB | 2.9985 | 1.54936 | 60 |
| SAL | 2.2102 | 1.26548 | 60 |
| SHIG | 2.3603 | 1.38139 | 60 |

**Correlations**

| | | Month | Point | FS | VIB | SAL | SHIG |
|---|---|---|---|---|---|---|---|
| Month | Pearson Correlation | 1 | .000 | -.468** | -.300* | -.170 | -.209 |
| | Sig. (2-tailed) | | 1.000 | .000 | .020 | .194 | .109 |
| | N | 60 | 60 | 60 | 60 | 60 | 60 |
| Point | Pearson Correlation | .000 | 1 | -.004 | .123 | -.072 | -.102 |
| | Sig. (2-tailed) | 1.000 | | .978 | .347 | .586 | .439 |
| | N | 60 | 60 | 60 | 60 | 60 | 60 |
| FS | Pearson Correlation | -.468** | -.004 | 1 | .258* | .358** | .300* |
| | Sig. (2-tailed) | .000 | .978 | | .046 | .005 | .020 |
| | N | 60 | 60 | 60 | 60 | 60 | 60 |
| VIB | Pearson Correlation | -.300* | .123 | .258* | 1 | .285* | .190 |
| | Sig. (2-tailed) | .020 | .347 | .046 | | .027 | .145 |
| | N | 60 | 60 | 60 | 60 | 60 | 60 |
| SAL | Pearson Correlation | -.170 | -.072 | .358** | .285* | 1 | .278* |
| | Sig. (2-tailed) | .194 | .586 | .005 | .027 | | .032 |
| | N | 60 | 60 | 60 | 60 | 60 | 60 |
| SHIG | Pearson Correlation | -.209 | -.102 | .300* | .190 | .278* | 1 |
| | Sig. (2-tailed) | .109 | .439 | .020 | .145 | .032 | |
| | N | 60 | 60 | 60 | 60 | 60 | 60 |

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

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

**Descriptive Statistics**

| | Mean | Std. Deviation | N |
|---|---|---|---|
| THB | 7.032 | 3.0218 | 60 |
| EC | 3.2235 | 1.32399 | 60 |
| TC | 3.2442 | 1.51548 | 60 |
| FC | 2.4672 | 1.48576 | 60 |
| FS | 3.2437 | 1.88688 | 60 |
| VIB | 2.9985 | 1.54936 | 60 |
| SAL | 2.2102 | 1.26548 | 60 |
| SHIG | 2.3603 | 1.38139 | 60 |

**Correlations**

| | | THB | EC | TC | FC | FS | VIB | SAL | SHIG |
|---|---|---|---|---|---|---|---|---|---|
| THB | Pearson Correlation | 1 | .753** | .843** | .627** | .549** | .344** | .405** | .496** |
| | Sig. (2-tailed) | | .000 | .000 | .000 | .000 | .007 | .001 | .000 |
| | N | 60 | 60 | 60 | 60 | 60 | 60 | 60 | 60 |
| EC | Pearson Correlation | .753** | 1 | .745** | .514** | .534** | .260* | .439** | .468** |
| | Sig. (2-tailed) | .000 | | .000 | .000 | .000 | .045 | .000 | .000 |
| | N | 60 | 60 | 60 | 60 | 60 | 60 | 60 | 60 |
| TC | Pearson Correlation | .843** | .745** | 1 | .662** | .618** | .366** | .343** | .533** |
| | Sig. (2-tailed) | .000 | .000 | | .000 | .000 | .004 | .007 | .000 |
| | N | 60 | 60 | 60 | 60 | 60 | 60 | 60 | 60 |
| FC | Pearson Correlation | .627** | .514** | .662** | 1 | .471** | .289* | .349** | .423** |
| | Sig. (2-tailed) | .000 | .000 | .000 | | .000 | .025 | .006 | .001 |
| | N | 60 | 60 | 60 | 60 | 60 | 60 | 60 | 60 |
| FS | Pearson Correlation | .549** | .534** | .618** | .471** | 1 | .258* | .358** | .300* |
| | Sig. (2-tailed) | .000 | .000 | .000 | .000 | | .046 | .005 | .020 |
| | N | 60 | 60 | 60 | 60 | 60 | 60 | 60 | 60 |
| VIB | Pearson Correlation | .344** | .260* | .366** | .289* | .258* | 1 | .285* | .190 |
| | Sig. (2-tailed) | .007 | .045 | .004 | .025 | .046 | | .027 | .145 |
| | N | 60 | 60 | 60 | 60 | 60 | 60 | 60 | 60 |
| SAL | Pearson Correlation | .405** | .439** | .343** | .349** | .358** | .285* | 1 | .278* |
| | Sig. (2-tailed) | .001 | .000 | .007 | .006 | .005 | .027 | | .032 |
| | N | 60 | 60 | 60 | 60 | 60 | 60 | 60 | 60 |
| SHIG | Pearson Correlation | .496** | .468** | .533** | .423** | .300* | .190 | .278* | 1 |
| | Sig. (2-tailed) | .000 | .000 | .000 | .001 | .020 | .145 | .032 | |
| | N | 60 | 60 | 60 | 60 | 60 | 60 | 60 | 60 |

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

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

**Descriptive Statistics**

| | Mean | Std. Deviation | N |
|---|---|---|---|
| THB | 7.032 | 3.0218 | 60 |
| EC | 3.2235 | 1.32399 | 60 |
| TC | 3.2442 | 1.51548 | 60 |
| FC | 2.4672 | 1.48576 | 60 |
| Somatic | 361.342 | 226.7375 | 60 |
| F-RNA | 262.467 | 192.7647 | 60 |

**Correlations**

| | | THB | EC | TC | FC | Somatic | F-RNA |
|---|---|---|---|---|---|---|---|
| THB | Pearson Correlation | 1 | .753** | .843** | .627** | .860** | .854** |
| | Sig. (2-tailed) | | .000 | .000 | .000 | .000 | .000 |
| | N | 60 | 60 | 60 | 60 | 60 | 60 |
| EC | Pearson Correlation | .753** | 1 | .745** | .514** | .804** | .780** |
| | Sig. (2-tailed) | .000 | | .000 | .000 | .000 | .000 |
| | N | 60 | 60 | 60 | 60 | 60 | 60 |
| TC | Pearson Correlation | .843** | .745** | 1 | .662** | .862** | .853** |
| | Sig. (2-tailed) | .000 | .000 | | .000 | .000 | .000 |
| | N | 60 | 60 | 60 | 60 | 60 | 60 |
| FC | Pearson Correlation | .627** | .514** | .662** | 1 | .707** | .732** |
| | Sig. (2-tailed) | .000 | .000 | .000 | | .000 | .000 |
| | N | 60 | 60 | 60 | 60 | 60 | 60 |
| Somatic | Pearson Correlation | .860** | .804** | .862** | .707** | 1 | .977** |
| | Sig. (2-tailed) | .000 | .000 | .000 | .000 | | .000 |
| | N | 60 | 60 | 60 | 60 | 60 | 60 |
| F-RNA | Pearson Correlation | .854** | .780** | .853** | .732** | .977** | 1 |
| | Sig. (2-tailed) | .000 | .000 | .000 | .000 | .000 | |
| | N | 60 | 60 | 60 | 60 | 60 | 60 |

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

**Descriptive Statistics**

| | Mean | Std. Deviation | N |
|---|---|---|---|
| FS | 3.2437 | 1.88688 | 60 |
| VIB | 2.9985 | 1.54936 | 60 |
| SAL | 2.2102 | 1.26548 | 60 |
| SHIG | 2.3603 | 1.38139 | 60 |
| Somatic | 361.342 | 226.7375 | 60 |
| F-RNA | 262.467 | 192.7647 | 60 |

**Correlations**

| | | FS | VIB | SAL | SHIG | Somatic | F-RNA |
|---|---|---|---|---|---|---|---|
| FS | Pearson Correlation | 1 | .258* | .358** | .300* | .569** | .509** |
| | Sig. (2-tailed) | | .046 | .005 | .020 | .000 | .000 |
| | N | 60 | 60 | 60 | 60 | 60 | 60 |
| VIB | Pearson Correlation | .258* | 1 | .285* | .190 | .403** | .405** |
| | Sig. (2-tailed) | .046 | | .027 | .145 | .001 | .001 |
| | N | 60 | 60 | 60 | 60 | 60 | 60 |
| SAL | Pearson Correlation | .358** | .285* | 1 | .278* | .519** | .437** |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | Sig. (2-tailed) | .005 | .027 | | .032 | .000 | .000 |
| | N | 60 | 60 | 60 | 60 | 60 | 60 |
| SHIG | Pearson Correlation | .300* | .190 | .278* | 1 | .501** | .489** |
| | Sig. (2-tailed) | .020 | .145 | .032 | | .000 | .000 |
| | N | 60 | 60 | 60 | 60 | 60 | 60 |
| Somatic | Pearson Correlation | .569** | .403** | .519** | .501** | 1 | .977** |
| | Sig. (2-tailed) | .000 | .001 | .000 | .000 | | .000 |
| | N | 60 | 60 | 60 | 60 | 60 | 60 |
| F-RNA | Pearson Correlation | .509** | .405** | .437** | .489** | .977** | 1 |
| | Sig. (2-tailed) | .000 | .001 | .000 | .000 | .000 | |
| | N | 60 | 60 | 60 | 60 | 60 | 60 |

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

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

**Descriptive Statistics**

| | Mean | Std. Deviation | N |
|---|---|---|---|
| Somatic | 361.342 | 226.7375 | 60 |
| F-RNA | 262.467 | 192.7647 | 60 |
| Month | 6.500 | 3.4812 | 60 |
| Point | 3.000 | 1.4261 | 60 |

**Correlations**

| | | Somatic | F-RNA | Month | Point |
|---|---|---|---|---|---|
| Somatic | Pearson Correlation | 1 | .977** | -.653** | -.010 |
| | Sig. (2-tailed) | | .000 | .000 | .940 |
| | N | 60 | 60 | 60 | 60 |
| F-RNA | Pearson Correlation | .977** | 1 | -.643** | -.011 |
| | Sig. (2-tailed) | .000 | | .000 | .931 |
| | N | 60 | 60 | 60 | 60 |
| Month | Pearson Correlation | -.653** | -.643** | 1 | .000 |
| | Sig. (2-tailed) | .000 | .000 | | 1.000 |
| | N | 60 | 60 | 60 | 60 |
| Point | Pearson Correlation | -.010 | -.011 | .000 | 1 |
| | Sig. (2-tailed) | .940 | .931 | 1.000 | |
| | N | 60 | 60 | 60 | 60 |

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

**Descriptive Statistics**

| | Mean | Std. Deviation | N |
|---|---|---|---|
| Month | 6.500 | 3.4812 | 60 |
| Point | 3.000 | 1.4261 | 60 |
| Temp | 22.867 | 2.8252 | 60 |
| pH | 7.0642 | .90434 | 60 |
| BOD5 | 4.5325 | 3.00596 | 60 |
| DO | 7.7203 | 1.12364 | 60 |
| COD | 28.333 | 41.5046 | 60 |

**Correlations**

| | | Month | Point | Temp | pH | BOD5 | DO | COD |
|---|---|---|---|---|---|---|---|---|
| Month | Pearson Correlation | 1 | .000 | -.663** | .361** | .130 | -.371** | .040 |
| | Sig. (2-tailed) | | 1.000 | .000 | .005 | .323 | .003 | .764 |
| | N | 60 | 60 | 60 | 60 | 60 | 60 | 60 |
| Point | Pearson Correlation | .000 | 1 | -.273* | -.331** | -.622** | .072 | -.324* |
| | Sig. (2-tailed) | 1.000 | | .035 | .010 | .000 | .585 | .012 |
| | N | 60 | 60 | 60 | 60 | 60 | 60 | 60 |
| Temp | Pearson Correlation | -.663** | -.273* | 1 | -.197 | -.061 | .224 | .180 |
| | Sig. (2-tailed) | .000 | .035 | | .132 | .645 | .086 | .168 |
| | N | 60 | 60 | 60 | 60 | 60 | 60 | 60 |
| pH | Pearson Correlation | .361** | -.331** | -.197 | 1 | .365** | .103 | .192 |
| | Sig. (2-tailed) | .005 | .010 | .132 | | .004 | .433 | .142 |
| | N | 60 | 60 | 60 | 60 | 60 | 60 | 60 |
| BOD5 | Pearson Correlation | .130 | -.622** | -.061 | .365** | 1 | -.093 | .048 |
| | Sig. (2-tailed) | .323 | .000 | .645 | .004 | | .479 | .719 |
| | N | 60 | 60 | 60 | 60 | 60 | 60 | 60 |
| DO | Pearson Correlation | -.371** | .072 | .224 | .103 | -.093 | 1 | -.074 |
| | Sig. (2-tailed) | .003 | .585 | .086 | .433 | .479 | | .575 |
| | N | 60 | 60 | 60 | 60 | 60 | 60 | 60 |
| COD | Pearson Correlation | .040 | -.324* | .180 | .192 | .048 | -.074 | 1 |
| | Sig. (2-tailed) | .764 | .012 | .168 | .142 | .719 | .575 | |
| | N | 60 | 60 | 60 | 60 | 60 | 60 | 60 |

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

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

**Descriptive Statistics**

| | Mean | Std. Deviation | N |
|---|---|---|---|
| Month | 6.500 | 3.4812 | 60 |
| Point | 3.000 | 1.4261 | 60 |
| Turbidity | 9.0845 | 8.16384 | 60 |
| E.C | 620.167 | 95.3966 | 60 |
| TDS | 350.317 | 366.7329 | 60 |
| Salinity | .2928 | .04521 | 60 |

**Correlations**

| | | Month | Point | Turbidity | E.C | TDS | Salinity |
|---|---|---|---|---|---|---|---|
| Month | Pearson Correlation | 1 | .000 | .240 | -.211 | -.078 | -.045 |
| | Sig. (2-tailed) | | 1.000 | .064 | .106 | .551 | .735 |
| | N | 60 | 60 | 60 | 60 | 60 | 60 |
| Point | Pearson Correlation | .000 | 1 | -.362** | .010 | .051 | -.103 |
| | Sig. (2-tailed) | 1.000 | | .005 | .937 | .697 | .436 |
| | N | 60 | 60 | 60 | 60 | 60 | 60 |
| Turbidity | Pearson Correlation | .240 | -.362** | 1 | .022 | -.015 | .055 |
| | Sig. (2-tailed) | .064 | .005 | | .866 | .912 | .676 |
| | N | 60 | 60 | 60 | 60 | 60 | 60 |
| E.C | Pearson Correlation | -.211 | .010 | .022 | 1 | .022 | .778** |
| | Sig. (2-tailed) | .106 | .937 | .866 | | .865 | .000 |
| | N | 60 | 60 | 60 | 60 | 60 | 60 |
| TDS | Pearson Correlation | -.078 | .051 | -.015 | .022 | 1 | -.146 |
| | Sig. (2-tailed) | .551 | .697 | .912 | .865 | | .266 |
| | N | 60 | 60 | 60 | 60 | 60 | 60 |
| Salinity | Pearson Correlation | -.045 | -.103 | .055 | .778** | -.146 | 1 |
| | Sig. (2-tailed) | .735 | .436 | .676 | .000 | .266 | |
| | N | 60 | 60 | 60 | 60 | 60 | 60 |

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

**Descriptive Statistics**

| | Mean | Std. Deviation | N |
|---|---|---|---|
| Temp | 22.867 | 2.8252 | 60 |
| pH | 7.0642 | .90434 | 60 |
| BOD5 | 4.5325 | 3.00596 | 60 |
| COD | 28.333 | 41.5046 | 60 |
| Turbidity | 9.0845 | 8.16384 | 60 |
| E.C | 620.167 | 95.3966 | 60 |
| TDS | 350.317 | 366.7329 | 60 |
| DO | 7.7203 | 1.12364 | 60 |
| Salinity | .2928 | .04521 | 60 |

**Correlations**

| | | Temp | pH | BOD5 | COD | Turbidity | E.C | TDS | DO | Salinity |
|---|---|---|---|---|---|---|---|---|---|---|
| Temp | Pearson Correlation | 1 | -.197 | -.061 | .180 | -.028 | .076 | .094 | .224 | -.111 |
| | Sig. (2-tailed) | | .132 | .645 | .168 | .830 | .565 | .474 | .086 | .398 |
| | N | 60 | 60 | 60 | 60 | 60 | 60 | 60 | 60 | 60 |
| pH | Pearson Correlation | -.197 | 1 | .365** | .192 | -.036 | .188 | -.051 | .103 | .297* |
| | Sig. (2-tailed) | .132 | | .004 | .142 | .786 | .150 | .697 | .433 | .021 |
| | N | 60 | 60 | 60 | 60 | 60 | 60 | 60 | 60 | 60 |
| BOD5 | Pearson Correlation | -.061 | .365** | 1 | .048 | .332** | .026 | -.127 | -.093 | .135 |
| | Sig. (2-tailed) | .645 | .004 | | .719 | .010 | .845 | .333 | .479 | .305 |
| | N | 60 | 60 | 60 | 60 | 60 | 60 | 60 | 60 | 60 |
| COD | Pearson Correlation | .180 | .192 | .048 | 1 | .128 | .293* | .012 | -.074 | .282* |
| | Sig. (2-tailed) | .168 | .142 | .719 | | .331 | .023 | .927 | .575 | .029 |
| | N | 60 | 60 | 60 | 60 | 60 | 60 | 60 | 60 | 60 |
| Turbidity | Pearson Correlation | -.028 | -.036 | .332** | .128 | 1 | .022 | -.015 | -.194 | .055 |
| | Sig. (2-tailed) | .830 | .786 | .010 | .331 | | .866 | .912 | .137 | .676 |
| | N | 60 | 60 | 60 | 60 | 60 | 60 | 60 | 60 | 60 |
| E.C | Pearson Correlation | .076 | .188 | .026 | .293* | .022 | 1 | .022 | .320* | .778** |
| | Sig. (2-tailed) | .565 | .150 | .845 | .023 | .866 | | .865 | .013 | .000 |
| | N | 60 | 60 | 60 | 60 | 60 | 60 | 60 | 60 | 60 |
| TDS | Pearson Correlation | .094 | -.051 | -.127 | .012 | -.015 | .022 | 1 | .133 | -.146 |
| | Sig. (2-tailed) | .474 | .697 | .333 | .927 | .912 | .865 | | .310 | .266 |
| | N | 60 | 60 | 60 | 60 | 60 | 60 | 60 | 60 | 60 |
| DO | Pearson Correlation | .224 | .103 | -.093 | -.074 | -.194 | .320* | .133 | 1 | .192 |
| | Sig. (2-tailed) | .086 | .433 | .479 | .575 | .137 | .013 | .310 | | .143 |
| | N | 60 | 60 | 60 | 60 | 60 | 60 | 60 | 60 | 60 |
| Salinity | Pearson Correlation | -.111 | .297* | .135 | .282* | .055 | .778** | -.146 | .192 | 1 |
| | Sig. (2-tailed) | .398 | .021 | .305 | .029 | .676 | .000 | .266 | .143 | |
| | N | 60 | 60 | 60 | 60 | 60 | 60 | 60 | 60 | 60 |

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

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

**Descriptive Statistics**

| | Mean | Std. Deviation | N |
|---|---|---|---|
| Month | 3.500 | 1.7370 | 30 |
| Point | 3.000 | 1.4384 | 30 |
| VLP | 486.0750 | 298.44084 | 30 |
| Somatic | 205.683 | 167.9165 | 30 |
| F-RNA | 119.433 | 103.9656 | 30 |

**Correlations**

| | | Month | Point | VLP | Somatic | F-RNA |
|---|---|---|---|---|---|---|
| Month | Pearson Correlation | 1 | .000 | -.909** | -.928** | -.913** |
| | Sig. (2-tailed) | | 1.000 | .000 | .000 | .000 |
| | N | 30 | 30 | 30 | 30 | 30 |
| Point | Pearson Correlation | .000 | 1 | -.080 | -.008 | .015 |
| | Sig. (2-tailed) | 1.000 | | .675 | .964 | .938 |
| | N | 30 | 30 | 30 | 30 | 30 |
| VLP | Pearson Correlation | -.909** | -.080 | 1 | .971** | .965** |
| | Sig. (2-tailed) | .000 | .675 | | .000 | .000 |
| | N | 30 | 30 | 30 | 30 | 30 |
| Somatic | Pearson Correlation | -.928** | -.008 | .971** | 1 | .991** |
| | Sig. (2-tailed) | .000 | .964 | .000 | | .000 |
| | N | 30 | 30 | 30 | 30 | 30 |
| F-RNA | Pearson Correlation | -.913** | .015 | .965** | .991** | 1 |
| | Sig. (2-tailed) | .000 | .938 | .000 | .000 | |
| | N | 30 | 30 | 30 | 30 | 30 |

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

**Descriptive Statistics**

| | Mean | Std. Deviation | N |
|---|---|---|---|
| VLP | 486.0750 | 298.44084 | 30 |
| THB | 4.670 | 1.8888 | 30 |
| TC | 2.1350 | 1.19819 | 30 |
| FC | 1.5993 | .74937 | 30 |
| EC | 2.5303 | 1.25084 | 30 |
| FS | 2.6140 | 1.84433 | 30 |
| VIB | 2.5947 | 1.22389 | 30 |
| SAL | 2.0903 | 1.42267 | 30 |
| SHIG | 2.0677 | 1.43926 | 30 |

**Correlations**

| | | VLP | THB | TC | FC | EC | FS | VIB | SAL | SHIG |
|---|---|---|---|---|---|---|---|---|---|---|
| VLP | Pearson Correlation | 1 | .847** | .832** | .467** | .747** | .787** | .515** | .744** | .547** |
| | Sig. (2-tailed) | | .000 | .000 | .009 | .000 | .000 | .004 | .000 | .002 |
| | N | 30 | 30 | 30 | 30 | 30 | 30 | 30 | 30 | 30 |
| THB | Pearson Correlation | .847** | 1 | .748** | .388* | .613** | .560** | .522** | .685** | .712** |
| | Sig. (2-tailed) | .000 | | .000 | .034 | .000 | .001 | .003 | .000 | .000 |
| | N | 30 | 30 | 30 | 30 | 30 | 30 | 30 | 30 | 30 |
| TC | Pearson Correlation | .832** | .748** | 1 | .464** | .678** | .705** | .429* | .558** | .570** |
| | Sig. (2-tailed) | .000 | .000 | | .010 | .000 | .000 | .018 | .001 | .001 |
| | N | 30 | 30 | 30 | 30 | 30 | 30 | 30 | 30 | 30 |
| FC | Pearson Correlation | .467** | .388* | .464** | 1 | .144 | .484** | .121 | .361* | .386* |
| | Sig. (2-tailed) | .009 | .034 | .010 | | .449 | .007 | .523 | .050 | .035 |
| | N | 30 | 30 | 30 | 30 | 30 | 30 | 30 | 30 | 30 |
| EC | Pearson Correlation | .747** | .613** | .678** | .144 | 1 | .592** | .341 | .598** | .372* |
| | Sig. (2-tailed) | .000 | .000 | .000 | .449 | | .001 | .066 | .000 | .043 |
| | N | 30 | 30 | 30 | 30 | 30 | 30 | 30 | 30 | 30 |
| FS | Pearson Correlation | .787** | .560** | .705** | .484** | .592** | 1 | .370* | .552** | .282 |
| | Sig. (2-tailed) | .000 | .001 | .000 | .007 | .001 | | .044 | .002 | .131 |
| | N | 30 | 30 | 30 | 30 | 30 | 30 | 30 | 30 | 30 |
| VIB | Pearson Correlation | .515** | .522** | .429* | .121 | .341 | .370* | 1 | .324 | .606** |
| | Sig. (2-tailed) | .004 | .003 | .018 | .523 | .066 | .044 | | .081 | .000 |
| | N | 30 | 30 | 30 | 30 | 30 | 30 | 30 | 30 | 30 |
| SAL | Pearson Correlation | .744** | .685** | .558** | .361* | .598** | .552** | .324 | 1 | .290 |
| | Sig. (2-tailed) | .000 | .000 | .001 | .050 | .000 | .002 | .081 | | .121 |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | N | 30 | 30 | 30 | 30 | 30 | 30 | 30 | 30 | 30 |
| SHIG | Pearson Correlation | .547** | .712** | .570** | .386* | .372* | .282 | .606** | .290 | 1 |
| | Sig. (2-tailed) | .002 | .000 | .001 | .035 | .043 | .131 | .000 | .121 | |
| | N | 30 | 30 | 30 | 30 | 30 | 30 | 30 | 30 | 30 |

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

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

**Table 17** Canonical correspondence analysis for the physico-chemical and bacterial indicators at the five sampling points from October 2013 to September 2014.

| Canonical properties | Axes | |
|---|---|---|
| | 1 | 2 |
| Eigenvalues | 0.010 | 0.006 |
| % cumulative variance of species data | 8.6 | 13.7 |
| % cumulative variance of species – environment relation | 45.2 | 72.2 |
| Species – environment correlations | 0.636 | 0.543 |
| Total inertia | 0.115 | |
| Total variance | 0.022 | |

**Table 18** Canonical correspondence analysis for the physico-chemical and coliphage populations at the five sampling points from October 2013 to September 2014.

| Canonical properties | Axes | |
|---|---|---|
| | 1 | 2 |
| Eigenvalues | 0.004 | 0.004 |
| % cumulative variance of species data | 46.6 | 100 |
| % cumulative variance of species – environment relation | 100 | 0.00 |
| Species – environment correlations | 0.683 | 0.00 |
| Total inertia | 0.008 | |
| Total variance | 0.004 | |

**Table 19** Canonical correspondence analysis for the coliphage and bacterial populations at the five sampling points from October 2013 to September 2014.

| Canonical properties | Axes | |
|---|---|---|
| | 1 | 2 |
| Eigenvalues | 0.037 | 0.003 |
| % cumulative variance of species data | 29.0 | 31.1 |
| % cumulative variance of species – environment relation | 88.7 | 95.1 |
| Species – environment correlations | 0.698 | 0.266 |
| Total inertia | 0.127 | |
| Total variance | 0.041 | |

**Table 20** Canonical correspondence analysis for the VLP, coliphage and physico-chemical parameters at the five sampling points from October 2013 to September 2014.

| Canonical properties | Axes | |
|---|---|---|
| | 1 | 2 |
| Eigenvalues | 0.014 | 0.00 |
| % cumulative variance of species data | 53.7 | 54.9 |
| % cumulative variance of species – environment relation | 97.9 | 100 |
| Species – environment correlations | 0.744 | 0.625 |
| Total inertia | 0.025 | |
| Total variance | 0.014 | |

**Table 21** Canonical correspondence analysis for the VLP, coliphage and bacterial populations at the five sampling points from October 2013 to September 2014.

| Canonical properties | Axes | |
|---|---|---|
| | 1 | 2 |
| Eigenvalues | 0.015 | 0.007 |
| % cumulative variance of species data | 21.3 | 31.0 |
| % cumulative variance of species – environment relation | 64.6 | 93.8 |
| Species – environment correlations | 0.515 | 0.823 |
| Total inertia | 0.072 | |
| Total variance | 0.024 | |

# Appendix III

# Nested PCR/RT-PCR analyses

## A. Adenovirus

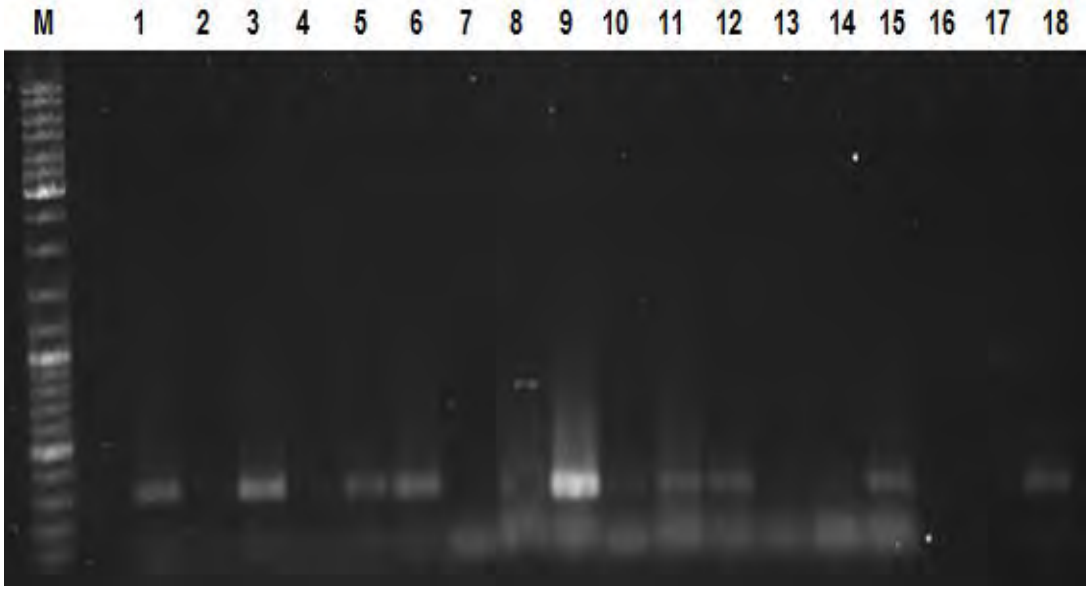


**Figure 1** PCR amplification of the hexon gene for 47 different adenovirus serotypes. M: molecular weight marker, L1 – L5: April, L6 – L10: May, L11 – L15: June, L16: blank well, L17: negative control and L18: positive control.

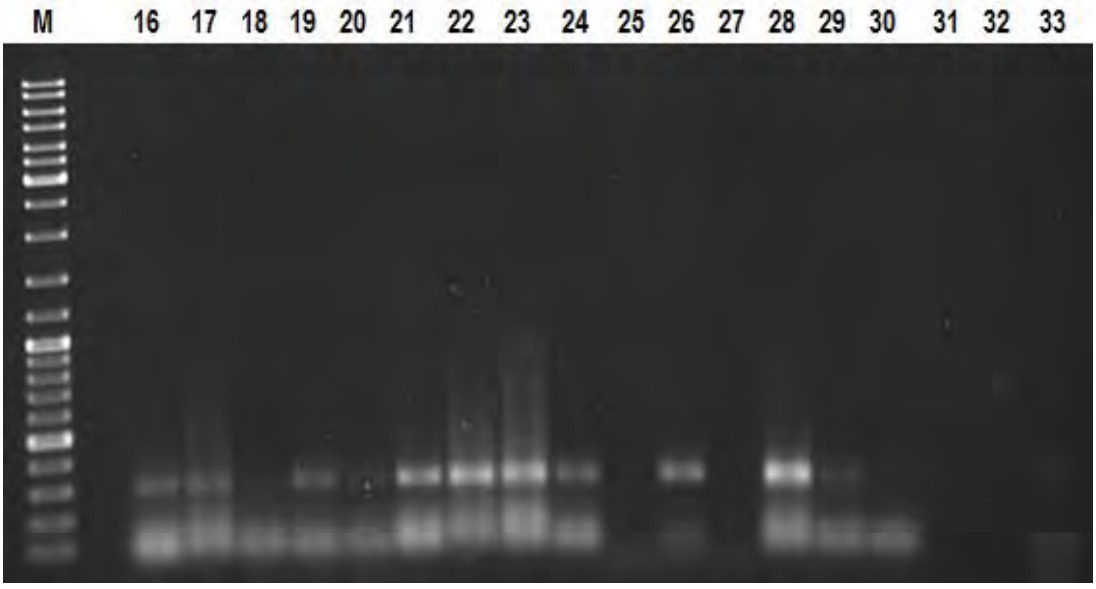


**Figure 2** PCR amplification of the hexon gene for 47 different adenovirus serotypes. M: molecular weight marker, L16 – L20: July, L21 – L25: August, L26 – L30: September, L16: blank well, L17: negative control and L18: positive control.

**B. Polyomavirus**

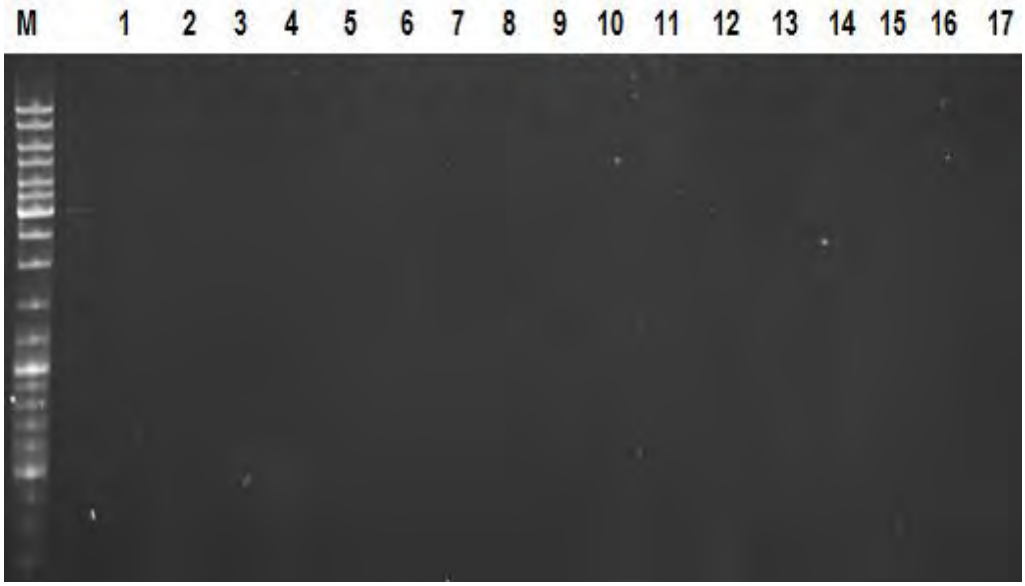


**Figure 3** PCR amplification of the BK and JC polyomaviruses. M: molecular weight marker, L1 – L5: April, L6 – L10: May, L11 – L15: June, L16: blank well, L17: negative control.

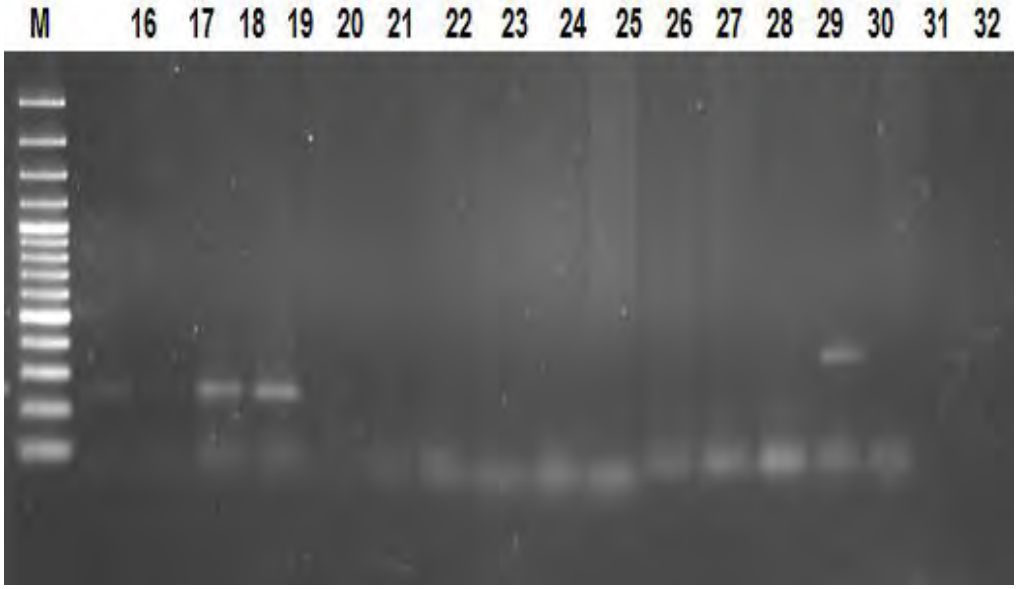


**Figure 4** PCR amplification of the BK and JC polyomaviruses. M: molecular weight marker, L16 – L20: July, L21 – L25: August, L26 – L30: September, L31: blank well, L32: negative control.

## C. Hepatitis A virus

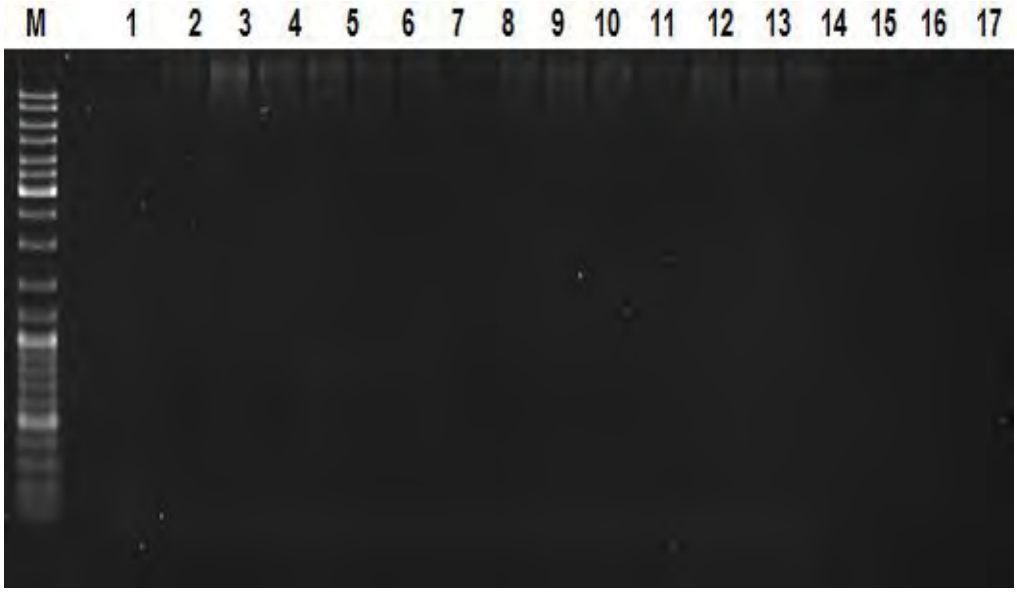


**Figure 5** PCR amplification of the VP1/VP2 region of hepatitis A viruses. M: molecular weight marker, L1 – L5: April, L6 – L10: May, L11 – L15: June, L16: blank well, L17: negative control.

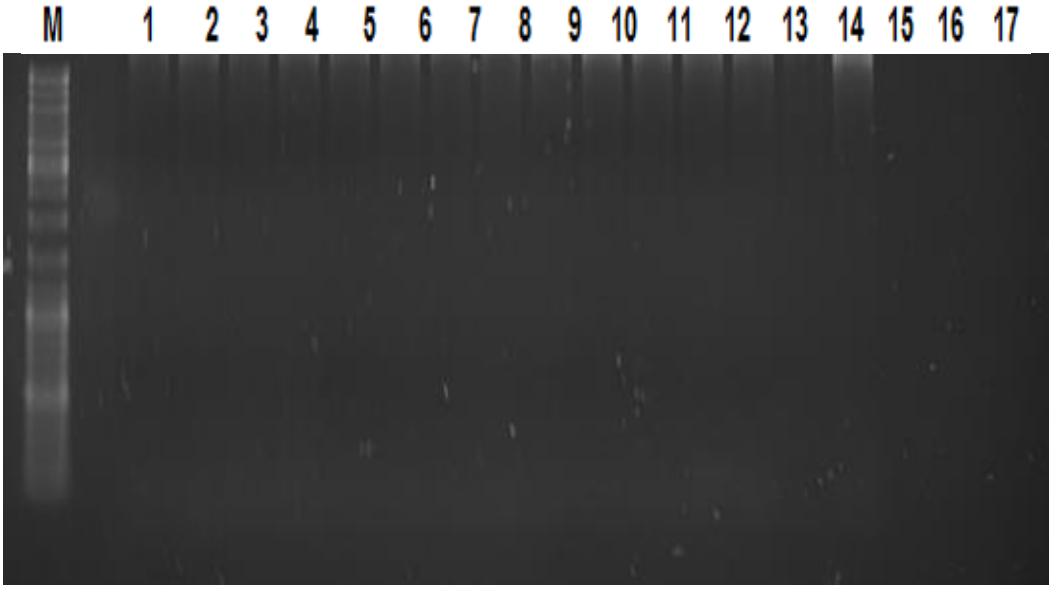


**Figure 6** PCR amplification of the VP1/VP2 region of hepatitis A viruses. M: molecular weight marker, L16 – L20: July, L21 – L25: August, L26 – L30: September, L31: blank well, L32: negative control.

**D. Hepatitis C viruses**

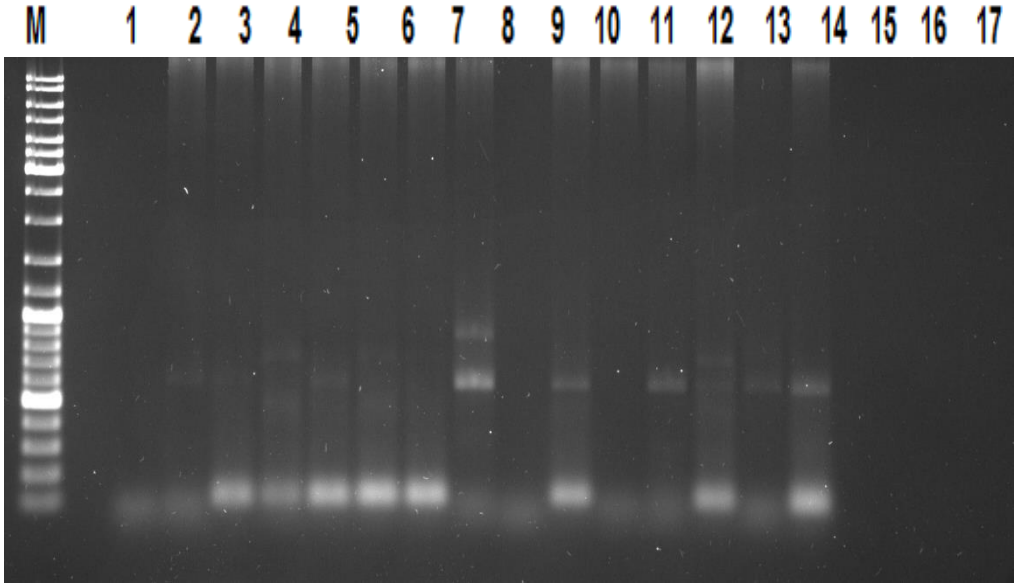


**Figure 7** PCR amplification of the 5'UTR region of hepatitis C viruses. M: molecular weight marker, L1 – L5: April, L6 – L10: May, L11 – L15: June, L16: blank well, L17: negative control.

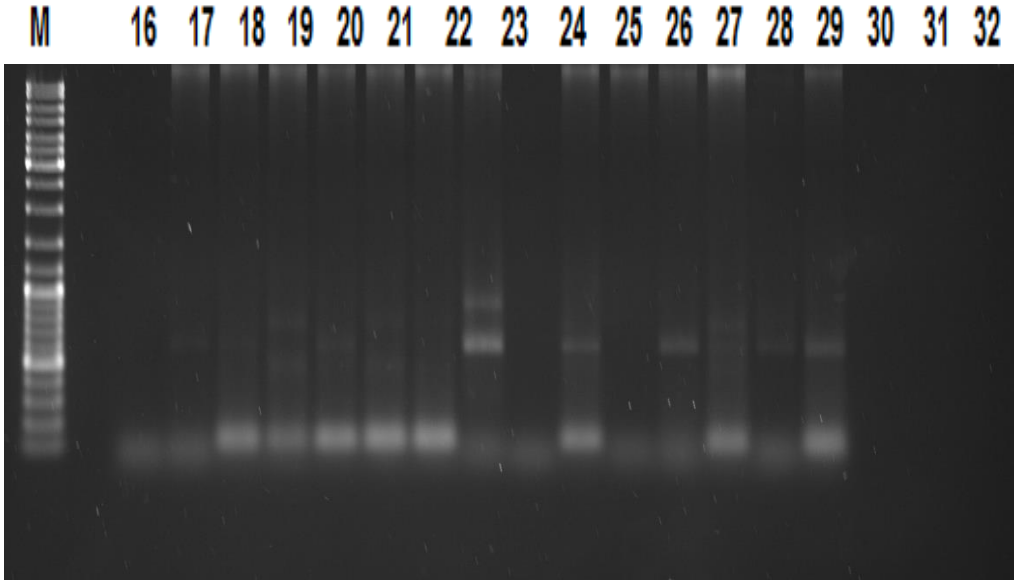


**Figure 8** PCR amplification of the 5'UTR region of hepatitis C viruses. M: molecular weight marker, L16 – L20: July, L21 – L25: August, L26 – L30: September, L31: blank well, L32: negative control.

**BLAST ANALYSIS OF EACH RAW SEQUENCE DATA**

**Adenovirus nested PCR sequence data**

**Sample 11 (June)**

ATAGGTTCACTGTGGCTCTACGCACGAYGTRACCACAGACCGGTCCAGCGTYTG
ACGCTGCGRTTCRTCSCKGTGGACCGCGAGGATACCGCGTACTCGTACAAAAMCS
CSGGSCSGGGGSCMMCSRGAMGKAMTTYARCCTGGGRAACMARTYTAGRAAMC
CCMCSGTGGCYCCSACSCMMGRWGTRACCACAGACMGGTYCCAGCGTTGACGC
TGCAGT

| Accession | Description | E-value | Max ident |
|---|---|---|---|
| KF268310.1 | Human adenovirus c strain | 2e-28 | 89% |

**Sample 16**

TTAGACCCACGTGTGGMTCYGACCACGAYGTAACCACAGACMGGTCACAGCGWYTGAC
GCTGCGRTTCRTMSCWGTSGACCGCGAGGATACCGCGTACTCGTACAAATTTGTTTGYTR
TGGTYRTSTCYYGCMTCSYCCCCGGGCAAAACGMCTTMCAMTTGACCSGTTTGTGGTTA
AGTCCTGGSTAGKGKCCACCGAGACGTACTTCAGCCTGRGRTAACAAGTTCTAGAAACCC
CACKGTGGCTACCKACSCACGAYGTAACCACAGACMGGTCACCAAGSSRTTGACGCTGC
RT

| Accession | Description | E-value | Max ident |
|---|---|---|---|
| KM205587.1 | Adenovirus 2 isolate AAU4 | 1e-26 | 86% |

**Polyomavirus nested PCR sequence data**

**Sample 4**

CACGRGTTAGTGTRCSAGTATSYWCAYGTTWWATSAWCMCTGGCAAACATTTCTTCATG
GCAAAACAGGYCTTMAYMCCACTTCTCATTAAATGTATTCCACCAGGATTCCCATTCATC
TGTTCCATAGGTTGGCACCTGTCTTTAGGGTCTTCTACCTTTTTTTTCTTCTTAGGTGGGGT
AGAGTGTTGGGATCCTGTGTTTTCATCATCACTGGCAAACATTTCTTCATGGCAAAACAG
GTCTTCATCCCACTCTCTATAAATKATCCMTMCSMG

| Accession | Description | E-value | Max ident |
|---|---|---|---|
| KM225765.1 | JC polyomavirus isolate GCN8, complete genome | 9e-53 | 98% |

**Sample 8**

GGGGGTAAGTGTTGGGATCCTGTGTTTTCATCATCACTGGCAAACATTTCTTCATGGCAA
AACAGGTCTTCATCCCACTTCTCATTAAATGTATTCCACCAGGATTCCCATTCATCTGTTC
CATAGGTTGGCACCCTTTTSTGTYTTTAGGGTCTTCTACCTTTTTTTTCTTCTTAGGTGGGG
TAGAGTGTTGGGATCCTGTGTTTTCATCATCACTGGCAAACATTTCTTCATGGCAAAACA
GGTCTTCATCCCACTTCTCATTAAATGATCCMTCA

| Accession | Description | E-value | Max ident |
|---|---|---|---|
| AB081021.1 | JC virus DNA, isolate ME-5 | 4e-61 | 99% |

**Hepatitis A nested PCR sequence data**

**Sample 19**

AYTTTCTCWKCCRCTCCATTGTACTAACTCGATGGTTGTTGCCGCAGAATGATGATKAGA
ATTGTTGCTGCTGGAGATTAGTCGTCATCGGAGGATCCTCCGACGTCAGAGGAGGATAA
ATTTTTTAGGAGTCACRTAKGTWGTAAACCACCMTAYAAAGAATTATTATTAGAAGTTG
GACAACRACTACTKTATGCCCCCCAGGARGAACTGWATGATGAACTTCTTCCTCCTCCGA
RGAAAATGGGAGGATTGTTTTCACMMRCYATTTTTTTTTTTTTTTATGCKGAGGAKSAWG
AAATGATGTTTTTTTGTTGAAGAGKARCTGCTGCTRCTACTGSCTTCTTGAGGAGGTTATT
CTTCTCYTTGGCCGCTGGAAGAGKTGGGWCGMCTCAAGAAAAACGATCCTCCTCTGACC
TAGGATCATCCACTSATGACTCCAAATCYCCASCTGCAAYTCYACWCMTCMTTSATYCAG
TSGACAACAYAKCATTTTAGTTGYTATTTGTCTGTCACAGAACAATCAGAATTTTACTTTC
CCAGAGCTCCATTGAACTCAAATGCCATGTTGTCCACTGAATCAATGATGAGTAGAATTG
CAGCTGGAGATTTGGAGTCATCAGTGGATGATCCTAGGTCAGAGGAGGATAAAAAATTT
GAGAGTCACATAGAATGTAGTAAACCATAYAAAGAATTGAGATTAGAAGTTGGGAAACA
AAGACTYAAGTATGCCCAGGAGGAAYTGTCAAATGAAGTACTTCCACCTCCCAGGAAAA
TGAAGGGATTGTTTTCACAAGCCAAAATTTCTCTTTTTTATACTGAGGAGCATGAAATAA
TGAAATTTTCTTGGAGAGGAGTGACTGCTGATACTAGGGCCTGAGGAGGTTCGATC

| Accession | Description | E-value | Max ident |
|---|---|---|---|
| HQ246217.1 | Hepatitis A virusCFH-HAV, complete genome | 3e-177 | 95% |

**Sample 22**

ATCYCCTTCARCTACCTTCRTACTCACACGCTGGTTGTCCTCGCTGAATGATGATTAGTAT
TGCTGCTGGTGATTTTTAGGATCATCGGAGGATCCTCCGTCGTCAGAGGAGGAAAAAAAT
TTAGTCGTCACRTAKGTWGTAAACCACCATATGAAGAATTATTATAAGAAGGGGGGAAA
CRACTACTSTATGCCCCCCAGGARGAACTGWATGAWGAACTTCTTCCTCCTCCCARGAA
AATGGGAGGGTTTTTTTCACMMRCCATTTTTTTTTTTTTTTATRCKGAGSAKSAWGAAAT
GATGTTTTTTTGTTGAAGAGGAACTGCTGCTRCTACKRCCKTCTTGAGGAGGGTAGTCTC
CTCCTTGGCCGCTRGAAGARKKKKGWSGMYTSAWRAAAAGAATGCTGAGTTCTTCCRGR
AGRMTGATTTAAGATGATGAARAGAAAAAAAAGATCATCCACTSATGACTCCAAATCYC
CASCTGCAAYTCYACWCATCATTCATTCAGTCGACAACAYCTCWTTTTAGTTGYTATTTG
TCTGTCACAGAACAATCAGAATTTTACTTTCCCAGAGCTCCATTGAACTCAAATGCCATG
TTGTCCACTGAATCAATGATGAGTAGAATTGCAGCTGGRGATTTGGAGTCATCAGTGGAT
GATCCTAGGTCAGAGGAGGAYAAAAAATTTGAGAGTCACATAGAATGTAGTAAACCATA
TAAAGAATTGAGATTAGAAGTKGGGAAACAAAGACTYAAGTATGCCCAGGAGGAACTG
TCAAATGAAGTACTTCCACCTCCCAGGAAAATGAAGGGATTRTTTTCACAAGCCAAAATT
TCTCTTTTTTATACTGAGGAGCATGAAATAATGAAATTTTCTTGGAGAGGAGTGACTGCT
GATACTAGRGCCTGAGGATGTTTGATTC

| Accession | Description | E-value | Max ident |
|---|---|---|---|
| FJ687513.1 | Hepatitis A isolate 9 polyprotein | 4e-166 | 96% |

**Hepatitis C nested PCR sequence data**

**Sample 7**

GGCKTCATCTGGCACTCACCCACKAATCCATCTCAACWAGGGTTTCKCCCTCSACCCTGA
TGTTCTGAAAGGGATGGTGCATCGCACCGTACACGGGCGCGACCTTGATAATGTTCTTGC
CCGTGTCGAAGATTTCGATGCGGGTGGTGTCCTGATAGGGTGCTTGCRAGTGCCCCGGGA
RAACGCAGAAAGCGTCTAGCCATGGCGTTAGTTTCCCGGCTTCAATCTKGCACTCAACCC
ACGAATCCATCTCAACAAGGGTTTCGCCCTCGACCCTGATGTTCTGAAAGGGATGGTGCA
TCGCACCGTACACGGGCGCGWCCTTGATAATGTTCTTGCCCGTGTCGAAGAGTGCGATG
YCCGGGT

| Accession | Description | E-value | Max ident |
|---|---|---|---|
| EF473252.1 | Hepatitis C isolate Ind-MN19 5‟UTR | 8e-05 | 100% |