Quantitative Investigation into The Link Between Irrigation Water Quality and Food Safety

VOLUME II: BASELINE STUDY ON EXTENT (TYPES AND QUANTITIES) OF CONTAMINATION FOUND IN IRRIGATION WATER AT SELECTED SITES

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EXECUTIVE SUMMARY

1. BACKGROUND AND MOTIVATION

Large parts of South Africa receive a moderate rainfall and thus rely heavily on irrigation systems to provide sufficient water for agricultural crops. Over the last decade, studies on many of South Africa's rivers revealed that the microbiological pollution has reached unacceptable levels. In many cases indicator and index organisms exceeded the Department of Water Affairs and Forestry (DWAF – now known as DWA – Department of Water Affairs) and the World Health Organisation (WHO) guideline of <1 000 *E. coli* per 100 mL water for irrigation of fresh produce. There have also been widespread public discussions over the last years with negative media headlines occurring nearly every week. As a result of the high pollution levels many South African rivers can be considered unsuitable for irrigation of fresh produce. Such highly polluted river systems could pose serious health risks since many of our rivers are, besides being used for irrigation purposes also used for drinking and recreational purposes.

The presence of pathogens in contaminated water sources is therefore a serious concern in view of irrigating fresh produce. Many of these organisms can cause gastro-enteric illnesses via the consumption of raw produce irrigated with contaminated water. According to the Department of Water Affairs (DWA) there is a correlation between the risk of being infected with the degree of produce contamination and the quantity of contaminated produce consumed. Therefore, higher counts of faecal coliforms in irrigation water can indicate an increased risk in contracting a waterborne disease, even if small quantities of this produce are consumed raw.

Another important aspect of concern for public health is the contamination of surface water with enteric viruses through disposal of human waste. In South Africa, entero, rota, adeno and astroviruses have been detected in polluted river waters. Human rotaviruses have also been detected on the surface of irrigated tomatoes. The surveillance of irrigation water is therefore essential to facilitate correct management procedures for the protection of fruit and vegetable growers and the health of farm workers and consumers. There is thus a crucial need for scientifically sound answers in terms of what the level of microbial contamination of water sources that are used for irrigation purposes is.

2. OBJECTIVES

The objective of this phase of the Water Research Commission solicited research project on "a quantitative investigation into the link between irrigation water quality and food safety" was to do a baseline study on the extent of contamination found in selected South African river water that is used specifically for irrigation purposes at selected sites.

3. FINDINGS

3.1 Selected Western Cape rivers and sampling sites

Microbial results indicated high concentrations of faecal indicators in the Plankenburg and Mosselbank, and to a lesser extent, in the Eerste and Berg Rivers. *Escherichia coli* concentrations exceeded the 1 000 cfu.100 mL^{-1} DWAF and WHO guideline at the main river sites with concentrations at times reaching log 7 levels. The presence of indicator organisms did not only indicate unsanitary conditions, but also the

presence of other potential pathogens such as *Staphylococcus*, *Klebsiella*, *Listeria* and *Salmonella*. No correlation was found between the environmental parameters. For the microbiological parameters, with the exception of the *E. coli*, no significant trends and no correlations between temperature and the dependent variables were found. For the faecal coliforms there was a significant temperature trend but not a good correlation. This is an important finding especially when the WHO and DWAF guidelines for faecal coliforms are applied as it indicates that temperature, even at low temperatures, does impact the faecal coliform numbers.

Based on these results the microbial quality of these rivers was found to be of an unacceptable standard and does not meet the WHO and DWAF guidelines for safe irrigation. It was also concluded that there is a high risk of exposure to human pathogens when water from these two rivers, especially the Plankenburg River, is used to irrigate produce that is consumed raw or without any further processing steps.

3.2 Accuracy of methods for the enumeration of coliforms and *Escherichia coli*

The accuracy of methods for the enumeration of coliforms and *Escherichia coli* present in river water intended for the irrigation of fresh produce was critically evaluated to determine whether the data from the traditional Multiple Tube Fermentation (MTF) method were reliable in indicating faecal pollution. The potential of the Colilert as a rapid alternative method was also explored. The work confirmed that MTF is reliable in the enumeration of coliforms and *E. coli*. Inaccuracies are primarily attributable to atypical organisms which are considered to make up a small proportion of the total bacterial population. Colilert was shown to be an acceptable alternative, and its rapid production of results can be advantageous in the monitoring of river water used for the irrigation of fresh produce.

3.3 Selected KwaZulu-Natal rivers and sampling sites

Samples from the Baynespruit River in Sobantu, Pietermaritzburg, showed that the faecal coliform counts frequently exceeded the recommended maximum values suggested by the WHO and DWAF for safe irrigation, as well as the recommended maximum values set by the Department of Health (DoH) for consumption of raw produce. Faecal coliform counts of up to 1.6×10^6 per 100 mL of irrigation water were observed. This indicates that faecal matter might have entered the Baynespruit River and that microbes present therein can be transferred via irrigation to fresh produce.

3.4 Selected Limpopo Province rivers and sampling sites

In the study on the microbiological quality of water used during subsistence farming in Limpopo to irrigate fresh produce it was found that the Mutshedzi River was contaminated with opportunistic and pathogenic bacteria and diarrhoea-causing viruses. This included heterotrophic bacteria, aerobic and anaerobic spore formers, *Staphylococcus aureus,* intestinal *Enterococcus*, different Gram negative coliforms, commensal and diarrhoeagenic *Escherichia coli* and norovirus G1 (NoV GI), norovirus GII (NoV GII) and hepatitis A virus (HAV). This showed that the river water used by subsistence communities as irrigation water is an important pre-harvest source of contamination and public health risk for the community.

The contamination in this study may have originated from both human and animal sewage disposal due to lack of proper sanitation and the fact that this water source is not protected from human and animal

contamination. This increases the risk of spreading disease causing microorganisms onto the irrigated pre-harvest produce which are usually eaten raw by the community in salads.

3.5 Selected Mpumalanga Province rivers and sampling sites

The microbiological quality of the irrigation canal from Loskop Dam, the two rivers (Olifants and Wilge) that feed it were investigated. The level of aerobic bacteria (3-4 log cfu.mL^{-1}), aerobic spore bacteria (1.6 log cfu.mL^{-1}) and anaerobic spore bacteria (1.5 log cfu.mL^{-1}) in the water was low. Levels of faecal coliforms and E. coli were higher than the WHO and DWAF guidelines for irrigation of fresh produce. *Staphylococcus aureus*, intestinal Enterococci, *Salmonella* and *Listeria monocytogenes* were recovered from the rivers and the canal. These results show that the rivers may contribute to the contamination in the irrigation canal and that may be a possible pre-harvest source of contamination of irrigated broccoli and cauliflower.

3.6 Cryptosporidium and Giardia in selected Mpumalanga, North West and Gauteng rivers

The aim of this study was to determine the microbial pollution level of irrigation water from the Loskopdam canal, Skeerpoort, Moses and Klip Rivers and to determine whether the presence of faecal indicators or bacterial pathogens can be linked to the presence of *Cryptosporidium* oocysts and/or *Giardia* cysts in irrigation water.

Levels of faecal coliforms and *E. coli* in all the samples analysed were higher than the WHO and DWAF guideline. *Staphylococcus aureus*, intestinal enterococci, *Salmonella*, *Listeria monocytogenes* were recovered from the two rivers and the canal. Of the 30 water samples analysed, 43% were positive for *Cryptosporidium* oocysts, 23% positive for *Giardia* cysts and 27% positive for *Salmonella* spp. No significant differences in the prevalence of *Cryptosporidium* and *Giardia* and indicator parameters were observed between rivers. The presence of *Cryptosporidium* oocysts and *Giardia* cysts in irrigation water indicates a risk of infection to consumers who consume produce irrigated with protozoan contaminated water. The use of only faecal indicator organism for monitoring surface water quality is not sufficient to accurately predict the presence of *Cryptosporidium* and *Giardia* and assess the microbiological safety of irrigation water.

3.7 Viruses in samples from selected rivers in Limpopo, North West, Western Cape and Mpumalanga Provinces

In this study 12.9% (13 of 101 samples) of irrigation water samples tested positive for one or more potentially pathogenic human viruses (NoV GII and HAV). Norovirus GII was the most predominant virus (~12%) detected in the irrigation water samples in this study with HAV being present ~4% of samples. Norovirus GI was not detected in any of the samples.

In terms of geographical areas compared, NoV GII was detected 4.5% (1/22) in samples from Limpopo, 0% from the North West, 11.7% (7/60) from the Western Cape and 22.2% (4/18) from Mpumalanga. Norovirus GII was detected at all three sites from the Phadzima farm in Mpumalanga suggesting that the irrigation water in Mpumalanga had a high level of contamination. NoV GII, and specifically Genotypes II.2 and II.4, was detected at seven sites in four Western Cape Rivers (Plankenburg, Eerste, Berg and Mosselbank).

Hepatitis A virus was detected in 4.5% (1/22) of samples from Limpopo, 0% from North West, ~5% from Western Cape and 0% from Mpumalanga. In the Western Cape, HAV (untypable) was detected at one site each in the Mosselbank and Plankenburg River. The Plankenburg sampling site is downstream of the Kayamandi informal settlement and the source might therefore be the untreated sewage from the informal settlement.

The detection of NoVs in the irrigation water samples from selected rivers was not unexpected as a number of other enteric viruses have been reported in surface water samples. NoVs have been implicated in a number of waterborne outbreaks of gastroenteritis. The detection of genetically related NoVs in the Western Cape and Gauteng suggests that these strains have a widespread distribution in SA. From the results it is evident that the NoV GII.2, GII.4 and GII.6 strains detected in the irrigation water are of clinical importance as they showed a high genetic relatedness to strains associated with sporadic gastroenteritis in South Africa and other regions of the world. The detection of closely related strains worldwide is of public health concern as they may be disseminated through a common vehicle such as the international food market.

4. CONCLUSIONS

Based on these results the microbial pollution levels of rivers monitored in different provinces of South Africa over a period of 3-4 years that are utilised for irrigation, these rivers were found to be of an unacceptable standard and did not meet the WHO and DWAF faecal guidelines for safe irrigation. Other potential waterborne pathogens (*Staphylococcus aureus*, intestinal enterococci, *Salmonella*, *Listeria monocytogenes, Cryptosporidium and Giardia*) were frequently recovered from the rivers studied. It was also found that of the 101 water samples analysed, 18% of river water samples, 0% of tap water samples, and 9% of irrigation canal samples were positive for the presence of one or more enteric viruses.

From the results it is evident that many of the bacterial, protozoan and virus strains detected in the irrigation water are of clinical importance as they show relatedness to species associated with gastroenteritis in South Africa and other regions of the world. The detection of closely related strains worldwide is of public health concern as they may be disseminated through a common vehicle such as the international food market. It was concluded that there is a high risk of exposure to human pathogens when water from these rivers is used to irrigate produce that is consumed raw or without any further processing steps.

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LIST OF ABBREVIATIONS

Abbreviation	Description
ACC	aerobic colony count
AnSF	anaerobic spore formers
API	analytical profile index
ASF	aerobic spore formers
CDC	Center for Disease Control and Prevention
cfu	colony forming units
COD	chemical oxygen demand
Crypto	*Cryptosporidium*
DAEC	diffusely adhering *E. coli*
DALY	disability-adjusted life year
DNA	deoxyribonucleic acid
DWA	Department of Water Affairs (previously known as DWAF)
DWAF	Department of water Affairs and Forestry
E. coli	*Escherichia coli*
EAEC	enteroaggregative *E. coli*
EC	*Escherichia coli*
EDTA	ethylene diamine tetra acetic acid
EHEC	enterohaemoragic *E. coli*
EIEC	enteroinvasive E. coli
Entero	intestinal enterococci
EPEC	enteropathogenic *E. coli*
ETEC	enterotoxigenic *E. coli*
EXPEC	extraintestinal *E. coli*
FC	faecal coliforms
FITC	fluorescein-iso-thiocyanate
Giar	*Giardia*
HastV	human astroviruses
HAV	hepatitis A virus
HIV	human immunodeficiency virus
HRV	human rotavirus
HuCV	human caliciviruses
HUS	haemolytic uremic syndrome
LM	*Listeria monocytogenes*
MPF	minimally processed fruits and vegetables
MPN	Most Probable Number
MTF	Multiple Tube Fermentation method
ND	not detected
NMMP	National Microbial Monitoring Programme of DWAF
NoV	norovirus
NoV GI	norovirus genotype GI
NoV GII	norovirus genotype GII
N-PCR	nested polymerase chain reaction
NT	not tested
PBS	phosphate buffered saline
PCR	polymerase chain reaction
QMRA	Quantitative Microbial Risk Assessment Modelling
RT-PCR	reverse transcriptase-polymerase chain reaction
SA	*Staphylococcus aureus*
Sal	*Salmonella* species
SANS	South African National Standards
TC	Total coliforms
TFTC	too few too count (<10 cfu)
TG	typical growth
TWQR	Target Water Quality Range
VTEC	Vero cytotoxigenic *E. coli*
WHO	World Health Organisation

CHAPTER 1

INTRODUCTION

Large parts of South Africa receive a moderate amount of rainfall with an annual average of less than 500 mm (Bennie & Hensley, 2001). It is therefore a country that relies heavily on irrigation systems to supplement rainfall in order to provide sufficient water to agricultural crops (Rahman *et al.*, 2002). Fortunately there are several large and reliable rivers with tributaries near to where many farms are situated. Because of the accessibility to the rivers as well as the availability of water, these rivers traditionally serve as a major source of irrigation water.

Over the last decade, studies on the quality of the water in many of South Africa's rivers revealed an increase in pollution levels (DWAF, 1996; 2000; Bezuidenhout *et al.*, 2002; DWAF, 2002; Griesel & Jagals, 2002; Barnes, 2003; Barnes & Taylor, 2004; Dalvie *et al.*, 2004; DWAF, 2004; Germs *et al.*, 2004; DWAF, 2005). The microbiological pollution levels have, in more recent years, reached unacceptable and dangerous levels. Food-borne infections have also been widespread and led to public discussions over the last years with media headlines such as: "*Kyk voor jy swem*; *Beware of badly polluted river;* and *Groundwater badly polluted with faecal matter*" (Barnard, 2008; Davids, 2008; Gosling, 2008), occurring nearly every week. As result of the high pollution levels many South African rivers can be considered unsuitable for irrigation of fresh produce. In many cases indicator and index organisms exceeded the DWAF and WHO guidelines of 1 000 *E. coli* per 100 mL water for irrigation of fresh produce (WHO, 1989b; DWAF, 1996). The uMngeni River in Kwazulu-Natal was reported to have *E. coli* counts of 1×10^6 cfu per 100 mL water (Olaniran *et al.*, 2009). The Diep, Berg and Plankenburg Rivers in the Western Cape have also been reported to have heavy loads of faecal indicators sometimes with *E. coli* loads of >500 000 per 100 mL water (Paulse *et al.*, 2009). Such highly polluted river systems will certainly pose serious health risks (Zamxaka *et al.*, 2004) since in the above examples the rivers are used for drinking, irrigation and recreational purposes.

Changes in consumer trends, consumer health awareness, population movements, increases in distance that food is transported, a growing immunocompromised population and increased microbial resistance to anti-microbial compounds are impacting the incidence of food-borne diseases. The occurrence of food-related illness outbreaks have increased globally (Johnston *et al.*, 2006; Matthews, 2009). There has also been an increased awareness of illnesses associated with food-borne pathogens as well as the carriers of these pathogens and the environmental conditions that lead to their survival and proliferation.

It has often been shown that poor quality irrigation water can serve as a source of food-borne pathogens on fruit and vegetables that are consumed fresh (Steele & Odumeru, 2004; Hamilton *et al.*, 2006; Johnston *et al.*, 2006; Kay *et al.*, 2008) Since this type of produce is consumed raw and no intervention practices are employed that will effectively control or eliminate potential pathogens prior to consumption it is a potential source of food-borne illness.

The presence of pathogens in contaminated water sources is therefore a serious concern in view of irrigating minimally processed crops. The presence of faecal coliforms is used to indicate the potential presence of other faecal pathogens such as *Salmonella* and *Shigella* species or *E.coli* pathogenic strains. Many of these organisms can cause gastro enteric illnesses via the faecal/oral route through the consumption of raw produce irrigated with contaminated water. According to South Africa's Department of Water Affairs there is a correlation between the risk of being infected with the degree of produce

contamination and the quantity of contaminated produce consumed. Therefore, higher counts of faecal coliforms in irrigation water can indicate an increased risk in contracting a waterborne disease, even if small quantities of this produce are consumed raw.

Another important aspect of a concern for public health is the contamination of surface water with enteric viruses through disposal of human waste, especially if these surface waters are used for recreation, irrigation and for drinking water (Rutjes *et al.*, 2005). In South Africa, entero, rota, adeno and astroviruses have been detected in polluted river water used for irrigation (Barnes & Taylor, 2004). Human rotaviruses have also been detected on the surface of irrigated tomatoes (Van Zyl *et al.*, 2006). The surveillance of irrigation water is therefore essential to facilitate correct management procedures for the protection of fruit and vegetable growers and the health of farm workers and the consumers. There is thus a crucial need for scientifically sound answers in terms of what the level of microbial contamination of water sources that are used for irrigation purposes is.

The aim of this phase of the on-going WRC study on “a quantitative investigation into the link between irrigation water quality and food safety” is to do a baseline study on the extent of contamination found in South African river water that is used specifically for irrigation purposes water at selected river sites.

CHAPTER 2
EXPERIMENTAL PROCEDURES

2.1 SELECTED AND SAMPLING SITES

2.1.1 Selected Western Cape rivers and sampling sites

Two rivers in the Stellenbosch region of the Western Cape (Plankenburg and Eerste Rivers) were monitored. The first site (Plank-1) in the Plankenburg River is located below the Kayamandi informal settlement and the second (Plank-2) 2 km downstream at the confluence between the Plankenburg and Eerste Rivers. The third site (Eerste-1) in the Eerste River (Jonkershoek river section) about 400 meters before the Plankenburg River merges to form the Eerste River. Local farmers upstream and downstream use these rivers as irrigation source points where they draw water for irrigation. A fourth sampling site (Plank-0) was selected about 10 km upstream from the Plank-1 site specifically to assess the impact of an informal settlement and an industrial area on the water quality of the Plankenburg River. However for large parts of each year, this site has no flowing water so it was not sampled at the same frequency. The rivers were sampled once a month for 42 months (September 2007 to March 2011) according to the SANS 5667-6 (SANS, 2006) guidelines.

2.1.2 Accuracy of methods for the enumeration of coliforms and *Escherichia coli*

Water was sampled from four rivers: the Plankenburg (2 sites), upper Berg (3 sites), Eerste (1 site) and Lourens (1 site) Rivers. 1 L water was drawn using the guidelines according to Standard Methods (APHA, 2005). The samples were analysed for coliforms and *E. coli* using the MTF and the Colilert methods (IDEXX Laboratories)(SANS, 2012). Results were reported as coliforms and *E. coli* MPN.100 mL^{-1}. The Spearman rank correlation coefficient (Estelberger & Reibnegger, 1995) between MTF and Colilert was calculated for both coliforms and *E. coli* for the four-river data set. Intra-class correlations (ICC) were also calculated for both coliforms and *E. coli* (Prof. M. Kidd, Center for Statistical Consultation, University of Stellenbosch, personal communication).

2.1.3 Selected KwaZulu-Natal rivers and sampling sites

The study site, Sobantu, is a suburban community in Pietermaritzburg which relies heavily on adjacent surface water for their daily water requirements and agricultural irrigation. The Baynespruit and Msunduzi Rivers run through a number of informal settlements in Pietermaritzburg, and are frequently used to irrigate agricultural land including both commercial and small scale market gardens. Sampling was done according to the SANS 5667-6 (SANS, 2006) guidelines.

2.1.4 Selected Mpumalanga rivers and sampling sites

The Loskopdam irrigation scheme was selected as sampling area and samples collected from: Loskop canal from which the farmers irrigate; and two rivers that feed the Loskop dam, the Olifants and Wilge Rivers. Water from the dam is released to Loskop canal system which is used to irrigate vegetables. Water from the three points was aseptically collected (SANS, 2006) during 12 intervals (November 2007 to October 2008).

2.1.5 Determination of the presence of *Cryptosporidium* and *Giardia* in water from selected Mpumalanga, North West and Gauteng rivers

Three sites were selected to determine the presence of *Cryptosporidium* and *Giardia* in irrigation water. The sites included the Moses River, Loskopdam in Mpumalanga, the Skeerpoort River in North West and the Klip River in Gauteng. These sites are used as water sources for the irrigation of fruits and vegetables. Additionally the sites were suspected to be contaminated with *Cryptosporidium* and *Giardia* as high levels of faecal coliforms were found in the previous studies. The presence of faecal coliforms in water indicates contamination with faecal matter. Since *Cryptosporidium* oocysts and *Giardia* cysts are spread through contaminated faeces (O'Donoghue, 1995) these waters were suspected to be contaminated with the two protozoa. Water samples were taken monthly for 10 months (June 2009 until April 2010) from the three sites. One litre samples were collected for bacteriological analyses and 50 L for *Cryptosporidium* and *Giardia* analyses.

2.1.6 Selected Limpopo Province rivers and sampling sites

Small-scale subsistence farming in rural communities in Limpopo Province provide sufficient means of survival for many communities. The communities living in the Vhembe district of the Limpopo Province consume cabbage and tomatoes grown in community field farming projects. The farming fields are situated next to surface water sources (rivers) which are surrounded by settlements and which are used by cattle and other domestic animals. The majority of the households next to the river are dependent on these river water sources for drinking, bathing, irrigation, recreation and food (e.g. fish). In addition, the community also uses the bush as a toilet area and when it rains these wastes are washed into these rivers. The same water from the rivers is then used for irrigation by the farming field projects.

The subsistent farming field used in this study was situated in the Phadzima region of the Vhembe region of the Limpopo province. The subsistent farmers used the Mutshedzi River as source of irrigation water and also by the surrounding communities who are dependent on this river for drinking water, water to irrigate crops, water their livestock as well as using the river to bath in or wash clothes. This site was thus an ideal site to study food safety issues in a subsistence farming scenario. The water from the Mutzhedzi River is directed to the farming field (Phadzima Farming field) through an irrigation canal. Aspects of importance for the selection of sampling sites are summarized in Table 1.

2.2 MOLECULAR ID OF COMMENSAL AND DIARRHOEAGENIC *E. COLI* PATHOTYPES

A sample (2 mL) was removed from presumptive positive *E. coli* MPN tubes and aliquoted into sterile Eppendorf tubes. The tubes were centrifuged for 5 min at 13 000 × g and the supernatant discarded. DNA was extracted using the guanidium thiocyanate/silica method (Boom *et al.*, 1990; Borodina *et al.*, 2003). The extracted DNA was used as template in all PCR reactions (Omar & Barnard, 2010).

2.3 ISOLATION OF *CRYPTOSPORIDIUM* OOCYSTS AND *GIARDIA* CYSTS

Cryptosporidium and *Giardia* were isolated using filtration, immuno-magnetic separation and staining with fluorescein-iso-thiocyanate (FITC) and 4',6-diamidino-2-phenylindole (DAPI) according to the Method 1623: *Cryptosporidium* and *Giardia* in water by Filtration/IMS/FA by United States Environmental Protection

Agency (Anonymous, 2005). Each 50 L water sample was filtered through a 1 µm HV filter capsule (Separation, Randburg). The Envirocheck^{TM} filter capsule was filled with 125 mL prepared elution buffer containing Laureth-12 (PPG Industries, Gurnee, IL), 1 M Tris pH 7.4, EDTA and silicone antifoam agent (Merck, South Africa) and shaken.

Table 1. Aspects considered when selecting sampling sites in the Limpopo Province.

Important Aspect	**Description**
Irrigation water sources	Local River and/or borehole water
Type of contamination	Possible faecal (human & animal) contamination in river or groundwater
Type of farming	Subsistence and small-scale commercial
Type of produce	Tomatoes, cabbages, garlic and chilli
Irrigation technologies	Canal distribution, short furrows or sprinkler irrigation
Irrigation usage periods	Throughout the year
Stages of growing season	Throughout the year
Pathways of contamination	Dense populations next to rivers, animal faeces, people using water sources for bathing and clothes washing and/or a wastewater sewage plant.
Availability of site	Possible sites for water sampling points
Produce selected for project	Tomatoes and cabbages

The pellet was re-suspended and transferred into flat-sided tubes and the IMS kit was used to fix the samples (Kyalami, South Africa). Samples on microscope slides were fixed with methanol and stained with 50 µl of *Cryptosporidium* FITC and 50 µl *Giardia* FITC stain (Davies Diagnostics, Randburg) and incubated at 37°C for 30 min. The samples were then stained with 50 µl DAPI working solution (Davies Diagnostic, Randburg) and left for 2 min at room temperature. Each slide was scanned under fluorescence microscopy (Zeiss Axiovert 200, Germany) at 65 X. Oocysts and cysts with apple green fluorescent cell walls and spherical or ovoid in shape were identified as positive for FITC. The UV filter block was then used for the DAPI examination. Oocysts and cysts that had been identified using the green filter were then examined under UV filter for confirmation. *Cryptosporidium* and *Giardia* were identified as DAPI positive if they exhibit up to 4 distinct sky-blue internal nuclei. Positive controls were used to verify the efficiency of the method. ColorSeed^{TM} and EasySeed^{TM} (BTF Pty Limited, Biomerieux, UK) were used as positive control to determine the recovery rate of the method. Each ColorSeed or EasySeed capsule contains an exact number of *Cryptosporidium* oocysts given with the certificate of analysis. Both kits were used to calculate the recovery rate according to the manufacturer's instruction.

Nested polymerase chain reaction – DNA was extracted using the Pinpoint slide DNA isolation System (Zymo Research, USA) followed by 15 cycles of freeze-thawing (1 min in liquid nitrogen followed by 1 min at 65ºC in thermocycler) to facilitate bursting of oocysts wall and extraction of DNA. Amplification of *Cryptosporidium* DNA isolated from microscope slides was performed according to the method described by Nichols and co-workers (Nichols *et al.*, 2003).

2.4 PHYSICO-CHEMICAL ANALYSIS

The parameters monitored according to Standard Methods (APHA, 1998) included water temperature (Digital thermometer – Hanna Instruments), pH, alkalinity, conductivity and chemical oxygen demand (COD). Conductivity ($mS.m^{-1}$) was measured with a Hanna (HI8733) conductivity meter. A DR2000 spectrophotometer (Hach Co. Loveland, CO) was used to determine the COD (Merck test kit 25-1 500 $mg.L^{-1}$) and data expressed as mg $O_2.L^{-1}$. Rainfall values were obtained from local meteorological stations.

2.5 MICROBIOLOGICAL ANALYSIS

The aerobic colony count was used as an indication of the size of the microbial population in the water. The aerobic and anaerobic spore formers were used to establish the presence of *Bacillus* and *Clostridium* strains. Total coliforms (TC), faecal coliform (FC), *E. coli* (EC) and intestinal enterococci (IE) were used as Indicator organisms for faecal contamination (Busta *et al.*, 2003). The “Index organisms”, *Staphylococcus*, *Salmonella* and *Listeria,* were used as to show the possible presence of related pathogens (Busta *et al.*, 2003). All methods applied were according to the standard methods prescribed by the South African National Standards (SANS) and International Standards Organisation (ISO) (South African Bureau of Standards, Pretoria, South Africa). All recommended quality control procedures were followed.

2.5.1 Aerobic Colony Count (ACC)

Dilutions of the sampled water were prepared according to standard methods (APHA, 2005) and analysis done on Plate Count Agar (Merck) according to the SANS method 4833 (SANS, 2007) and incubated at 30°C for 72 h and results were reported as $cfu.mL^{1}$ of sample (colony forming units)(Merck, 2007).

2.5.2 Aerobic and anaerobic spore formers (ASF and AnSF)

Dilutions in BPW were prepared and then placed at 75°C for 20 min (Austin, 1998). Pour plates using Trypticase Soy Agar (Merck) were prepared in duplicate and incubated at 35°C for 48 h. A set of duplicate plates were incubated anaerobically at 37°C for 48 h and the data reported as $cfu.mL^{-1}$ of sample (Merck, 2007).

2.5.3 Coagulase-positive Staphylococci (Staph)

Set plates were prepared according to the SANS 6888-1 method (SANS, 2000) using Baird-Parker Agar with egg yolk tellurite emulsion (Merck) and incubated at 37°C for 24 h. Typical colonies were identified as being black with a clear zone and the data reported as $cfu.mL^{-1}$ of sample (Merck, 2007). Catalase test was performed on positive colonies and confirmed with Staphylase test (Oxoid Ltd.). Atypical colonies were identified by the coagulase test and the API Staph test kit (BioMerieux, F-69280 Marcy l’Etoile, France).

2.5.4 Salmonella (Sal)

The presence of *Salmonella* spp. was tested according to the SANS procedure 6579 (SANS, 2003) . Streaking onto pre-dried Xylose Lysine Deoxycholate (Merck) plates was used and incubated at 35°C for 24 h. Typical colonies were identified and the presence of *Salmonella* reported as either present or absent

(Merck, 2007). Typical colonies were confirmed on *Salmonella* chromogenic medium (Oxoid, Basingstoke, Hampshire, England).

2.5.5 Listeria (LM)

The detection of *Listeria monocytogenes* was according to the SANS method 11290-1 (SANS, 2001). Plates were incubated anaerobically at 37°C for 48 h. The presence of *Listeria* reported as either present or absent (Merck, 2007).

2.5.6 Intestinal Enterococci (Entero)

A 0.45 µm membrane filter was used to filter 100 mL of sample according to the SANS 7899-2 method (SANS, 2004) and the filter aseptically placed on a pre-dried Slanetz & Bartley Agar plate (Merck). The plates were inverted and incubated at 35°C for 44 h. The filter was then placed on a Bile Esculin Azide Agar plate and incubated at 44°C for 2 h. Typical colonies were reported as cfu.100 mL^{-1} of sample (Merck, 2007).

2.5.7 Coliforms, faecal coliforms and E. coli

Detection of these organisms was done using the multiple tube fermentation (MTF) method (Cristensen *et al.*, 2002) and Standard Methods (APHA, 1998). The coliform and faecal coliform counts were determined using the Most Probable Number (MPN) tables. Typical *E. coli* colonies (Merck, 2007) on Levine Eosin Methylene Blue (L-EMB) (Oxoid) were identified as having a metallic green sheen. These were then streaked on *E. coli* chromogenic agar (Oxoid), and identity confirmed with API 20E (BioMerieux, F-69280 Marcy l'Etoile, France). The dilution factor of the positive EC-MUG tubes that resulted in typical colonies was used to determine the *E. coli* count from the MPN tables (APHA, 1998). The MTF results were expressed as coliforms (TC), faecal coliforms (FC) or *E.coli* MPN.100 mL^{-1}.

2.6 VIROLOGICAL ANALYSIS

2.6.1 Selection of sites and sampling of river water

Irrigation water samples – Irrigation water samples (10 L) included untreated river and irrigation canal water used by subsistence and/or small scale farmers, and surface river water used by commercial farmers for irrigating fresh produce. Water samples were collected in a sterile container and transported in cooler bags to the laboratory and stored at 4°C until further processing.

Selection of sites – In Limpopo, the irrigation water sites included: i) Gobe River in the Vhembe region at Farmer 1's farm, ii) Mvudi River in the Vhembe region at Farmer 2's farm, and iii) irrigation canal water from the Phadzima River used by the Phadzima community farm.

In Mpumalanga, the irrigation water sites included: (i) surface water from an irrigation canal fed from the Loskop Dam (Site A); (ii) surface water from the Olifants River (Site B), and (iii) surface water from the Wilge River (Site C).

In the North West Province irrigation water was collected at a single site on the Crocodile River (Site WIII).

In the Western Cape, the irrigation water sites included: i) a branch of the Mosselbank River, about 1 km downstream from a sewage works; (ii) water from the Mosselbank River used for irrigating vegetables; (iii) the Plankenburg river just downstream of an industrial area and the Kayamandi informal settlement; (iv) the Plankenburg river 2 km before the merger of the Plankenburg and Eerste Rivers; (v) the Plankenburg river at the confluence; (vi) an irrigation canal fed from the Eerste River just after the confluence with the Plankenburg River; (vii) water drawn from a dam filled from the Plankenburg River; (ix) Berg River downstream of the confluence of the Berg and Franschhoek River; (x) Berg river 10 km downstream between Franschhoek and Paarl and (xi) Berg River downstream at the Lady Loch Bridge close to Wellington.

2.6.2 Viral recovery from irrigation water

Prior to the viral recovery process each water sample was seeded with 10 µl (1 x 10^5 $TCID_{50}$) mengovirus as a process control. Viruses were recovered by means of a glass wool adsorption-elution technique (Wolfaardt (Wolfaardt *et al.*, 1995; Grabow *et al.*, 1996; Vivier *et al.*, 2004), and further optimised by Venter (Venter, 2004). Samples (10 L) were filtered through the positively charged glass wool columns using negative pressure at a rate of 10 $L.h^{-1}$. The negatively charged viruses, which adsorbed to the glass wool, were eluted twice with 50 mL of GBEB (0.05 M glycine [Merck, Darmstadt, Germany], 0.5% beef extract [BBL™ Becton Dickinson and Co., Sparks, MD] pH 9.5). The eluting solution was left in contact with the glass wool for 5 min before being passed through the filter under pressure, where after the pH was adjusted to pH 7 using 1 M HCl (Merck). The viruses were further concentrated to a final volume of 500 µl in phosphate buffered saline (PBS, pH 7.2; Sigma-Aldrich Co., St Louis, MO) using a polyethylene glycol 6000 (Merck, Darmstadt, Germany)/sodium chloride (Merck) precipitation method (Minor, 1985; Vilaginès *et al.*, 1997). The resulting pellet was re-suspended in 20 mL of PBS pH 7.2 and recovered virus samples were stored at -20ºC.

Viral detection – Nucleic acid extraction – Genomic viral nucleic acid was extracted directly from 1 mL of the recovered virus concentrate using the MagNA Pure LC Total Nucleic Acid Isolation Kit (large volume)(Roche Diagnostics GmbH, Mannheim, Germany), in a MagNA Pure LC instrument (Roche), following the manufacturer's instructions. The purified nucleic acid (100 µl) was aliquoted and stored at -70°C before use. Five microliters of the eluted nucleic acid was used for virus nucleic acid amplification.

Viral amplification – Primers and Probes: The primers and probes used for the real-time RT-PCR assays were those recommended by the European Committee of Standardisation (CEN) TC275/WG6/TAG4 Technical Committee. *Hepatitis A virus* (HAV): The primers and probes as described before (Loisy *et al.*, 2005; Costafreda *et al.*, 2006; Da Silva *et al.*, 2007; Svraka *et al.*, 2007) were used for the Norovirus genogroups I and II (NoV GI and GII).

Viral detection – Molecular amplification and real-time RT-PCR detection of NoV GI and NoV GII were done using the QuantiTect Probe® RT-PCR kit (Qiagen GmbH, Hilden, Germany) on the LightCycler 1.5 (Roche). The Quantitect Probe kit provides a one-step real-time RT-PCR amplification of RNA genomes. Five microliters of extracted viral RNA is added to the Quantitect Probe Master Mix, containing 4 pmol each of the forward primer and reverse primers and labelled TaqMan. The cycling conditions were used as described by the manufacturer. For the detection of HAV, the RNA Ultrasense™ One-step qRT-PCR system (Invitrogen, Carlsbad, CA) was applied as it was found to be more sensitive than the Quantitect Probe® RT-PCR kit (Qiagen) for the detection of HAV. For the reaction 5 µl RNA, 10 pmol of

the forward primer, 18 pmol of the reverse primer and 5 pmol of the labelled probe was added to the Master Mix. The cycling conditions were customised for HAV. The real-time RT-PCR amplification for mengovirus was done using the QuantiTect Probe® RT-PCR kit (Qiagen) using five microliters of nucleic acid and 10 pmol each of the forward primer, 18 pmol of the reverse primer and 5 pmol of the labelled TaqMan probe. The reaction parameters were the same as for HAV. Included in each set or batch of real-time RT-PCR reactions were a negative RNA extraction control, a negative real-time RT-PCR control (nuclease-free water: Promega Corp.), and a real-time RT-PCR positive control comprised of RNA from the target virus.

2.7 STATISTICAL ANALYSIS

Relationship between temperature and other parameters was done by doing regressions with temperature as independent predictor and logACC, COD, pH, logFaecal, logColiforms as dependent predictor variables. The resulting regression coefficients quantified the type of association between the predictor and respective dependent variable. As this was a time series data set, the Durbin-Watson (d) test was done to determine the serial correlation for all the above parameters. This value must be near 2 which would then indicate that there is no serial correlation. Additionally a TSCREG (Time Series Cross-Sectional Regression) was done. Results are interpreted as a normal regression. A p value of <0.05 was considered statistically significant.

Analysis of variance, $P>0.05$, was used to determine whether there were significant differences between the levels of turbidity, COD, aerobic plate count, aerobic spore former counts and anaerobic spore former counts in water samples from the Olifants and Wilge rivers and Loskop canal (n = 12) as well as between the bacterial counts determined on the cauliflower and broccoli from three farms and the Loskop canal (n = 3). Statistica Version 9 (Statsoft, Tulsa, OK, 1984-2009) was used for the statistical analysis.

CHAPTER 3

RESULTS, DISCUSSIONS AND CONCLUSIONS

(Please note that the following abbreviations were used in the text and tables: ASF = aerobic spore formers; AnSF = anaerobic spore formers; IE = Intestinal Enterococci; SA = Staphylococcus aureus; LM = Listeria monocytogenes; Sal = Salmonella spp.; TC = total coliforms; FC = faecal coliforms; ACC = aerobic colony count; Crypto = Cryptosporidium; and Giar = Giardia)

3.1 WESTERN CAPE PROVINCE

3.1.1 Specific aims

The aim of this study was to quantify the pollution levels in selected Western Cape Rivers (Plankenburg, Eerste, Berg and Mosselbank) by doing a base-line microbial study of water from selected sites over an extended period. The presence of indicator and index organisms (coliforms, faecal coliforms, *E. coli*, *Staphylococcus*, *Salmonella* and Enterococci) will be monitored in terms of presence and concentrations to give some insight into the type and extent of pollution that exists in these rivers.

3.1.2 Physico-Chemical results

The environmental and chemical data obtained during the research period (September 2007-March 2011) for each of the different sites, is summarised in Table 2.

Temperature – As a whole it was found that the water temperature from the sites showed similar profiles at all the sampling points with variation between 9° and 25.6°C. For the summer periods (Sept to April) they varied from 18° and 21°C and specifically for the Mosselbank and Berg-3 sites reaching up to 25°C. As expected for the winter sampling was between 9° and 15°C.

pH and alkalinity – The pH ranged from 5.63 to 7.24 with, on average, values mostly above 6.0 (Table 2). These values are within DWAF's Target Water Quality Range (TWQR) of 6.0 to 9.0 for pH (DWAF, 1996). The pH values obtained for the Mosselbank River were always above 6.0 and the values for the other sites varied from 4.3 to 7.24 with on average above 6.0. Berg-1 and Berg-2 sites had at times minimum values below 5.0 and no explanation can be given as to why this was so. The alkalinity ranged from 13 to 1 125 mL^{-1} $CaCO_3$ and the COD from zero to 296 $mg.L^{-1}$. The higher values were mostly found at the Plank-1 site where values of above 100 were regularly recorded. Since this site is just below the Kayamandi informal settlement and the Plankenbrug industrial area the fluctuations could be caused by nutrient cycling or effluent discharges.

Conductivity – The conductivity was found to range from 2 to 160 $mS.m^{-1}$ and is with two exceptions is within DWAF's TWQR of 0 to 70 $mS.m^{-1}$ for conductivity (DWAF, 1996).

Chemical oxygen demand – With one exception the COD values for most of the sites were found to be less than 100 $mg.L^{-1}$. The exception was for Plank-2 site where values of above 100 were regularly was recorded.

Overall it was concluded that according to physico-chemical of the DWAF guidelines the Plankenburg, Eerste, Berg and Mosselbank river water will normally have no adverse effect on the crop yield production and based on the above parameters measured the water should be suitable to be used for irrigation of fresh produce.

3.1.3 Microbiological evaluations

The microbiological data obtained during the research period for each of the different sites, is summarised in Table 3 and the *E.coli* and faecal coliform levels found for the different sites are given in Figs. 1 and 2.

Aerobic colony counts (ACC) – the counts were found to vary from very low values (none detected per mL) too high (below 100 000 cfus) to high (150 000-500 000 cfus), to extremely high 12 800 000 000 cfu per mL for the Mosselbank site in February 2008. The ACC data is generally taken as representative of the total microbial content including spoilage microorganism (Sela & Fallik, 2009). However, DWAF guidelines only give values for the heterotrophic bacterial counts of less than log 2.0 cfu per mL for safe domestic use.

Table 2. The environmental and chemical data obtained for the Plankenburg, Eerste, Berg and Mosselbank River sites for the period September 2007 to March 2011 (n = 20-42 sampling per site).

Site	Temperature (°C)		pH		Alkalinity ($mg.L^{-1}$)		Conductivity ($mS.m^{-1}$)		COD ($mg.L^{-1}$)	
	Min	Max	Min	Max	Min	Max	Min	Max	Min	Max
Plank-1	10.2	20.8	5.97	7.20	14	1 125	47	62	0	69
Eerste-1	10.1	21.8	5.72	7.05	13	250	8	87	0	46
Plank-3	9.9	21.8	5.78	7.24	25	1 000	30	89	0	208
Berg-1	11.9	21.6	4.30	7.08	5	625	9	84	0	81
Berg-2	12.2	21.6	4.75	6.98	5	250	8	93	0	40
Berg-3	12.3	25.6	5.10	7.01	13	625	14	160	0	67
Mosselbank	14.7	25.4	6.07	6.82	60	1 500	60	91	25	63

Endospore formers – The counts for both aerobic (ASF) and anaerobic endospore formers (AnSF) either were found to be absent, very low, or with values below 20 000 (Table 3), and surprising showed no correlations to the aerobic colony count values. In many cases no spore formers were detected.

Coliforms – The coliform counts varied from low <200 to an unacceptably high value of 16 000 000 cfu.100 mL^{-1}. The Eerste river counts were on average lower than found for the Plankenburg sites (Figure 1) with the Berg values the lowest (Figure 2). For most of the Plankenburg sites values were much higher, and in cases reaching unacceptable values of >500 000 cfu.100 mL^{-1}. This was especially true for the two sites from the Plankenburg River, the Mosselbank River and the lower Berg River (Berg-3). On average, but not always, it was found that counts were much higher in the warmer summer samples.

Faecal coliforms and E. coli – The thermotolerant faecal coliform counts varied form undetectable, low 100-500 cfu.100 mL^{-1}, to unacceptably high values of >1 000 000 cfu.100 mL^{-1}. The faecal coliforms

dilutions were all confirmed as containing typical *E. coli* on L-EMB plates (Merck, 2007). Therefore the maximum *E. coli* count was also considered >1 000 000 MPN.100 mℓ^{-1} of water (Figures 1 and 2). When applying the DWAF and WHO guideline of <1 000 faecal coliform.100 mℓ, irrigation water, all four rivers can be considered as unsuitable for irrigation of fresh produce intended to be consumed raw (WHO, 1989b; DWAF, 1996). The high *E. coli* counts also suggest high faecal contamination of the water at all sampling sites as the presence *E. coli* is indicative of animal or human faecal pollution sources (Busta *et al.*, 2003). This is consistent with previous studies (Barnes & Taylor, 2004; Paulse *et al.*, 2009; Lötter, 2010).

Salmonella – Typical growth of *Salmonella* was found in the samples from most of the sites (Table 3). Typical colonies on the Baird-Parker plates were identified as round black colonies with a clear zone (Merck, 2007). The data could also be an indication that enterotoxin-producing *Staphylococcus aureus* strains that can cause food poisoning may possibly be present in the water

Listeria – Typical growth of *Listeria* was found in many of the samples and some were identified as *L. monocytogenes*.

Staphylococcus – The counts for *Staphylococcus* were found to be absent to >90 000 cfu.100 mL^{-1}.

Intestinal Enterococci – The counts were low and varied from not detectable to >300 cfu.100 mL^{-1}. The presence of intestinal enterococci was taken as an additional indicator of faecal contamination of the water from these rivers.

3.1.4 Microbial diversity in water samples from the different sites

The following bacterial species were also found to be present in the water samples from the different sites: *Bacillus sp., Citrobacter freundii, Clostridium sp., Escherichia sp., E. coli, Enterococcus sp., E. cloacae, E. aerogenes, E. hirae, Klebsiella pneumoniae, K. pneumoniae spp. pneumoniae, Listeria sp., L. seeligeri, L. ivanovii, L. grayi, L. monocytogenes, Micrococcus sp., Proteus mirabilis, Pseudomonas sp., Salmonella sp., S. enteritidis, S. typhimurium, non-typical-Salmonella, Serratia fonticola, S. marcescens, Shigella sp., S. sonnei, Staphylococcus sp., S. aureus, S. saprophyticus and S. epidermidis.*

The above shows a list of other microbes identified during the sampling period from the sampling sites on the four rivers. Most importantly, is that the isolates did not only include faecal Indicators such as faecal coliforms, *E. coli* and Enterococci, but among the Index organisms' also potential pathogens such as *Klebsiella pneumoniae*, *Listeria monocytogenes*, *Salmonella* and *Staphylococcus aureus*. The presence of such Index organisms in river water is disturbing as most of them, if not all, have been implicated in both waterborne and food-borne outbreaks in the past (Wilkes *et al.*, 2009). It can therefore again be concluded that the water quality from these sites is of unacceptable microbiological quality and could potentially present a threat to the health of any person or animal that is exposed to it. This highlights the importance of Indicator and Index organisms as the types and levels that they are present reflects the state of contamination in river water, without it being necessary to test the water separately for each microbial genus or species.

3.1.5 Discussion and Conclusions

Studies on the three rivers as part of the WRC project showed that they were unsuitable for irrigation of fresh produce because of the high levels of indicator and index organisms. Although no disease outbreak as a direct or indirect use of water from these has been reported, it was considered important to monitor the river for faecal pollution over a longer period so that corrective actions to prevent disease outbreaks can be

implemented based on longer term data. The data obtained showed that all sites continuously had high faecal contamination levels that exceeded DWAF and WHO guidelines of <1 000 *E. coli* per 100 mL water for irrigation of fresh produce intended to be consumed raw. The Plankenburg River had higher faecal contamination levels (*E. coli* = 1 400 000 MPN. 100 mL^{-1}) than the Eerste River (*E. coli* = 79 000 MPN.100 mL^{-1}). The data from this study clearly shows that the Plankenburg River was more polluted than the Eerste and Berg Rivers as both Plankenburg sampling sites (Plank-1 and 3) always had higher *E. coli* loads than the other three rivers sites. Intestinal *Enterococci* loads were also higher for the Plankenburg sites than for the Eerste River site. Furthermore *Staphylococcus* was absent from the Eerste and Berg rivers but always present in the Plankenburg and Mosselbank rivers. The Kayamandi informal settlement just above the Plank-1 site is suspected to be the main source of the faecal pollution as the highest faecal contamination levels were present at this sampling site. Therefore because of the extremely high coliform, faecal coliform and *E. coli* levels which exceeded the DWAF and WHO guidelines of 1 000 cfu.100 mL^{-1} water, and the presence of other index organisms both rivers must be considered unsuitable as water sources for the irrigation of fresh produce intended to be consumed raw.

Although the Eerste and Berg Rivers had in most cases lower faecal levels, they showed the highest incidence of index microorganisms (*Salmonella* and *Listeria*). There were also incidences of intestinal enterococci, Salmonella and endospore formers for both these rivers. Therefore, the high occurrence of faecal indicator and index organisms suggest the presence of potential pathogens that can be carried-over to fresh produce during irrigation. Pathogens are known to survive minimal processing and the likelihood of contaminated fresh produce reaching the consumer is very possible. Also the consumption of contaminated fresh produce may lead to disease outbreaks that can result in death for children, the elderly and those that are medically stressed. Therefore, it was concluded that irrigation with water from both rivers poses a health hazard and should not be allowed until preventative measures have been implemented to minimize faecal pollution of the rivers. We cannot ignore the fact the data clearly identifies potential pathogens of the same genus and species in both water and product and at times the loads are far higher than are acceptable in terms of food safety.

Table 3. The microbiological data for the Plankenburg, Eerste, Berg and Mosselbank sites for period Sept 2007 to March 2010.

Site*	ACC		ASF		AnSF		Coliforms		FC / *E. coli*		Sal		LM		Staph		Entero	
	(cfu.mL^{-1})		(cfu.mL^{-1})		(cfu.mL^{-1})		(MPN.100 mL^{-1})		(MPN.100 mL^{-1})						(cfu.mL^{-1})		(cfu.100 mL^{-1})	
	Min	Max	Min	Max	Min	Max	Min	Max	Min	Max	Min	Max	Min	Max	Min	Max	Min	Max
Plank-1	520	2 760 000	0	16 000	0	20 000	490	7 000 000	350	7 000 000	ND	TG	ND	TG	ND	94 000	20	6 993
Eerste-1	0	7 100 000	0	440	0	0	2 200	35 000	330	3 300	ND	TG	ND	TG	ND	72 000	9	169
Plank-3	9 400	412 000	0	410	0	230	310	2 300 000	36	130 000	ND	TG	ND	TG	ND	68 000	11	2 760
Berg-1	250	32 000	0	0	0	16	23	700 000	23	540 000	ND	TG	ND	TG	ND	ND	ND	209
Berg-2	320	85 000	0	1 370	0	15	440	172 000	49	160 000	ND	TG	ND	TG	ND	TG	ND	405
Berg-3	410	440 000	0	1 000	0	110	230	1 700 000	110	1 700 000	0	TG	ND	TG	ND	600	11	332
Mossel bank	12400	128 000 000	0	9 000	0	670	1 100	16 000 000	440	1 600 000	TG	TG	ND	TG	0	6 100	ND	267

ND = None Detected; TG = Typical growth

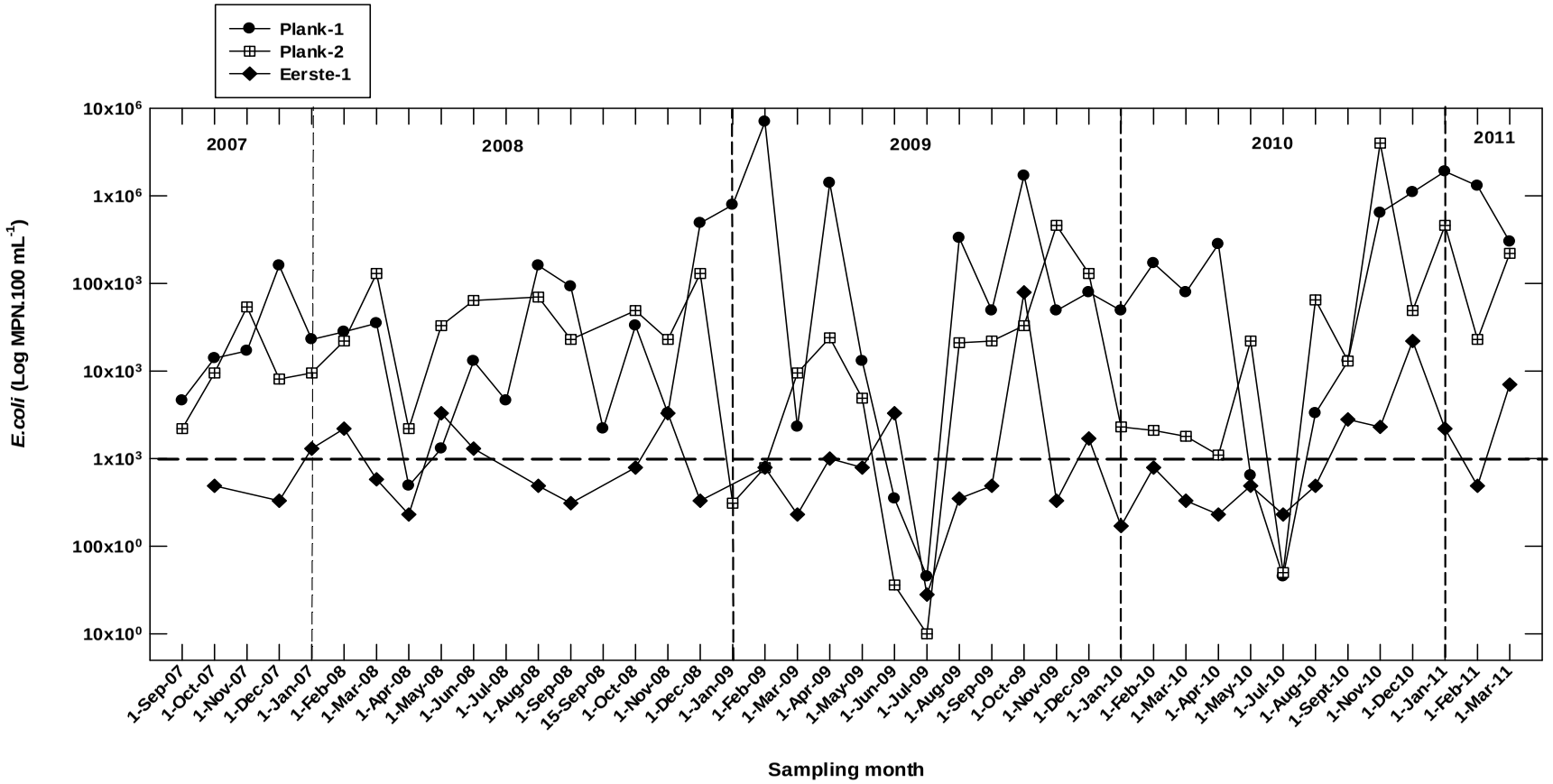

Figure 1. The *E.coli* data obtained for the Plankenburg (Plank-1 and 2) and Eerste River sites for the period September 2007 to March 2011. The dashed line indicates the WHO and DWAF guideline limit for the acceptable presence of faecal coliforms in irrigation water.

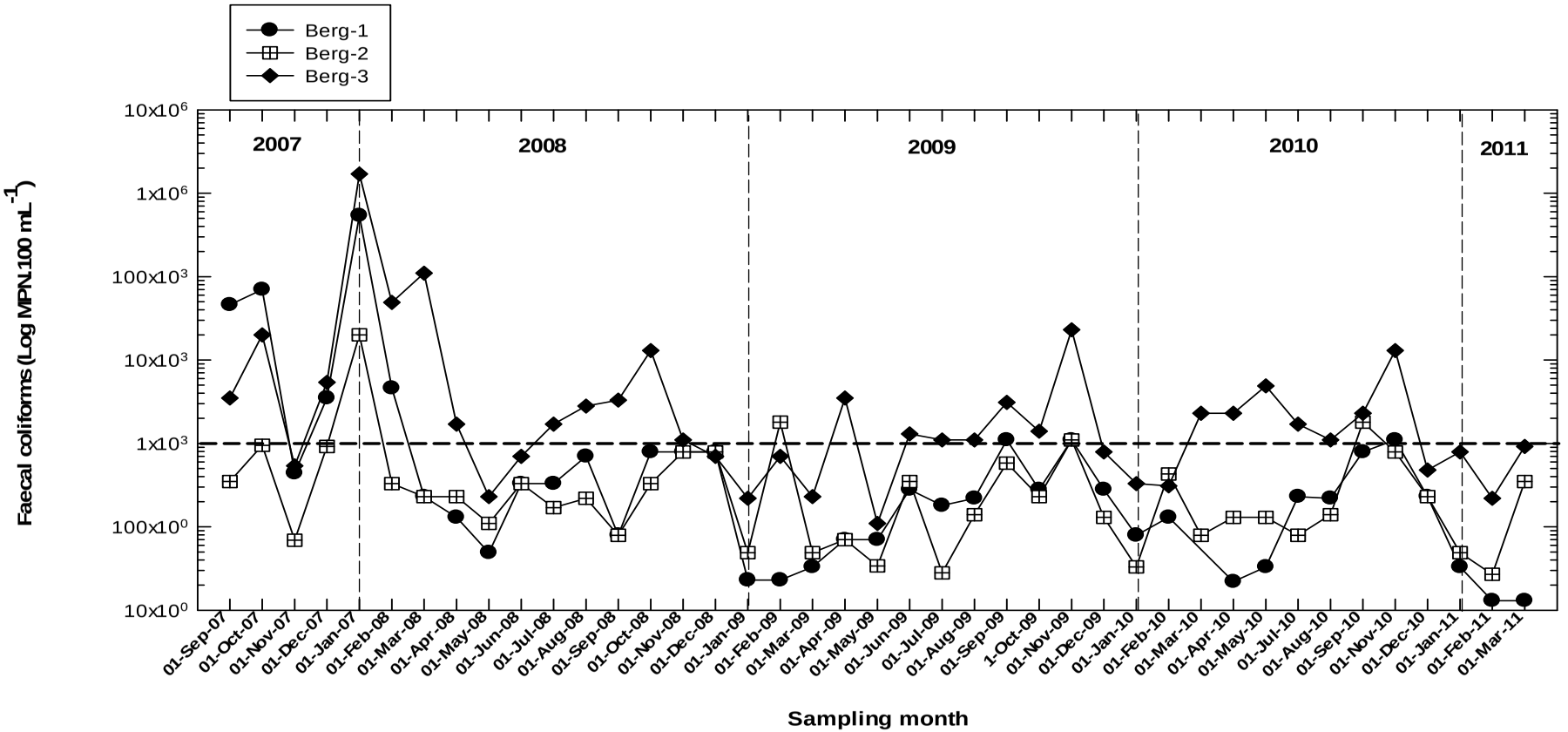

Figure 2. The faecal coliform data obtained for the three Berg River sites for the period September 2007 to March 2011. The dashed line indicates the WHO and DWAF guideline limit for the acceptable presence of faecal coliforms in irrigation water.

3.2 ACCURACY OF METHODS FOR THE ENUMERATION OF COLIFORMS AND *ESCHERICHIA COLI*

3.2.1 Specific aim

The aims were to determine whether Colilert is an appropriate rapid alternative for MTF in the microbial analysis of river water; and to determine whether there is a difference in the performance of Colilert at high, intermediate and low levels of faecal pollution.

3.2.2 Background

The use of the Multiple Tube Fermentation (MTF) method to accurately enumerate coliforms, faecal coliforms and *E. coli* is the standard method for the analysis of faecal contamination. The accuracy of the method is attributable to selective hurdles. However, the method has several practical drawbacks. A large number of coliforms and 5% of *E. coli* (Moberg, 1985) do not produce gas during lactose fermentation (Fricker *et al.*, 1997). In addition, the method is expensive, labour intensive, and the results are only available after 4 days (Maheux *et al.*, 2008). This has resulted in a need for more rapid enumeration methods for water and food products (Anonymous, 2000). The Colilert method has only recently gained popularity for the rapid enumeration of coliforms and *E. coli*, after automation was improved and the Quanti-Tray for enumeration was developed. This method has several practical advantages over MTF, as analysis is more automated and results are available within 18 hours (Fricker *et al.*, 2008; Wohlsen *et al.*, 2008). Furthermore, anaerogenic coliforms (Fricker *et al.*, 1997) do not affect the results (Wohlsen *et al.*, 2008) as is the case with MTF, which relies on the production of gas through the fermentation of lactose.

3.2.3 Comparison of methods

Coliforms – The data set for coliforms consisted of 56 MTF and corresponding Colilert values for each sample. Mean, median and standard deviation values were calculated for each set. The coliform data for the Berg River was found to vary from 200-198 630 MPN.100 mL^{-1}. When the calculated means were compared for each site, it was clear that Colilert tends to produce higher values than for MTF for all sites in the upper Berg River. This was attributed to the ability of Colilert to include anaerogenic coliforms (Fricker *et al.*, 1997; Wohlsen *et al.*, 2008), whereas MTF did not. For Berg-1, the least faecally polluted of the three sites in the upper Berg River, the mean Colilert value was 7 times higher than the mean value for MTF. For Berg-2, also lightly faecally polluted, this was lower, with the mean for Colilert 3.5 times higher than that for MTF. The mean Colilert value for water obtained from Berg-3, the most polluted, was only around 2.5 times higher than the mean value calculated for MTF. These observations are interesting, since it alludes to the possibility that Colilert enumerations for coliforms tend to agree more closely with MTF values at higher pollution levels.

The coliform data for the Plankenburg River showed variations between 33 000 and 2 419 600 MPN.100 mL^{-1}. Mean, median and standard deviation values were also calculated. In this case the results showed that the mean Colilert value was nearly double the mean value for MTF for the Plank-1 site. The values for Plank-3 showed that the Colilert mean value was >4 times higher than the mean value for MTF. The differences between mean values for the two sites followed the same trend with respect to pollution levels which was observed previously for the Berg River. This trend shows a closer agreement between Colilert and MTF coliform enumerations in rivers which are heavily polluted, when compared to river water with a low level of faecal pollution.

The coliform data sets for the Eerste and Lourens rivers showed a variation of 330 to 178 500 MPN.100 mL^{-1}. The mean Colilert value for the Eerste River was >3 times higher than the mean value for MTF. It did appear that higher Colilert enumerations were consistent in their occurrence for this site and that the difference between Colilert and MTF mean values was not a function of occasional high value spikes in Colilert values.

The conclusions from the results on the Lourens River should be drawn with caution due to the small data set. The only conclusion to be drawn from the data obtained was as previously found that Colilert tended towards higher coliform levels than MTF.

The results show that Colilert consistently gave higher counts than MTF in the coliform enumeration. This is in agreement with results reported in literature (Eckner, 1998; Noble *et al.*, 2004; Kampfer *et al.*, 2008; Al-Turki & El-Ziney, 2009). These results indicate that Colilert outperforms MTF in the recovery of coliforms from river water.

Escherichia coli – The *E. coli* data set for the Berg River varied from 20 to 23 000 MPN.100 mL^{-1}. Mean, median and standard deviation values were calculated. During the study several values were found to be higher than the DWAF and WHO guideline of <1 000 faecal coliforms per 100 mL for water used for the irrigation of produce likely to be consumed raw (WHO, 1989b; DWAF, 1996).

The mean values for Colilert were >2 and 4 times higher than MTF values for Berg-1 and Berg-2, respectively. The mean value for Colilert at Berg-3 was slightly lower than the mean value for MTF. This is indicative of a similar trend observed for the coliforms enumerations by Colilert to count higher than MTF when *E. coli* is analysed. In addition the Berg-1 and 2 sites are considered to be less polluted than Berg-3. This indicates the possibility that the agreement of Colilert to MTF when more polluted water is studied may be a phenomenon found in the enumeration of both coliforms and *E. coli*.

The *E. coli* data set for the Plankenburg River showed a variation between 740 and 1 300 00 MPN.100 mL^{-1}. The MTF values again exceeded the WHO and DWAF guideline of <1 000 faecal coliforms per 100 mL for water used in the irrigation of fresh produce (WHO, 1989b; DWAF, 1996). The mean values in this case indicated, contrary to the previous results, that Colilert tended towards lower *E. coli* enumeration when compared to MTF. The mean value for Colilert at Plank-1 was less than half of the mean value for MTF, while the mean value for Colilert at Plank-3 was 7 times lower than the mean value for MTF. The reason for this reaction is not clear but this could possibly be ascribed to the composition of the water as this river flows through the Plankenbrug industrial area. The composition of the effluents which reach the river is unknown but it can be speculated that some chemical compound in the industrial effluents could have caused interference with the Colilert detection of *E. coli*.

The mean values for the Eerste River showed the same trend as found for the Plankenburg River; with the MTF mean <1.5 times the mean value for Colilert. This was attributed to the higher enumeration by MTF and not due to one isolated instance of a considerably higher value. These results are surprising as the Eerste River is considered a “less polluted” river. The *E. coli* counts for the Eerste River showed only two instances of exceeding the WHO and DWAF guideline.

In the case of the Lourens River two cases were found that could indicate sewage intrusion. If this intrusion is taking place upstream of agricultural activities which use the Lourens River as irrigation source, this river could also pose a health risk when used to irrigate fresh produce.

The *E. coli* results showed that while Colilert tended towards higher enumeration for the Berg River, this method was consistently lower when compared to MTF for the Plankenburg, Eerste and Lourens

Rivers. The results are in agreement with the work by Noble (Noble *et al.*, 2004), who reported lower Colilert values when enumerating *E. coli* from coastal waters. In contrast studies in Saudi Arabia on drinking water reported that Colilert had a significantly higher ($p>0.05$) recovery rate (13.7%) when compared to MTF (7.5%) (Al-Turki & El-Ziney, 2009). It is possible that high levels of pollution interfere with the recovery rate of *E. coli* when using Colilert. This was also reported by (Olstadt *et al.*, 2007), who found that the recovery rate of *E. coli* when using Colilert decreased when microbial concentrations in spiked groundwater were increased from 1-10 to 50-100 bacteria.

3.2.4 Conclusions

Colilert as rapid alternative for enumeration of coliforms – The preliminary results of the evaluation of Colilert as an alternative method for MTF indicated that Colilert mostly showed low (upper Berg River) to intermediate (Plankenburg River) levels of agreement with MTF enumerations and that the disagreement between the two methods were primarily due to a tendency by Colilert to enumerate higher coliform values than MTF. It was shown by the Bland and Altman scatterplot that Colilert agreed well with MTF at lower levels of pollution with coliforms (0 to 100 000 coliforms MPN.100 mL^{-1}) and became increasingly prone to error towards higher enumeration as pollution levels increased beyond 100 000 MPN.100 mL^{-1}. The r^2 value (Spearman coefficient) was acceptable enough ($r^2=0.69$) to recommend that Colilert can be used for the rapid detection of coliforms in river water.

Colilert as rapid alternative for enumeration of E. coli – The preliminary results of the evaluation of Colilert as an alternative method for MTF enumeration of *E. coli* showed that low (Plankenburg River) to intermediate (Berg River) levels of agreement between the two methods were obtained. When disagreement occurred between the methods for samples from the upper Berg River, Colilert tended towards higher enumeration than MTF. In contrast, disagreement between the methods for water from the Plankenburg, Eerste and Lourens Rivers resulted in Colilert tending towards lower enumeration when compared to MTF.

The *E. coli* data revealed (Bland and Altman scatterplot), that Colilert values agreed well with MTF values in the range 0 and 50 000 *E. coli* MPN.100 mL^{-1}. In contrast with the results for coliforms, Colilert tended strongly towards lower enumeration than MTF when the *E. coli* numbers exceeded 50 000 MPN.100 mL^{-1}. These results are a reflection of the trend observed in water samples from the Plankenburg, Eerste and Lourens Rivers and are further evidence to support the theory that the Berg River differed due to its lower faecal pollution levels. Despite these discrepancies, the results indicate that Colilert can be used confidently in place of MTF, in water samples with *E. coli* levels ranging from 0 to 50 000 MPN.100 mL^{-1}. The r^2 value is high enough to recommend the use of Colilert as a rapid alternative for MTF in water analyses where immediate action based on the results is imperative. However, the 26.0% risk of inaccurately representing the MTF enumeration will more than likely manifest in an under-estimation by Colilert, a point which needs to be kept in mind when the threat posed for the irrigation of fresh produce is assessed.

These results show that river water samples with low to intermediate levels of faecal pollution can be confidently analysed, for both coliforms and *E. coli*, with Colilert instead of MTF. However, in the analysis of high faecally polluted water (>100 000 coliforms MPN.100 mL^{-1} or >50 000 *E. coli* MPN.100 mL^{-1}) the results for coliforms will most likely be over-estimated, while the results for *E. coli* are probably under-estimated. In addition, the observation that Colilert tends towards increasingly inaccurate

enumerations above certain levels of both coliforms and *E. coli* indicates that this method may have upper operational limits above which enumerations are no longer reliable.

3.3 KWAZULU-NATAL PROVINCE

3.3.1 Specific aims

To enable a microbial risk assessment, this research project intended to verify the seasonal microbial burden of river water used for irrigation in local community gardens in Sobantu, Pietermaritzburg, KwaZulu-Natal.

3.3.2 Physico-Chemical results

The physico-chemical analyses of the Baynespruit River, Pietermaritzburg, KwaZulu-Natal over 13 months revealed highest river water temperatures of >22°C during the months of January 2010 to March 2010 and October 2010 to January 2011 (Table 4). The maximum value of 27.2°C was measured in February 2010 while the lowest value was measured in June 2010 with only 11.4°C. These temperatures represent the changing seasonal temperatures. The pH values oscillated within a range of 7.05-8.40 with a maximum pH value of 8.40 measured in both January 2010 and May 2010. According to the DWAF (DWAF, 1996) guidelines, the pH of raw water is acceptable for irrigation within a pH range of 6.50-8.50. The COD (chemical oxygen demand) values determined stayed in a range of 14-107 $mg.L^{-1}$ as was reported for other rivers in South Africa (de la Rey *et al.*, 2004).

Table 4. Physico-chemical data for river water samples from the Baynespruit River over 13 months.

Date	pH	Temperature (°C)	COD ($mg.L^{-1}$)
January 2010	8.40	22.4	96
February 2010	7.81	27.2	54
March 2010	7.66	22.2	45
April 2010	7.67	20.2	56
May 2010	8.40	14.6	44
June 2010	7.58	11.4	107
July 2010	7.38	16.3	88
August 2010	7.56	17.7	39
September 2010	7.05	20.4	34
October 2010	7.38	26.0	14
November 2010	7.29	26.2	26
December 2010	7.40	23.4	56
January 2011	8.30	24.7	20

3.3.3 Microbiological evaluations

The microbiological quality of the Baynespruit River was assessed by establishing the *E. coli* count, the count for aerobic and anaerobic endospore formers and the presence of *Salmonella* spp., *Staphylococcus aureus*, intestinal enterococci, and *Listeria monocytogenes* (Table 5). In addition, the aerobic plate count and total and faecal coliform counts were determined (Figure 3). For all months but August 2010 the *E. coli* counts of river water samples were exceeding 1 000 MPN.100 mL^{-1}. In addition, *Salmonella* spp. and intestinal enterococci were detected in river water samples for each individual month, while *S. aureus* was Present in 6 out of the 13 monthly water samples analysed. The counts for aerobic and anaerobic endospore formers did not exceed a value of 1 800 cfu.mL^{-1} over 13 months. The aerobic plate count for the Baynespruit River ranged from 7 300 (October 2010) to 301 000 (July 2010) cfu.mL^{-1} These results are in a similar range as data reported for polluted rivers in the Venda region in South Africa (Obi *et al.*, 2002). Further corroborating the poor state of the river quality are the total and faecal coliform counts detected for example for March 2010, which are in fact in a range reported in the literature for raw sewage (Bell *et al.*, 1981; Vilanova *et al.*, 2004).

As indicated by the *E. coli* counts (Table 5), faecal coliform counts exceeded the WHO guideline value of 1 000 faecal coliforms per 100 mL (WHO, 2006b)(Fig. 1) over 13 months, indicating that the Baynespruit River water is not suitable for irrigation.

Similar to the data established for the Baynespruit River (Table 4), the Msunduzi River exhibited river water temperatures of ≥22°C during the months of January 2010 to March 2010 and September 2010 to January 2011 (Table 6). The maximum value of 26.3°C was measured in February 2010 while the lowest value of 14.6°C was measured in June 2010. As for the Baynespruit River, these temperatures are a reflection of the changing seasonal temperatures. Msunduzi River water pH values ranged from 7.18 to 8.47, with the maximum pH value of 8.47 detected in January 2010. As for the Baynespruit River, these pH values indicate the principal suitability of Msunduzi River water for irrigation (DWAF, 1996). For the Msunduzi River, COD values were established in a range of 17-118 mg.L^{-1}, again indicating a similarity to the Baynespruit River in view of the physico-chemical properties determined over 13 months.

Table 5. Microbiological data from river water samples from the Baynespruit River over 13 months.

Date	*E. coli* (MPN.100 mL^{-1})	ASF/AnSF (cfu.mL^{-1})	IE	SA	LM	Sal
January 2010	**	720/840	TG	TG	ND	TG
February 2010	4 900	TFTC/TFTC	TG	ND	ND	TG
March 2010	350 000	190/700	TG	ND	ND	TG
April 2010	1 300	TFTC/TFTC	TG	ND	ND	TG
May 2010	17 000	TFTC/1800	TG	ND	ND	TG
June 2010	6 800	600/600	TG	ND	ND	TG
July 2010	33 000	TFTC/TFTC	TG	ND	ND	TG
August 2010	240	120/140	TG	TG	ND	TG
Sept. 2010	3 300	TFTC/250	TG	ND	ND	TG
October 2010	1 700	TFTC/150	TG	TG	ND	TG
November 2010	110 000	TFTC/150	TG	TG	ND	TG
December 2010	23 000	360/300	TG	TG	ND	TG
January 2011	7 000	TFTC/150	TG	TG	ND	TG
TG=typical growth; TFTC= number <10 at lowest dilution; ND=not detected; **=*E. coli* present						

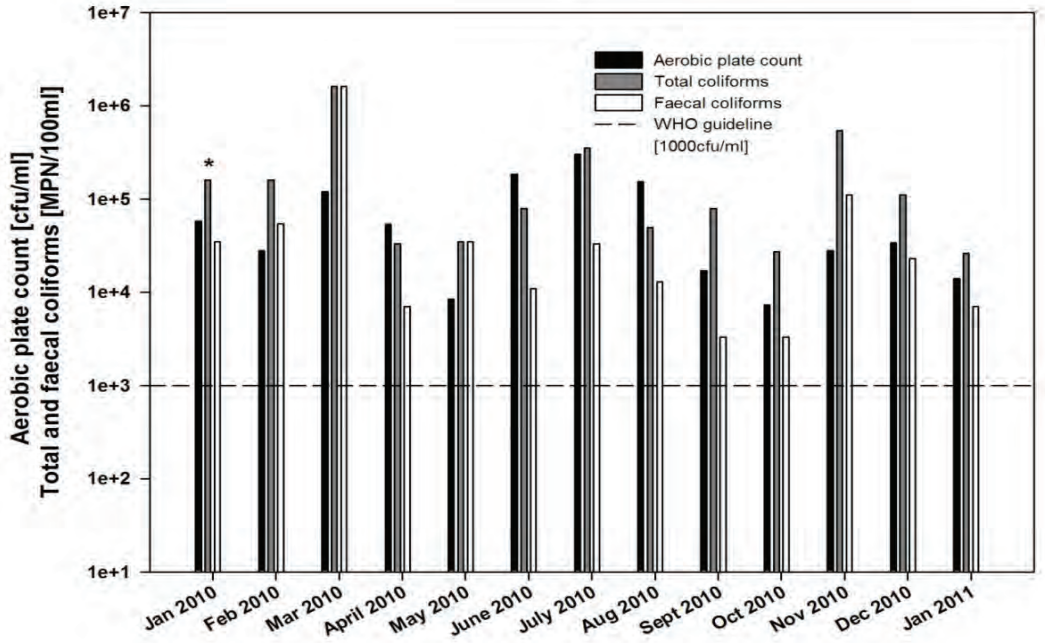

Figure 3. ACC count and the TC and FC counts for the Baynespruit River over 13 months (y-axis: log scaling). * = TC count exceeded 160 000 MPN.100 mL^{-1}. The dotted line indicates the WHO guideline limit for the acceptable presence of faecal coliforms in irrigation water.

Table 6. Physico-chemical data for river water samples collected from the Msunduzi River over 13 months.

Date	pH	Temperature (°C)	COD ($mg.L^{-1}$)
January 2010	8.47	22.0	118
February 2010	7.99	26.3	99
March 2010	7.72	22.8	62
April 2010	7.71	19.6	84
May 2010	7.55	16.5	114
June 2010	7.58	14.6	69
July 2010	7.28	17.1	49
August 2010	7.83	19.0	45
September 2010	7.18	22.5	25
October 2010	7.22	24.9	17
November 2010	7.29	26.0	21
December 2010	7.52	23.7	20
January 2011	7.80	26.1	27

Table 7. Microbiological data obtained from samples from the Msunduzi River over 13 months.

Date	*E. coli* (MPN.100 mL^{-1})	ASF ($cfu.mL^{-1}$)	Entero	SA	LM	Sal
January 2010	0	4 200/2 200	TG	TG	ND	TG
February 2010	3 300	TFTC/TFTC	TG	TG	ND	TG
March 2010	3 500	500/500	TG	ND	ND	TG
April 2010	790	TFTC/TFTC	ND	ND	ND	ND
May 2010	110	TFTC/750	ND	ND	ND	ND
June 2010	<18	600/500	ND	ND	ND	ND
July 2010	180	TFTC/TFTC	TG	ND	ND	ND
August 2010	2 400	TFTC/120	TG	TG	ND	TG
September 2010	3 300	TFTC/100	TG	ND	ND	TG
October 2010	4 900	TFTC/TFTC	TG	TG	ND	TG
November 2010	7 900	150/165	TG	TG	ND	TG
December 2010	7 900	185/190	TG	TG	ND	TG
January 2011	1 700	TFTC/TFTC	TG	TG	ND	TG

TG=typical growth; TFTC= number was <10 at lowest dilution; ND=not detected; **=*E. coli* Present but not quantified

The *E. coli* counts established for Msunduzi River water samples exceeded 1 000 MPN.100 mL^{-1} (Table 7) for 8 months. In addition, *Salmonella* spp. and intestinal enterococci were detected in river water samples for 9 out of 13 and 10 out of 13 months, respectively. The presence of *S. aureus* in Msunduzi River water samples was shown for 7 out of 13 months analysed while *L. monocytogenes* was not detected. The highest counts for aerobic and anaerobic endospore formers were established for January 2010 with 4 200 (aerobic endospore formers) and 2 200 (anaerobic spore formers) $cfu.mL^{-1}$. However, in the 12 subsequent months analysed these values did not exceed a value of 750 $cfu.mL^{-1}$. The aerobic plate count for the Msunduzi River ranged from a maximum value of 18 000 $cfu.mL^{-1}$ in January 2010 to 2 800 $cfu.mL^{-1}$ in September 2010 (Fig. 4). These results are much lower than those established for the Baynespruit River over the same period of time (Fig. 3). However, the mostly unsatisfactory quality of the Msunduzi River water is indicated by the fact that faecal coliform counts exceeded 1 000 MPN.100 mL^{-1} in 9 out of the 13 months analysed (January 2010 to March 2010 and August 2010 to January 2011). The highest value for the faecal coliform count was established for March 2010 with 63 000 MPN.100 mL^{-1} (Fig. 4). Hence for most of the time faecal coliform counts exceeded the WHO guideline value of 1 000 faecal coliforms per 100 mL^{-1} (WHO, 2006b).

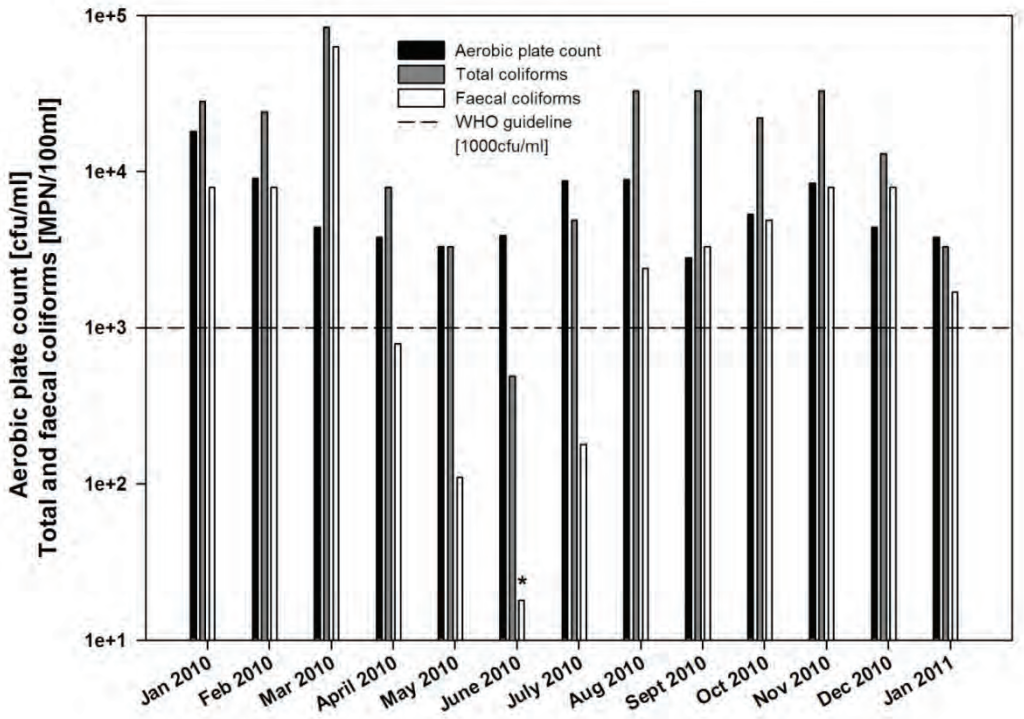

Figure 4. Aerobic plate count and total and faecal coliform counts for the Msunduzi River over 13 months (y-axis: log scaling). *Faecal coliform count <18. The dotted line indicates the WHO guideline limit for the acceptable presence of faecal coliforms in irrigation water.

3.3.4 Discussion and Conclusion

Based on the microbiological analyses of Baynespruit and Msunduzi River water samples over a period of 13 months, the level of faecal coliforms Present in these water samples used for irrigation of produce mostly exceeded the WHO recommendation value for safe irrigation of 1 000 faecal coliforms per 100 mL (WHO, 1989b; 2006b). The microbiological analyses showed that in addition to *E. coli* (established values mostly exceeding 1 000 MPN per 100 mL river water over 13 months), the most reliable indicator for faecal contamination and a potential pathogen (i.e. as in case of toxin producing strains such as STEC), intestinal enterococci and other potentially pathogenic bacteria such as *Salmonella* spp. and *S. aureus* were frequently detected in water samples collected from both rivers. The use of Baynespruit and Msunduzi River water is therefore not recommended for produce irrigation.

3.4 VENDA PROVINCE

3.4.1 Specific aims

This study will investigated the microbiological quality of water used by subsistence farmers for irrigation of MPF products in the Limpopo province of South Africa.

3.4.2 Physico-Chemical results

The pH values for river water varied between 6.00 and 8.41 (Table 8). The pH values fell within the South African water quality pH guideline range of 6.0 to 9.0 (DWAF, 1996). Several studies have indicated that

pH could play a role in the survival of microorganisms during disinfection (Engelbrecht *et al.*, 1980; Schaper *et al.*, 2002).

The South African recommended guideline values for temperature ranged between 18ºC to 24ºC (DWAF, 1996). In this study the temperatures for all water source samples fell well within this range. Several studies have shown that temperature plays an important role in the survival of microorganisms.

Turbidity measurements give a general indication of the concentration of suspended clay, silt, organic matter, inorganic matter, plankton and other microscopic organisms in a water source (DWAF, 1996). In this study the river water source samples had turbidity values which exceeded the recommended South African guideline value of 0.1 NTU (Table 8) (SABS-ISO, 2001). High turbidity values are associated with the survival of microorganisms due to association of the microorganisms with particulate matter in the water (DWAF, 1996).

3.4.3 Microbiological evaluations

Total Coliforms, faecal coliforms and E. coli – The minimum total coliform counts (TC) in water was 14 cfu.100 mL^{-1}, the maximum Total coliform counts was >2 400 cfu.100 mL^{-1} (Fig. 5). The minimum faecal coliform counts (FC) in water was 14 cfu.100 mL^{-1}, the maximum faecal coliform counts was >2 400 cfu.100 mL^{-1}. The presence of faecal coliform in water is the indication of faecal contamination.

Diarrhoeagenic E.coli strains – It was found that many of the samples contained more than one diarrhoeagenic *E.coli* strain (Table 9). A total of 27 water samples over the study period from the MPN isolation showed *E. coli* presence and of these 37% were positive for the commensal *E. coli* gene. Enteropathogenic *Escherichia coli* (EPEC) are an important cause of diarrhoea in young children in developing countries. EPEC strains are grouped on the basis of genotype as atypical EPEC when they possess eae gene only, typical EPEC when they possess eae and bfpA genes, STEC when they possess stx1 and/or stx2, EHEC when they possess eae and stx1 and/or stx2 and non-pathogenic *E. coli* does not possess either of these genes eae, bfpA, stx1 and stx2 (Bugarel *et al.*, 2011). Other studies (Trabulsi *et al.*, 2002) have shown that humans are the only reservoir for typical EPEC, whereas both animals and humans are the reservoirs for atypical EPEC. In the studies by Nataro and co-workers (Nataro & Kaper, 1998) typical EPEC is well recognized as a cause of gastroenteritis in infants in developing countries (Nataro & Kaper, 1998). In this study the presence of typical EPEC is of health risk to humans as they are able to cause infection to human. In this study 48% of the samples showed the presence of pathogenic atypical EPEC strains and 22% tested positive for the prevalence of typical EPEC strains. In addition, 56% of the samples had Enteroaggregative *E. coli*, 11% of the samples contained Enteroinvasive *E. coli* and 30% respectively of the samples tested positive for Enterohaemorrhagic *E. coli* and Enterotoxigenic *E. coli* bacteria (Table 6). Enterotoxigenic *E. coli* is the major cause of traveller's diarrhoea worldwide. Infection with ETEC leads to watery diarrhoea which lasts up to a week, but can be treated. Abdominal cramps, sometimes with nausea and headache occur and fever is usually absent (Harris *et al.*, 2003; Ahmed *et al.*, 2007a; Ahmed *et al.*, 2007b).

Table 8. Physico-chemical data for irrigation water from the Phadzima irrigation canal, Limpopo Province.

Date	pH	Temperature (°C)	Conductivity ($\mu S.m^{-1}$)	Turbidity (NTU)
October 2008	7.90	22.3	56.6	15.46
November 2008	7.70	ND	ND	ND
December 2008	8.14	19.4	66.5	30.4
January 2009	ND	ND	ND	ND
February 2009	7.43	ND	84.3	7.52
March 2009	8.02	ND	37.02	8.06
April 2009	6.45	20.0	187.6	27.0
May 2009	7.28	ND	36.0	2.16
June 2009	6.00	13.4	23.3	3.18
July 2009	7.00	11.7	96.7	1.81
August 2009	7.21	14.0	103.2	1.83
Sept. 2009	7.53	18.7	56.7	2.30
October 2009	7.91	20.2	70.5	6.30
November 2009	7.80	17.6	20.3	1.50
December 2009	No water	No water	No water	No water
January 2010	ND	ND	ND	ND
February 2010	ND	ND	ND	ND
March 2010	ND	ND	ND	ND
April 2010	8.09	21.2	65.3	5.03
May 2010	ND	ND	ND	ND
June 2010	7.14	18.3	66.3	2.05
July 2010	6.19	18.7	150.2	0.83
August 2010	6.63	21.1	109.0	1.25
September 2010	8.15	19.3	119.1	0.58
October 2010	8.27	19.8	114.1	1.06
November 2010	8.41	18.5	113.4	1.11
December 2010	8.07	20.3	109.9	1.59
January 2011	7.20	19.2	78.3	2.30
February 2011	8.21	20.3	105.4	1.30
March 2011	7.93	18.3	11.3	2.54
April 2011	8.30	19.8	97.5	1.12
May 2011	8.03	20.2	65.3	0.72
June 2011	7.30	17.8	109.2	2.86

Table 9. Prevalence of commensal and diarrhoeagenic *Escherichia coli* bacteria obtained from irrigation water from the Phadzima irrigation canal, Limpopo Province.

Date	Sample	*E. coli*
October 2008	Water	-
November 2008	Water	AP / I
December 2008	Water	A / AP / I
January 2009	Water	-
February 2009	Water	AP / T / I
March 2009	Water	AP

April 2009	Water	AP / T / A
May 2009	Water	AP
June 2009	Water	A / H
July 2009	Water	AP / A
August 2009	Water	AP / H / A
October 2009	Water	H / A / AP
November 2009	Water	AP / A
December 2009	Tomato	TP
April 2010	Water	TP / H / T / A
June 2010	Water	AP / T / A / H
July 2010	Water	C
August 2010	Water	TP / C
September 2010	Water	TP / T / A / AP / H
October 2010	Water	C / AP
November 2010	Water	C / T / A / H / TP
December 2010	Water	C / TP / T / A
February 2011	Water	C / A
March 2011	Water	C / A
April 2011	Water	C / T / H
May 2011	Water	C / A
June 2011	Water	C

C = commensal *E. coli;* A = Enteroaggregative *E. coli;* T = Enterotoxigenic *E. coli;* I = Enteroinvasive *E. coli;* H = Enterohaemorrhagic *E. coli;* AP = Atypical Enteropathogenic *E. coli;* TP = Typical Enteropathogenic *E. coli*

Enteroinvasive *E. coli* are transmitted through the faecal-oral route and even minimal contact is adequate for transmission. EIEC *E. coli* have been associated with diarrhoeal illness in infants and travellers and linked to outbreaks associated with acute or persistent diarrhoea especially in developing countries. Infection is typically followed by a watery mucous, diarrheal illness with little to no fever and an absence of vomiting (Ahmed *et al.*, 2007a). Entero-haemorrhagic *E. coli* strains are produced from domestic animals and humans and are spread in the environment by faeces thus can remain viable in the soil or water for months (Nataro & Kaper, 1998). EHEC was implicated to cause diarrhoea in infants, haemorrhagic colitis and haemolytic uremic syndrome which is a severe clinical manifestation of infections with Shiga-like toxin producing *E. coli* (Blank *et al.*, 2003).

Aerobic colony counts (ACC) – The minimum ACC counts in water were 100 cfu.mL^{-1}, the maximum aerobic/heterotrophic plate counts were 30 000 000 cfu.mL^{-1}. According to DWAF and FAO guidelines the maximum limits of ACC counts in irrigation water is 100 cfu.mL^{-1}, which means that the values of aerobic/heterotrophic plate counts in the present study for water were much higher than the acceptable maximum limits (DWAF, 1996; WHO, 2006b; 2006c). The presence of aerobic/heterotrophic plate counts in water indicated the presence of potential pathogenic and opportunistic microorganisms which could be a health risk to consumers (Lye & Dofour, 1991). Generally aerobic/heterotrophic plate counts are considered harmless.

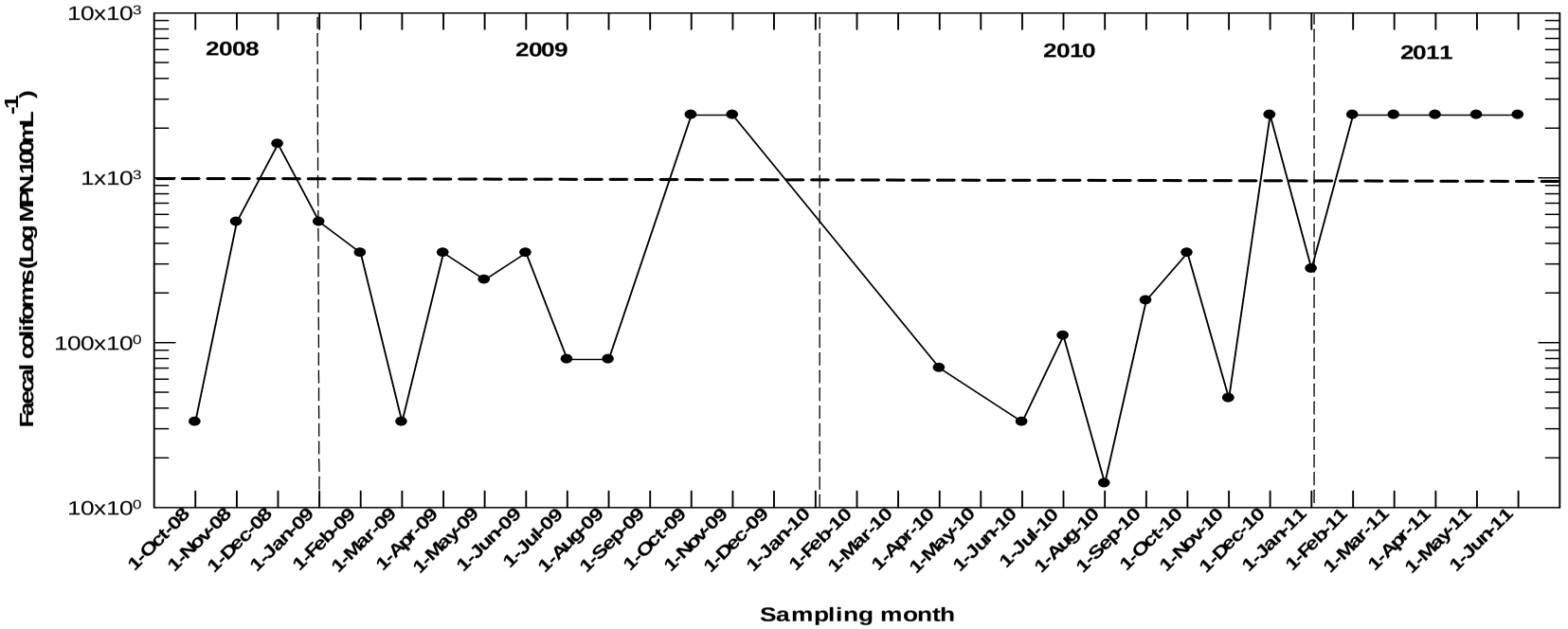

Figure 5. The *E.coli* data obtained for the from irrigation water from the Phadzima irrigation canal, Limpopo Province.

The dashed line indicates the WHO and DWAF guideline value. The dashed line indicates the WHO and DWAF guideline value.

The predominant heterotrophic bacterial species that have been shown by a number of studies are *Acinetobacter*, *Aeromonas*, *Alcaligenes*, *Comamonas*, *Enterobacter*, *Flavobacterium*, *Klebsiella*, Legionella, *Moraxella*, *Pseudomonas*, *Sphingomonas*, *Stenotrophomonas*, atypical *Mycobacterium*, *Bacillus* and *Nocardia* (WHO, 2003; 2006a).

However studies have showed that some aerobic/heterotrophic plate counts are opportunistic pathogens (Payment *et al.*, 1991). Opportunistic pathogens such as *Flavobacterium* sp., *Klebsiella pneumonia*, *Bacillus* sp., and *Enterobacter* sp. have been associated with diseases in immunocompromised individuals, infants, and elderly during exposure to or consumption of contaminated water (Payment *et al.*, 1991). *Legionella* sp can cause a potential fatal disease characterized by more severe form of the infection and produces high fever and pneumonia. *Mycobacteria sp* can cause infections such as abscesses, septic arthritis, and osteomyelitis. Sometimes they can also infect the lungs, lymph nodes, gastrointestinal tract, skin, and soft tissues. *Acinetobacter* can cause pneumonia, skin and wound infections, urinary tract infection blood infections. Diseases caused by *Klebsiella* sp include pneumonia, urinary tract infections, ankylosing spondylitis septicemia and wound infections. The infectious disease of heterotrophic bacteria becomes more severe in immune-compromised individuals (WHO, 2003).

In *Pseudomonas genus*, *Pseudomonas aeruginosa* is the most important species for public health considerations, which can cause infections of many body parts, including skin, ears, eyes, wounds, bones and joints, the lungs, heart, central nervous system and the urinary tract. The genus *Pseudomonas* is a gastrointestinal pathogen and routinely enumerated in aerobic/heterotrophic plate counts determination and considered by some as an opportunistic pathogens when found in water and possesses the required number of virulence factors to cause infection. However it will not proliferate on normal tissue but requires previously damaged organs.

The risk to human health is that only certain specific hosts are at risk, including patients with profound neutropenia, cystic fibrosis, severe (Hardalo & Edberg, 1997). *Aeromonas sp* has been associated with diarrhoeal disease in many cases (WHO, 1984). *Aeromonas* sp. is found in drinking water and it has been suggested as an opportunistic pathogen when Present in drinking water. It is a gastrointestinal pathogen by ingestion (Payment *et al.*, 1991). A small percentage of *Aeromonas hydrophila isolates* can cause gastroenteritis and enteritis and produce modest, self-limited infection. Although most cases are food-borne, few waterborne cases were associated with ingestion of untreated drinking water from swallow wells. Only small percentage of an *Aeromonas hydrophila* isolates possess human virulence factors (Rusin *et al.*, 1997).

Proteus genus has four species of which three cause disease. All strains are urease positive and motile. They may swarm on blood agar, producing concentric zones or an even film. They are resistant to polymyxin B and colistin. *Proteus* species can resemble non-motile *Salmonella* biochemically and can agglutinate in polyvalent *Salmonella* antisera. Genus *Providencia* was originally established for organisms similar to *Proteus* species that were urease negative.

Pathogenic *Enterobacter* species cause health problems which include vaginal infections, urinary tract infections, and pelvic inflammatory disease. *Enterobacter sakazakii* is a Gram negative, motile, peritrichous non spore forming, facultative anaerobic bacterium; it is an opportunistic pathogen in infants. *Enterobacter* sp has an eleven species, but only eight have isolates from clinical materials. They grow readily on ordinary agar, ferment glucose with the production of acid and agar and are motile by peritrichous flagella. Some strains with a k antigen possess a capsule, *Enterobacter sakazakii* can cause

bacteraemia and meningitis in infants and been isolated from infants in association with nerotising enterocolitis (WHO, 2006a).

Pantoea sp refers to Gram negative bacteria that are usually found in plants and in the faeces of humans and animals. *P. agglomerans* is associated with the most common infection known as arthritis or synovitis whereas other reported infections caused by *Pantoea sp* include ostitis, cholelithiasis, occupational respiratory infections, skin allergy and blood stream infection in an elderly person. Other species than *P. agglomerans* rarely cause human infection. According to studies done by Habsah (Habsah *et al.*, 2005) *Pantoea* sp have been implicated in more than 95% of all outbreaks and sporadic cases of nosocomial bloodstream infections related to contaminated parenteral admixtures. *Serratia sp* has been reported to be responsible for about 2% of nosocomial infections of the bloodstream, lower respiratory tract, surgical wounds, and skin and soft tissues in adult patients (Basilio, 2007).

Klebsiella pneumoniae is a Gram negative opportunistic human pathogen that is usually found everywhere. It has been associated with pneumonia, urinary tract infections, ankylosing spondylitis septicemia soft body infections and diarrhoea in humans. It has been isolated from food with high starch and environmental sources such as soil, vegetation and water (Cabral, 2010).

Citrobacter youngae is a Gram negative bacterium that is widely distributed in the environment such as water, soil, sewage, and cornstalks which serve as the sources of contamination. It is considered as an opportunistic pathogen that may be spread by person-to person contact and has been associated with diarrhoea in children and it has been isolated from water, fish, animals and food (Cabral, 2010).

Enterobacter aerogenes is a Gram negative pathogenic bacterium that causes opportunistic infections. It has been found to live in various wastes chemicals, and soil. *E. aerogenes* is generally found in the human gastrointestinal tract and does not generally cause disease in healthy individuals. *Enterobacter cloacae* are found in water, sewage, soil, skin, the intestinal tracts of humans and animals and in hospital environments. Health problems associated with *E. sakazakii* include vaginal infections, urinary tract infections, pelvic inflammatory disease. *Enterobacter gergoviae* is a Gram negative, rod-shaped organism that is peritrichous when motile that rarely causes human infection (Cabral, 2010). A study done by Obi and co-workers (Obi *et al.*, 2002) in the Levubu, Mutale, Ngwedi, Tshinane, Makonde, Mutshindudi and Mudaswali Rivers in rural Venda communities in South Africa, showed that the range of counts with regard to all the water sources investigated for HPC were between 180 and 1 300 000 cfu.mL^{-1}. Comparing the results of the present study with the results of Obi and co-workers (Obi *et al.*, 2002), the results showed that river waters in the Venda communities including the Mutzhedzi River are of poor microbiological quality for irrigation purposes (Table 10).

Salmonella – Typical growth of *Salmonella* isolates were seen on several of the XLD and Brilliant Green plates. However when these isolates were identified further with API 20E kits, no *Salmonella* spp. were identified, although several opportunistic Gram negative *Enterobacteriaceae* were identified (Table 11). Although *Salmonella* species were not present in this study, the species that were found as shown in Table 11 were of potential health risk as described under aerobic/heterotrophic plate counts.

Spore formers – The minimum and maximum counts of aerobic spore formers in irrigation water were zero and 80 000 000 cfu.mL^{-1}, respectively. The minimum and maximum counts for anaerobic spore formers in the water were zero and 3 000 000 cfu.mL^{-1}, respectively (Table 10). The high count of spore formers in irrigation water raises a cause for concern because a large number of spores could be produced.

Table 10. Bacterial counts for irrigation water from the Phadzima irrigation canal, Limpopo Province.

Date	ASF (cfu.mL^{-1})	AnSF (cfu.mL^{-1})	Entero (cfu.100 mL^{-1})	SA	LM	Sal
October 2008	400	30 000	ND	ND	ND	ND
November 2008	2	2	ND	ND	ND	ND
December 2008	10	8	ND	ND	ND	ND
January 2009	7	3	ND	ND	ND	ND
February 2009	20	4	ND	ND	ND	ND
March 2009	3	10	ND	ND	ND	ND
April 2009	200	20	ND	ND	ND	ND
May 2009	1	1	ND	ND	ND	ND
June 2009	1	10	ND	ND	ND	ND
July 2009	0	0	ND	ND	ND	ND
August 2009	0	2	129	ND	ND	ND
Sept. 2009	20 000	200	0	ND	ND	Absent
October 2009	10	1	192	Present	ND	Present
November 2009	2 000	1	1 400	Present	ND	Present
December 2009	No water	No water	No water	No water	No water	No water
January 2010	ND	ND	ND	ND	ND	ND
February 2010	ND	ND	ND	ND	ND	ND
March 2010	ND	ND	ND	ND	ND	ND
April 2010	300 000	30 000	0	Present	Absent	Absent
May 2010	ND	ND	ND	ND	ND	ND
June 2010	2 000	500	>3000	Absent	Absent	Present
July 2010	40 000	4 000	229	Absent	Absent	Present
August 2010	300 000	800	>3 000	Present	Absent	Absent
Sept. 2010	300 000	10 000	>3 000	Present	Absent	Present
October 2010	800 000	2 000	>3 000	Present	Absent	Present
November 2010	400 000	30 000	>3 000	Present	Absent	Present
December 2010	7 000 000	3 000 000	>3 000	Present	Absent	Present
January 2011	8 000 000	4 000	>3 000	Present	Absent	Present
February 2011	90 000	500	183	Present	Absent	Present
March 2011	1 000 000	200 000	>3 000	Present	Absent	Present
April 2011	90 000	1 000	>3 000	Present	Absent	Present
May 2011	700 000	40 000	>3 000	Present	Absent	Present
June 2011	7 000 000	ND	>3 000	Absent	Absent	Present

ND = not done; Present = typical growth; Absent = no growth

Endospore formers such as *Bacillus cereus* are often found in soil and are likely to be detected in river water if they are present in the soil. Therefore irrigation of minimally processed food (MPF) produce with water contaminated with spore forming bacteria may pose a greater health risk to consumers since the food is not processed at all (Table 10).

Table 11. Identification of Gram negative isolates from irrigation water from the Phadzima irrigation canal, Limpopo Province (the identification was based on data from the specific API kit).

Date	Sample	API Identification
October 2009	Water	*Pseudomonas spp.* *Aeromonas spp.*
November 2009	Water	*Enterobacter spp.*
June 2010	Water	*Enterobacter spp.* *Citrobacter spp.*
July 2010	Water	*Pantoea spp.* *Enterobacter spp.* *Proteus spp.*
September 2010	Water	*Aeromonas spp.* *Providencia alcalifaciens*
October 2010	Water	*Enterobacter spp.* *Citrobacter spp.*
November 2010	Water	*Aeromonas spp.* *Pseudomonas spp.*
December 2010	Water	*Pantoea spp.* *Aeromonas spp.*
January 2011	Water	*Enterobacter spp.* *Citrobacter spp.*
February 2011	Water	*Pseudomonas spp.* *Aeromonas spp.*
March 2011	Water	*Enterobacter spp.* *Pseudomonas lutea*
April 2011	Water	*Serratia marcescens* *Pantoea spp.*
May 2011	Water	*Aeromonas spp.* *Enterobacter spp.* *Serratia marcescens*
June 2011	Water	*Enterobacter spp.* *Klebsiella spp.*

Intestinal Enterococci – High microbial counts of intestinal *Enterococcus* were obtained frequently throughout the period of the study in the irrigation water (Table 10). The minimum Enterococci counts in water was zero and the maximum >3 000 cfu.100 mL^{-1}.

Listeria – No *Listeria monocytogenes* were isolated from any of the water samples tested during the whole study period (Table 10).

Staphylococcus – Typical growth of *Staphylococcus strains* were observed on the selection plates (Table 10). The identity of these isolates was further confirmed with the Staphylococcus Latex Agglutination test kits and samples from two of the months tested positive for *Staphylococcus* (Table 12).

Virology – The virus isolation studies were positive for three months (Table 13) indicating the prevalence of Norovirus GI, Norovirus GII and Hepatitis A virus, respectively. From the data in Table 13 it is evident that irrigation canal water used by the Phadzima community farm for the production of fresh produce was contaminated with potentially pathogenic viruses.

Table 12. Latex agglutination identification of presumptive positive *Staphylococcus* isolates from irrigation water from the Phadzima irrigation canal, Limpopo Province.

Date	**Sample**	**Staph agglutination**
October 2009	Water	Neg
November 2009	Water	Neg
April 2010	Water	Neg
August 2010	Water	Neg
September 2010	Water	Neg
October 2010	Water	Neg
November 2010	Water	Pos
December 2010	Water	Neg
January 2011	Water	Pos
February 2011	Water	Neg
March 2011	Water	Neg
April 2011	Water	Neg
May 2011	Water	Neg

Pos = positive; Neg = negative; ND = not done

3.4.4 Discussion and Conclusions

The contamination of the irrigation water source with aerobic/heterotrophic place count bacteria, aerobic and anaerobic spore formers, *Staphylococcus aureus,* intestinal *Enterococcus*, different Gram negative opportunistic bacteria, commensal and diarrhoeagenic strains of *E. coli* bacteria and viruses such as Norovirus GI, Norovirus GII and Hepatitis A virus, clearly showed that the river water used by this community as irrigation water is an important pre-harvest source of contamination and public health risk for the community. According to Ijabadeniyi (2010), South African's irrigation water sources are alleged to be at risk of contamination with human bacterial pathogens because of pollution caused by informal settlement. The surface water contamination in this study may have originated from both human and animal sewage disposal due to lack of proper sanitation and the fact that this water source is not protected from human and animal contamination.

Table 13. Prevalence of specific viruses in the irrigation water from the Phadzima irrigation canal, Limpopo.

Date	**Real-time RT-PCR analysis**		
	NoV G1	**NoV GII**	**HAV**
October 2008	Neg	Pos	Neg
November 2008	Neg	Neg	Neg
December 2008	Neg	Neg	Neg
January 2009	Neg	Neg	Neg
February 2009	Neg	Neg	Neg
March 2009	Neg	Neg	Neg
April 2009	ND	ND	ND
May 2009	Neg	Neg	Neg

June 2009	Neg	Neg	Pos
July 2009	Neg	Neg	Neg
August 2009	Neg	Neg	Neg
September 2009	Neg	Neg	Neg
October 2009	Neg	Neg	Neg
November 2009	Neg	Neg	Neg
December 2009	No water	No water	No water
January 2010	ND	ND	ND
February 2010	ND	ND	ND
March 2010	ND	ND	ND
April 2010	Neg	Neg	Neg
May 2010	ND	ND	ND
June 2010	Neg	Neg	Neg
July 2010	Pos	Neg	Neg
August 2010	ND	ND	ND
September 2010	ND	ND	ND
October 2010	ND	ND	ND
November 2010	ND	ND	ND
December 2010	Neg	Neg	Neg
January 2011	Neg	Neg	Neg
February 2011	Neg	Neg	Neg
March 2011	Neg	Neg	Neg
April 2011	Neg	Neg	Neg
May 2011	Neg	Neg	Neg
June 2011	Neg	Neg	Neg

3.5. MPUMALANGA PROVINCE

3.5.1 Specific aims

The aim of this study was to determine the microbiological quality of irrigation water in the Mpumalanga Province (Loskopdam canal, Wilge and Olifants Rivers) which could serve as a potential pre-harvest source of bacterial contamination of vegetables.

3.5.2 Physico-Chemical results

The turbidity of water samples differed significantly ($p \leq 0.05$) during the 1st 12 sampling intervals (Tables 14, 15, 16). During the sampling period, the Wilge River had the highest mean turbidity of 19.1 NTU followed by the Olifants River with 14.7 NTU and Loskop Canal with the lowest mean turbidity of 5.4 NTU. The mean turbidity level at all three sampling locations was higher than the international turbidity (1 NTU) standard for water (DWAF, 1996). At some sampling intervals, there was a high variation between the NTU in both rivers and the Canal. For example, the NTU for both rivers was very high at intervals 2, 5, 6, 7 and 12. However, no such trend was observed for the Canal. The COD of water samples also differed

significantly ($p \leq 0.05$) during the 1st 12 sampling interval (Tables 14, 15, 16). The Wilge River had the highest mean COD of 54.2 $mg.L^{-1}$ followed by the Olifants River with 53.5 mg per L and the Loskop Canal with the lowest COD of 50.4 $mg.L^{-1}$.

The pH of the water samples from the Olifants River ranged between 7.02-7.88 (data not shown) for the 1st 12 sampling intervals. The pH of water samples from the Wilge River and the Loskop Canal ranged between 7.00-7.62 and 7.03-9.71 respectively (Tables 14, 15, 16). In the Canal, it was however unusually high during sampling intervals 1 and 2, 9.71 and 9.45 respectively. The average water temperature of the Loskop Canal ranged between 16-19°C while it ranged between 17-23°C for the Olifants River and 16-22°C for the Wilge River during 12 sampling intervals (data not shown).

Table 14. Physico-chemical parameters of the Loskopdam Canal.

Date	pH	Temperature (^{0}C)	COD ($mg.L^{-1}$)
November 2007	9.66	17.9	68.45
November 2007	9.76	17.9	64.17
December 2007	9.4	15.9	66.31
December 2007	9.5	15.9	62.04
December 2007	7.38	18.3	24.56
January 2008	7.4	18.3	29.13
February 2008	7.2	19	111.21
February 2008	7.19	19	72.73
March 2008	7.04	17.6	77.34
March 2008	7.02	17.6	70.46
April 2008	7.23	22.5	58.46
April 2008	7.25	22.5	60.75
May 2008	7.1	18.7	71.84
May 2008	7.08	18.7	72
July 2008	7.28	14.6	41.15
July 2008	7.31	14.6	39.5
August 2008	7.09	14.5	14.81
August 2008	7.06	14.5	18.1
September 2008	7.05	15.5	30.62
September 2008	7.03	15.5	28.95
October 2008	7.13	16.8	20.22
October 2008	7.15	16.8	25.45
November 2008	7.35	26.9	42.3
November 2008	7.33	26.9	39.8
June 2009	7.2	12.6	
July 2009	7.47	12	
August 2009	7.42	13.3	
September 2009	6.59	27.8	
October 2009	7.7	23.5	
December 2009	7.96	25.5	
January 2010	7.29	25.8	
February 2010	8.21		
March 2010	7.5	23.4	
April 2010	6.23	21.8	
February 2011	5.44	13.3	
March 2011	7.8	20.5	
April 2011	7.35	18.3	
May 2011	7.96	20	
June 2011	7.44	15.5	

Date	pH	Temperature (^{0}C)	COD (mg.L^{-1})
July 2011	9.92	13.6	
August 2011	7.4	12.5	
September 2011	6.69	16.6	
October 2011	7.3	8.5	
November 2011	6.75	14.9	

Table 15. Physico-chemical parameters of the Olifants River.

Date	pH	Temperature (^{0}C)	COD (mg.L^{-1})
November 2007	7.86	19.2	57.76
November 2007	7.9	19.2	59.9
December 2007	7.9	17	59.9
December 2007	7.75	17	58.83
January 2008	7.6	20.1	38.28
January 2008	7.76	20.1	40.56
February 2008	7.52	25	141.14
February 2008	7.55	25	135.18
March 2008	7.19	22.4	68.17
March 2008	7.17	22.4	63.58
April 2008	7.45	24.2	60.75
April 2008	7.43	24.2	67.63
May 2008	7.06	18.8	88.19
May 2008	7.03	18.8	90.63
July 2008	7.23	14	26.33
July 2008	7.25	14	24.69
August 2008	7.01	16.8	26.33
August 2008	7.03	16.8	24.69
September 2008	7.5	21.2	26.14
September 2008	7.48	21.2	22.48
October 2008	7.21	24.3	15.78
October 2008	7.2	24.3	17.56
November 2008	7.21	27.1	30.49
November 2008	7.09	27.1	28.1

Table 16. Physico-chemical parameters of the Wilge River.

Date	pH	Temperature (^{0}C)	COD (mg.L^{-1})
November 2007	7.56	16.5	62.04
November 2007	7.65	16.5	57.76
December 2007	7.65	16.3	62.04
December 2007	7.58	16.3	65.24
January 2008	7.58	18.8	51.99
January 2008	7.6	18.8	47.42
February 2008	7.54	24	96.24
February 2008	7.56	24	109.07
March 2008	7.48	17.6	65.87
April 2008	7.47	17.6	66.51
April 2008	7.61	21.6	76.81
April 2008	7.59	21.6	80.57
May 2008	7.52	16.4	92.18
May 2008	7.5	16.4	95
July 2008	7.57	10.5	44.44

Date	pH	Temperature (^{0}C)	COD (mg.L^{-1})
July 2008	7.55	10.5	41.15
August 2008	6.99	13.9	16.46
August 2008	7.01	13.9	14.81
September 2008	7.26	19.9	36.12
September 2008	7.25	19.9	40.88
October 2008	7.51	23.2	18.34
October 2008	7.49	23.2	22.66
November 2008	7.3	25.3	35.35
November 2008	7.31	25.3	32.86

3.5.3 Microbiological evaluations

The mean APC count of water samples ranged between 2.9-3.2 log cfu.mL^{-1} and differed significantly (P≤0.05) over time (Tables 17, 18, 19). Similar to turbidity and COD, the Wilge River had the highest mean APC counts of 3.2 log cfu.mL^{-1} followed by Olifants River with 3 log cfu.mL^{-1} and Loskop Canal with the lowest APC counts of 2.9 log cfu.mL^{-1} during the 12 sampling intervals (Tables 17, 18. 19). The APC counts of the two rivers and the Canal during the sampling period followed the same trend with higher and lower counts noted at the same time at the three locations. Also, the lowest APC levels at interval 9 correspond with low COD and turbidity levels determined at interval 9. ASF at the three locations differed significantly (p≤0.05) during the 12 sampling intervals (Tables 17, 18, 19). The Wilge River had the highest mean ASF count of 2 log cfu mL^{-1} followed by the Olifants River with 1.66 log cfu.mL^{-1} and the Loskop Canal's mean ASF was 1.23 log cfu.mL^{-1} (Tables 17, 18, 19). While ASF was detected in the water samples from the Wilge River during all the sampling intervals, it was not detected at sampling interval 8 in the Olifants River and intervals 8 and 11 in the Loskop Canal. The mean AnSF count for both the Loskop Canal and the Olifants River was 1.23 log cfu.mL^{-1} while the mean AnSF count for the Wilge River was 1.93 log cfu.mL^{-1}. Similar to the ASF, AnSF was detected during all the sampling intervals in the Wilge River but it was not detected at sampling intervals 9, 11 and 12 in the Olifants River and at 10 and 12 in the Loskop Canal

Table 17. Microbiological parameters of the Loskopdam Canal.

Date	E.coli	ASF	AnSF	IE	SA	LM	Sal	FC	ACC	Crypto	Giar
	(MPN.100 mL^{-1})	(log cfu. mL^{-1})	(log cfu.mL^{-1})		(log cfu.mL^{-1})			(MPN.100 mL^{-1})	(log cfu.mL^{-1})		
November 2007	+	0.9	0.85	+	0	-	-	1 600	4.15		
November 2007	+	1.11	0.95	+	0	-	-	1 600	4.1		
December 2007	+	1.53	2.85	+	0	-	+	16 000	3.04		
December 2007	+	1.68	2.89	+	0	-	+	16 000	3.08		
December 2007	+	2.61	1.7	+	1.7	+	+	16 000	1.69		
January 2008	+	2.65	1.48	+	1.48	+	+	16 000	1.48		
February 2008	+	1.9	1.3	+	1.6	+	+	1 600	3.3		
February 2008	+	1.78	1.48	+	1.3	+	+	1 600	3.48		

Date	E.coli	ASF	AnSF	IE	SA	LM	Sal	FC	ACC	Crypto	Giar
March 2008	+	1.7	1.9	-	0	-	+	1 600	3		
March 2008	+	1.6	2.15	-	0	-	+	1 600	3.12		
April 2008	+	1.85	2	+	1	-	-	16 000	3.85		
April 2008	+	1.78	2.23	+	0	-	-	16 000	3.9		
May 2008	+	1.48	2.23	+	0	-	-	16 000	3.73		
May 2008	+	1	1.3	+	0	-	-	16 000	3.9		
July 2008	+	0	0	-	0	+	-	250	2.43		
July 2008	+	0	1.3	-	1	+	-	250	2.46		
August 2008	+	1	0	+	0	+	-	16 000	1.9		
August 2008	+	1.3	0	+	0.7	+	-	16 000	1.48		
September 2008	+	1	1	+	0	+	-	16 000	2.3		
September 2008	+	0	0	+	0	+	-	16 000	2.3		
October 2008	+	0	0	+	0.48	+	+	16 000	2.51		
October 2008	+	0	0	+	0	+	+	16 000	2.4		
November 2008	+	1	0	-	0	-	+	16 000	3.2		
November 2008	+	1	0	-	1.3	-	+	16 000	3.3		
June 2009	+						-	1 700		-	-
July 2009	+						-	1 700	840	-	-
August 2009	+						+	350	250	-	+
September 2009	+						-	700	2105	+	+
October 2009	+						-	3 500	7950	-	+
December 2009	-						+	70 000	1860	-	-
January 2010	-						+	11 000	1910	-	-
February 2010	+						+	350	2400	-	-
March 2010	+						+	2100	935	+	-
April 2010	+						+	16 000	32350	+	-
February 2011		0.0	0.0		0.0			3.3	2.7		
March 2011		1.8	0.0		0.0			0.0	4.1		
April 2011		1.5	3.3		1.5			0.0	3.7		
May 2011		5.1	3.1		1.7			1.1	3.8		
June 2011		0.0	0.0		0.0			2.1	3.6		
July 2011		0.0	0.0		0.0			1.1	2.8		
August 2011		4.0	2.9		3.2			0.3	3.7		
September 2011		0.0	0.0		2.8			1.9	2.8		
October 2011		3.4	3.6		0.0			1.1	2.0		
November 2011		5.1	5.1		0.0			1.7	2.7		

Table 18. Microbiological parameters of the Olifants River.

Date	E.coli	ASF	AnSF	Entero	SA	LM	Sal	FC	ACC	Crypto	Giar
	(MPN.100 mL^{-1})	(log cfu. mL^{-1})	(log $cfu.mL^{-1}$)		(log $cfu.mL^{-1}$)			(MPN.100 mL^{-1})	(log $cfu.mL^{-1}$)		
November 2007	+	1.81	1	+	0	-	-	16 000	2.62		
November 2007	+	1.84	1	+	0	-	-	16 000	2.67		
December 2007	+	2.06	2.58	-	0	-	+	16 000	3.26		
December 2007	+	2.08	2.63	-	0	-	+	16 000	3.29		
January 2008	+	2.18	1.78	+	0	+	+	540	2.4		
January 2008	+	2.7	1.78	+	0	+	+	540	2.38		
February 2008	+	2	1.6	+	2.9	+	+	1 600	4.23		
February 2008	+	1.7	1.3	+	2.7	+	+	1 600	3.85		
March 2008	+	1.9	2	-	0	-	-	1 600	3.33		
March 2008	+	2.42	2	-	0	-	-	1 600	3.24		
April 2008	+	1.78	1.3	+	1	-	-	16 000	3.62		
April 2008	+	1.78	1.85	+	0	-	-	16 000	3.69		
May 2008	+	1.48	2.41	+	0	-	-	16 000	3.54		
May 2008	+	1.78	1.48	+	0	-	-	16 000	3.4		
July 2008	+	0	0	+	0	+	-	250	2.6		
July 2008	+	0	1	+	0	+	-	250	2.52		
August 2008	+	0	0	-	0	+	+	16 000	2.4		
August 2008	+	1.48	1	-	0	+	+	16 000	2.28		
September 2008	+	1.48	0	+	1	+	-	240	3		
September 2008	+	1.48	0	+	0	+	-	240	3.04		
October 2008	+	1.3	1	+	0	+	-	16 000	2.95		
October 2008	+	1.48	0	+	0	+	-	16 000	2.72		
November 2008	+	1.85	0	-	0	-	+	920	2.53		
November 2008	+	2.04	0	-	0	-	+	920	2.49		

Table 19. Microbiological parameters of the Wilge River.

Date	E.coli	ASF	AnSF	Entero	SA	LM	Sal	FC	ACC	Crypto	Giar
	(MPN.100 mL^{-1})	(log cfu. mL^{-1})	(log $cfu.mL^{-1}$)		(log $cfu.mL^{-1}$)			(MPN.100 mL^{-1})	(log $cfu.mL^{-1}$)		
November 2007	+	1.41	2.12	+	0	-	-	16 000	5.15		
November 2007	+	1.61	2.23	+	0	-	-	16 000	5.18		
December 2007	+	2.57	2.83	+	0	+	+	16 000	3.19		
December 2007	+	2.6	2.88	+	0	+	+	16 000	3.21		
January 2008	+	2.32	2.18	+	1	+	+	920	2.18		
January 2008	+	2.4	2.34	+	0	+	+	920	2.3		
February 2008	+	2.2	1.85	+	0	-	-	1 600	3		
February 2008	+	2.48	2	+	0	-	-	1 600	3.15		
March 2008	+	2.91	2.61	+	1.6	+	+	16 000	3.8		
April 2008	+	2.92	2.4	+	1.9	+	+	16 000	4.31		
April 2008	+	2.83	2.32	+	1.3	-	-	16 000	4.05		
April 2008	+	2.82	2.63	+	1	-	-	16 000	4.1		
May 2008	+	1.85	2.72	+	0	-	-	16 000	3.9		
May 2008	+	1.6	2.93	+	0	-	-	16 000	3.54		
July 2008	+	1.48	1.3	+	0	+	-	250	2.36		
July 2008	+	1.6	1.48	+	0	+	-	250	2.26		
August 2008	+	1.3	1.3	+	0	+	-	16 000	1.85		
August 2008	+	1.6	0	+	0	+	-	16 000	1.95		
September 2008	+	1.48	1	+	1	+	-	240	2.3		
September 2008	+	1.78	1	+	0	+	-	240	2.6		
October 2008	+	1.3	1.6	+	0	+	-	16 000	2.67		
October 2008	+	1	1.78	+	0	+	-	16 000	2.59		
November 2008	+	2.18	1	+	0	-	+	16 000	2.83		
November 2008	+	2.23	1	+	0	-	+	16 000	2.79		

Of the water samples collected during the 12 sampling intervals, 25% of the samples from the Olifants River, 33% from the Wilge River and 58% of the samples from the Loskop Canal were positive for *S. aureus* (Fig. 6). However, the average *S. aureus* counts of water from the three surface water sampling sites were very low <1 log $cfu.mL^{-1}$. Incidence of *S. aureus* did not correspond between the sampling locations and only at interval 6 was *S. aureus* detected at all three locations (data not shown). *E. coli* was recovered from the two rivers and the Loskop Canal during every sampling interval (Fig. 6). Furthermore coliform and faecal coliform levels for the surface water met the international standard (1 000 MPN $100.mL^{-1}$) only once during the 12 sampling intervals in Loskop Canal water while at the Wilge River and Olifants River, the water samples met the standard during 25% and 30% of the 12 sampling intervals respectively. IE was present in all the water samples collected from the Wilge River while incidence was

lower in the Olifants River (67%) and the Loskop Canal (75%) (Fig. 6). Incidence of *Salmonella* (50%) was higher in the Loskop Canal than in the Wilge River and the Olifants River (33% and 42% respectively). However, the incidence of *L. monocytogenes* (58%) in the Wilge River was higher than the 50% incidence observed in both the Loskop Canal and the Olifants River during the 12 sampling intervals (Fig. 1).

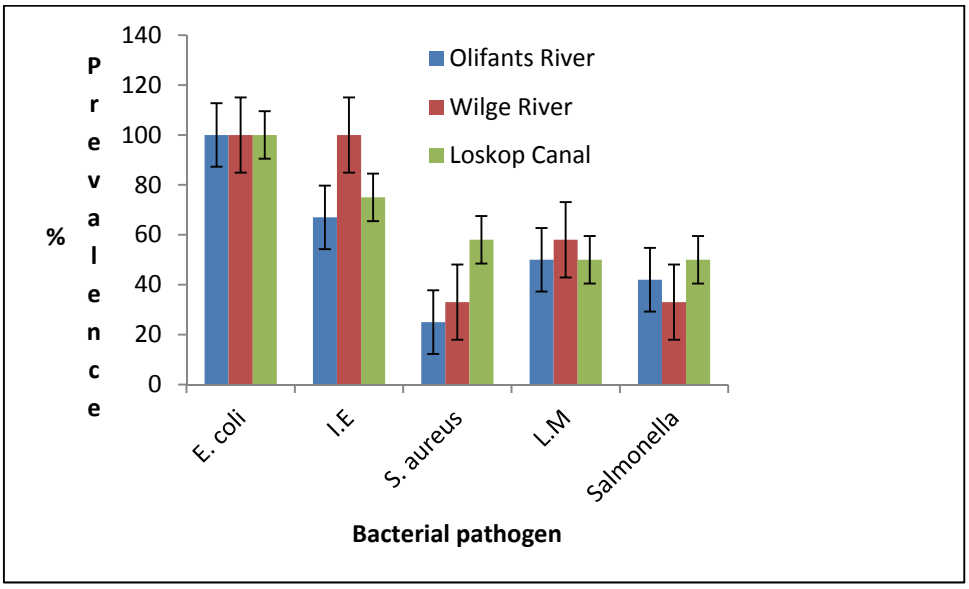

Figure 6. Prevalence of bacterial pathogens in the three water sources.

3.5.4 Discussion

The temperature and pH values of the Loskop Canal and the two rivers that were conducive for bacterial growth may have influenced the survival of aerobic bacteria and bacterial pathogens in the water sources. These two parameters could influence the level of faecal coliforms and intestinal *Enterococci*. The turbidity of the three water samples did not meet the SA water quality range for domestic water supply, 0 to 1 NTU (DWAF, 1996). The turbidity range for water of good quality should be between 0 to 1 NTU. The high turbidity level of surface water in this work corresponds with the river turbidity results of (Fatoki *et al.*, 2003). They also found high turbidity levels in surface water indicated that soil erosion and run-off could be a source of high turbidity in the water system. The soil erosion and run-off could have been caused by the informal settlements around the two rivers. The COD results for all three water samples from Loskop Dam, Olifants River and Wilge River also did not meet the WHO standard of 10 mg/litre. This shows that the surface water contains organic pollutants that may have originated from the informal settlements and mines around the region where rivers are located.

Although the level of aerobic bacteria in both water and vegetable samples was low, a high prevalence of bacterial pathogens was observed in this study. This shows that aerobic bacteria levels are not a good determinant of the microbiological quality of irrigation water and produce.

The recovery of aerobic spore formers from the three water samples is similar to work from Alaska water at the same low level. However, the level of anaerobic spore formers observed in our water samples was lower than has been reported by (Molongoski & Klug, 1976). Molongosk and Klug (Molongoski & Klug, 1976) recovered up to 6 log of anaerobic spore formers from freshwater lakes. Although low level of

aerobic spore formers were observed in the water samples, it may be unsuitable for the irrigation of fresh produce because of the possibility of microbial growth and cell division after attachment and infiltration on the vegetables.

The reason for a higher level of aerobic bacteria, aerobic spore formers and anaerobic spore formers in the Wilge River and the Olifants River, compared with those in Loskop Canal may be because the floor of the Canal is cemented. It was noticed from the result that the higher difference was lower than 1 log and fell within the same level. This indicates that the Loskop Canal could have been contaminated by the two rivers namely, Wilge and Olifants Rivers. The average aerobic bacteria, aerobic spore formers and anaerobic spore formers in the water samples and on the vegetables were also within the same level, indicating that Loskop Canal could have contributed to the microbiota and contamination of the vegetables.

Although recovery of *S. aureus* from water samples is low, it may still pose a problem if such irrigation water is used for the production of produce that are eaten raw. *S. aureus* was not expected to be recovered from the Loskop Canal, Wilge River or the Olifants River because its primary reservoir is the nasal cavity of humans. The presence of *S. aureus* in the two rivers and Loskop Canal also shows that the rivers may have contributed to the contamination level in the Canal.

The result of heavy contamination of the three water sources, with *E. coli* and faecal coliforms corresponds to the work of Tshivhandekano (Tshivhandekano, 2005) on the Apies River, South Africa. This shows that the concern regarding contamination of surface water sources in SA may be valid and widespread. The two rivers may have been polluted with human faeces since *E. coli* and faecal coliforms are indicators of faecal pollution (Garcia-Armisen & Servais, 2007). Human faeces contain higher faecal coliform counts, while animal faeces contain higher levels of faecal *Enterococci*. The high incidence of *E. coli*, faecal coliforms and intestinal *Enterococcus* in the two rivers and the Loskop Canal indicate that the rivers are potential sources of contamination of the Canal. In addition, the source of this contamination may be the informal settlements along the two rivers.

Contamination of water sources with other bacterial pathogens, namely, *L. monocytogenes* and *Salmonella* show that the two rivers and Canal are of poor microbiological quality possibly as a result of faecal pollution. It also indicates that the two rivers are potential sources of contamination of the Loskop Canal. Other workers have reported the widespread contamination of faecal polluted surface water with these pathogens and this is a public health concern especially when water is used for agricultural purposes (Tymczyna *et al.*, 2000; Garcia-Armisen & Servais, 2007; Lyautey *et al.*, 2007). According to Bhagwat in 2006, the greatest concerns with human pathogens on fresh and minimally processed vegetables are *E. coli* O157:H7, *Salmonella and L. monocytogenes.* The first two have low infective doses while *L. monocytogenes* grow very well under refrigeration storage conditions (Bhagwat, 2003). Another safety concern with these pathogens is that they can form biofilms on the produce thereby making sanitizers' ineffective (Somers *et al.*, 1994; Fonseca, 2006).

L. monocytogenes was not recovered from the Loskop Canal during the sampling intervals when incidence in the irrigation water source and vegetables were compared. However, it was recovered at previous sampling intervals. This signifies that *L. monocytogenes* may survive on the surface of broccoli for a long time after contact with irrigation water.

The study clearly indicates the potential effect of raw sewage spillage, informal settlements and wastewater from mines and industries on irrigation water sources and pre-harvest vegetables.

The high pH observed in Loskop Canal over the intervals was also noted during previous years of sampling (Ijabadeniyi, 2010) and could have been due to mining activities as the Canal also provides water to mines within the area (Mr Ferreira, Loskop Irrigation Board, personal communication October, 2011).

Although counts for aerobic bacteria, anaerobic and aerobic spore formers in Loskop Canal and the Skeerpoort river differed significantly ($p<0.05$) in the two rivers and over each month during the study, counts were usually less than 1 log unit and therefore carried no practical importance. These low counts probably indicated low bacterial contamination. Low counts of these indicator bacteria in Loskop Canal were probably due to its cemented floor and side surface which limited contamination from runoff. Water from the Skeerpoort River used for irrigation was diverted and collected in a farm dam prior to irrigation which probably limited contamination from external sources. Low counts for aerobic bacteria were similarly observed in previous studies carried out in the Loskop Canal (Ijabadeniyi *et al.*, 2011) and the Skeerpoort River. Low counts of aerobic and anaerobic spore formers were also observed in Loskop Canal (Ijabadeniyi *et al.*, 2011). The changes in counts for indicators over the study in the two irrigation water sources could have been attributed to the sporadic nature of pollution events (Doyle & Erickson, 2008). Higher aerobic bacteria on lettuce could be attributed to its closeness to the ground and rough leaf surface which could have enhanced attachment of bacteria (Islam *et al.*, 2004). Similar high counts of aerobic bacteria were noted on parsley, spinach and cauliflower irrigated with water from the Baynespruit River in a suburban community of KwaZulu-Natal (Gemmell & Schmidt, 2012). Results for aerobic bacteria, anaerobic spore formers and aerobic spore formers in the Skeerpoort River and on lettuce did not follow similar patterns over the whole 10 months indicating that their presence on produce could be from soil which is a major reservoir.

Staphylococcus aureus is usually associated with animal skins and humans, hence its low numbers and prevalence in the two water sources. The higher counts on lettuce could have been due to handling by workers. The hands of workers were noted to be a source of bacteria on produce (Materon *et al.*, 2007).

Faecal coliforms and intestinal enterococci indicate faecal contamination and possible presence of food-borne pathogens. Low counts for both faecal indicators in Loskop Canal and the Skeerpoort River probably indicates low faecal contamination. However previous studies have shown higher levels in Loskop Canal (Ijabadeniyi *et al.*, 2011) and the Skeerpoort River (Duhain, 2011). This difference probably arose because the level of indicator organisms changes seasonally and pollution events are usually sporadic in nature (da Silva *et al.*, 2011). Pollution from informal settlement through disposal of sewage and influence of contamination from the rivers that feed the Canal were attributed to the high contamination levels observed in the Skeerpoort river (Duhain, 2011) and Loskop Canal (Ijabadeniyi *et al.*, 2011), respectively. The high faecal coliform counts noted in the Skeerpoort could have been due to high rainfall precipitation experienced during that period. Rainfall has been noted to increase runoff and sewage drainage flows which carry all sort of contamination that later ends up in surface water sources (Beuchat, 2002). Additionally rainfall turns sediment in water sources which harbours a lot of faecal bacteria (Walters *et al.*, 2011). The low rainfall precipitation levels noted for Loskop Canal area over the 10 month study indicate it was not a major source of contamination and rather influence was more from the Wilge and Olifants rivers which feed into it. Incidence of *E.coli* gives a more definite indication of faecal contamination since it naturally proliferates in human and animal intestines. Therefore its presence in both irrigation water sources indicates faecal contamination and such water could pose a risk of contaminating irrigated produce with food-borne pathogens. Higher prevalence of *E.coli* (100%) was noted in the Loskop Canal in a

previous study (Ijabadeniyi *et al.*, 2011) and similar prevalence as in the current study in the Skeerpoort River (Duhain, 2011). Although counts of faecal coliforms were low throughout the study the incidence of *E.coli* was not. This was also observed in previous studies carried out on water sources (Gemmell & Schmidt, 2012). This shows that indicator bacteria might not give a good indication of incidence of bacterial pathogens in irrigation water. Presence of *E.coli* on both lettuce and water from the Skeerpoort River indicates that it's a source of bacterial food-borne pathogens on fresh produce. Contaminated irrigation water was noted as a source of pathogens on fresh produce grown on a farm in Texas, USA (Materon *et al.*, 2007). Additionally *E.coli* was isolated from both Loskop Canal and irrigated cauliflower and broccoli during a 3 month study (Ijabadeniyi *et al.*, 2011). The Department of Health (DoH) in South Africa recommends that since fresh produce in the country is usually eaten raw, *E.coli* should be absent per gram of food product (DoH, 2000). Therefore since fresh produce contaminated with *E.coli* in was noted in this study it could have the potential to cause food related illnesses.

Salmonella enterica subsp. *salamae* has previously been isolated from reptiles. Prevalence of Salmonella spp. in Loskop Canal was higher in a previous study (Ijabadeniyi *et al.*, 2011) but not isolated in the Skeerpoort River (Duhain, 2011). High prevalence of Salmonella spp in water shed has been linked to contamination with animal faeces and urban sewage with increase after rainfall events (Polo *et al.*, 1999). High prevalence of Salmonella spp was linked to human and animal sewage disposal in a Nigerian irrigation water source (Okafo *et al.*, 2003).The results from Loskop Canal and the Skeerpoort river show that they had not probably not been heavily impacted by human and animal sewage during the duration of the study.

Studies previously carried out to determine the microbiological quality of surface water sources in South Africa that have shown that their quality is deteriorating (Obi *et al.*, 2002; Olaniran *et al.*, 2009). The isolation of common food-borne pathogens known to cause illness in Loskop Canal and the Skeerpoort River confirmed contamination with faecal material raising concerns on fitness for irrigating fresh produce. Additionally isolation of *E.coli* in both irrigation water from the Skeerpoort River and lettuce shows that contaminated irrigation water poses a risk of food-borne pathogen transfer onto produce. However results from this study did not ably distinguish levels of faecal contamination in the two water sources as was initially hoped. Also incidence of food-borne pathogens did not help in clarifying which of the water sources was more contaminated with faecal material. This was probably because bacterial indicators suffer from a number of limitations such as short survival times, origin other than faecal sources and regrowth outside the host (Sidhu & Toze, 2009). Through understanding the ecology of enteric bacterial pathogens in irrigation systems, the risks to produce can be better defined.

3.5.5 Conclusions

The water used for irrigation in this study is a likely source of contamination of vegetables produced in this area with bacterial pathogens and constitutes a food safety risk. The water should be properly treated when used for produce that may be eaten raw. This safety measure should be combined with Good Agricultural Practices (GAPs) and HACCP during the production of fresh vegetables.

3.6. CRYPTOSPORIDIUM AND GIARDIA IN WATER FROM SELECTED MPUMALANGA, NORTH WEST AND GAUTENG RIVERS

3.6.1 Specific aims

The first aim of this study is to determine the microbial pollution level of irrigation water from the Skeerpoort river, Loskopdam canal, Moses River and Klip rivers. The second aim was to determine whether the presence of faecal indicators or bacterial pathogens such as *Salmonella* spp. can be linked to the presence of *Cryptosporidium* oocysts and/or *Giardia* cysts in irrigation water.

3.6.2 Physico-Chemical results

The minimum and maximum temperature of the three rivers ranged from 13.4º, 12º and 8.4ºC in winter to 22.5º, 27.8º and 26.1ºC summer for the Klip, Moses and Skeerpoort rivers respectively (Tables 20 and 21). No significant differences in temperature between rivers was observed ($p>0.05$). The pH of the Klip River and Moses River were within normal range and varied between 6.58 and 8.02 for the Klip River and between 6.23 and 8.21 for the Moses River. The average pH of the Skeerpoort river was significantly higher ($p=0.003$) than the pH of the Moses and Klip river and ranged from 7.2 to 8.63 .

The pH ranged from 5.6 to 9.9 and 7.9 to 9.1 in Loskop Canal and the Skeerpoort River respectively (Tables 20 and 21). The pH in Loskop Canal exceeded national guidelines ref for irrigation water once (June, 2011 at pH 9.9) during the 10 month study. Temperature in Loskop Canal ranged from 8.4 to 20.0°C with the lowest and highest noted in September and April respectively. Temperature in the Skeerpoort river ranged from 10.7 to 26.7^{o}C with the lowest and highest noted in May and March, respectively.

Table 20. Physico-chemical parameters of the Skeerpoort River.

Date	pH	Temperature (^{0}C)
June 2008	8.48	11.8
July 2008	8.51	12.4
June 2009	8.23	13.3
July 2009	8.23	11.2
August 2009	8.5	8.4
September 2009	8.63	19.1
October 2009	8.12	23.9
December 2009	7.62	26.1
January 2010	8.2	25.9
February 2010	8.23	25.7
March 2010	7.6	24.9
April 2010	7.2	21.2
February 2011	8.42	26
April 2011	7.93	26.7
May 2011	8.91	20.5
June 2011	8.42	10.7
July 2011	9.18	10.9
August 2011	8.08	13

Date	pH	Temperature (^{0}C)
September 2011	8.6	13.2
October 2011	8.35	16
November 2011	7.91	22.3
March 2011	8.42	23

Table 21. Physico-chemical parameters of the Klip River.

Date	pH	Temperature (^{0}C)
July 2009	7.5	13.4
August 2009	6.8	14
September 2009	7.7	17.7
October 2009	7.21	21.3
December 2009	8.02	22.5
January 2010	7.51	20.5
February 2010	6.91	21.6
March 2010	6.58	21.5
April 2010	7.5	18.6
May 2010	7.42	16.4

3.6.3 Microbiological evaluations

The APC in the Klip River ranged from 2.3 to 6 log cfu.mL^{-1} with an average of 5 log cfu.mL^{-1} (Table 22). Levels of faecal coliforms and *E. coli* ranged from 3.2 to 5.5 log counts.100 mL^{-1}. *E. coli* was not found in 5 of the samples despite high faecal coliform counts. From the 10 water samples taken from the Klip River, 7 samples were positive for at least one pathogen and 2 samples were positive for 2 pathogens. *Salmonella* spp., *Salmonella* ser. Enteritidis and *Salmonella* ser. Adeoye were isolated from 2 out of the 10 samples taken from the Klip River during the summer months. *Cryptosporidium* oocysts were present in 5 samples and *Giardia* cysts in 2 samples taken from the Klip River. *Cryptosporidium* and *Giardia* were isolated from the Klip River during both winter and summer months. Water samples positive for *Cryptosporidium* oocysts were confirmed with PCR. The recovery efficiency for *Giardia* was significantly lower ($p<0.05$) than the recovery efficiency for *Cryptosporidium* with 15.3% recovery for *Cryptosporidium* and 9.2% recovery for *Giardia*. The average recovery efficiency for the 10 positive controls for the isolation of *Cryptosporidium* and *Giardia* was lower than reported in some studies (Robertson & Gjerde, 2001b).

The APC counts in the Skeerpoort River ranged from 2.4 to 4.7 log cfu.mL^{-1} (Table 23). Faecal coliforms counts in the Skeerpoort River varied greatly between samples and ranged from 0.9 log 100 mL^{-1} in winter to more than 9.2 log 100 mL^{-1} in summer. *E. coli* was isolated from 4 out of the 10 samples and was found in samples with both high and low faecal coliform counts. The *E. coli eae intimin* was detected in the Skeerpoort river in March 2010 although *E. coli* O157:H7 was not detected (Table 23). The *eae Intimin* must have originated from other entero-haemorrhagic *E. coli* (EHEC) not included in the Gene Disc analysis. None of the samples tested positive for *Salmonella* spp. but 5 samples tested positive for *Cryptosporidium* oocysts. *Giardia* cysts were found in 2 of the samples. *Cryptosporidium* oocysts were found during both winter and summer while *Giardia* cysts were only isolated in summer. *Cryptosporidium* oocysts were found in water samples containing low (0.9 log counts.100 mL^{-1}) as well as high (>9.2 log counts. 100 mL^{-1}) levels of faecal coliforms.

The APC in the Moses River ranged from 2.4 to 4.5 log cfu.mL^{-1}. Faecal coliforms ranged from 2.5 to 4.8 log counts.100 mL^{-1} and 7 out of the 10 samples had faecal coliform levels above 3 log counts.100 mL^{-1}. An increase in faecal coliforms was observed during the beginning of summer (sampling interval 4-6) which coincides with the first rainfall after a dry winter. *E. coli* was isolated from 8 out of the 10 samples. No *E.coli* was found in two of the samples for which the faecal coliforms counts were high (4 and 4.8 log counts. 100 mL^{-1}). *E. coli* shigatoxin 1 was detected in the Moses river in April 2010 although *E. coli* O157:H7 was not detected. Out of the 10 water samples analysed, 8 tested positive for at least one pathogen and 4 samples were positive for 2 pathogens. *Salmonella* spp. was found in 6 samples. *Salmonella* ser. Gaminara*, Salmonella* ser. Fulica*, Salmonella* ser. Infantis*, Salmonella* subsp. I and *Salmonella* subsp. II were isolated from the Moses River. *Cryptosporidium* oocysts and *Giardia* cysts were isolated from 3 of the samples. The two protozoa were isolated from different water samples and only one sample contained both *Cryptosporidium* oocysts and *Giardia* cysts. Both protozoa were isolated from water that contained both low and high levels of faecal coliforms and were found in the Moses River during winter as well as summer months.

APC counts were on average higher in Klip River than in the Skeerpoort and Moses River (Tables 22 and 23). Faecal coliform counts in Skeerpoort River were lower on average than in the Moses and Klip River. These differences were however not statistically significant ($p>0.05$). Fewer pathogens were isolated from the Skeerpoort River than from the other two rivers. Only 50% of the samples taken from the Skeerpoort River contained at least one pathogen and 2 samples tested positive for 2 pathogens. There were however no significant difference ($p>0.05$) between the three rivers in terms of incidence of pathogens and levels of indicator organisms. This ten month study took place over three seasons, starting in winter 2009 and finishing in summer 2010. More samples were found positive for *Cryptosporidium* in summer than in winter and spring while more positive *Giardia* samples were found in spring than in winter and summer. However, those differences in incidence between seasons were not statistically significant ($p>0.05$) (data not shown).

Two water samples contained less than 100 faecal coliforms per 100 mL. *Salmonella* spp. and *Giardia* were absent from those samples and only *Cryptosporidium* oocysts were isolated from those 2 samples. Between 100 and 1 000 faecal coliforms.100 mL^{-1} were found in 7 samples. The incidence of pathogens in those samples was low. The highest incidence of *Cryptosporidium* oocysts was found in samples containing between a 1 000 and 10 000 faecal coliforms.100 mL^{-1}. *Giardia* cysts were however found at equal rate in samples containing between 100 and 1 000 faecal coliforms.100 mL^{-1} and between 1 000 and 10 000 faecal coliforms.100 mL^{-1}. The incidence of *Salmonella* spp. was the highest in samples with faecal coliform counts above 10 000 counts.100 mL^{-1}. However, the overall incidence of pathogens was lower in samples containing more than 10 000 faecal coliforms.100 mL^{-1} than in samples containing between 1 000 and 10 000 faecal coliforms.100 mL^{-1} although more than 50% of the samples containing more than 10 000 faecal coliforms.100 mL^{-1} were positive for either *Salmonella* spp., *Cryptosporidium* or *Giardia*.

Level of *E. coli* of less than 1 count.100 mL^{-1} was found in 40% of the samples. In those samples the incidence of pathogens was low, and no *Salmonella* spp. was found. However, half of those samples tested positive for at least one pathogen. Levels of *E.coli* between one and 1 000 counts.100 mL^{-1} water were found in 20% of the samples. The incidence of pathogen in those samples was high and 83% of the samples tested positive for at least one pathogens. Between 1 000 and 10 000 *E. coli* 100 mL^{-1} were found

in 30% of samples. The highest incidence of *Giardia* cysts was observed in these samples. The highest incidence of *Cryptosporidium* oocysts was observed in samples containing more than 10 000 *E. coli* 100 mL^{-1}.

3.6.4 Discussion and Conclusions

Cryptosporidium oocysts, *Giardia* cysts and *Salmonella* spp. were isolated from surface water used for irrigation of vegetables. The presence of these human pathogens in South African irrigation water may have serious public health implications as irrigation water can be a potential source of contamination of fresh produce as the pathogens can come in contact and attach to the surface of the crops (Armon *et al.*, 2002; Macarisin *et al.*, 2010).

Table 22. Microbiological parameters of the Klip River.

Date	E. coli (MPN.100 mL^{-1})	Sal	FC (MPN.100 mL^{-1})	ACC	Crypto	Giar
July 2009	-	-	3 500	899 000	+	-
August 2009	+	-	7 900	1 370	+	-
September 2009	+	-	1 700	32 700	+	+
October 2009	+	-	350 000	27 250	-	-
December 2009	+	+	4 900	29 750	-	-
January 2010	-	+	4 600	19 400	-	-
February 2010	-	-	2 400	65 500	+	-
March 2010	+	-	5 400	78 000	+	+
April 2010	-	-	3 500	52 000	-	-
May 2010	-	-	92 000	21 700	-	-

The aerobic plate counts and faecal coliform counts of the Klip River where higher than those of the Skeerpoort and Moses rivers which indicates higher microbiological pollution in the Klip River (DoH, 2000). All samples from the Klip River were above the WHO guideline for irrigation water which limits the level of faecal coliforms to 1 000 counts per 100 mL (WHO, 1989a) while the Moses and Skeerpoort River had faecal coliform level above the WHO standard in many but not all samples. This means that the Klip River has been contaminated with higher levels of faecal matter from human or animal origin than the two other rivers although the faecal contamination levels of the Moses and Skeerpoort were also high. The Klip, Moses and Skeerpoort rivers could have become contaminated due to run off water from informal settlement located near the rivers and from run off from nearby farms. The presence of high level of faecal coliforms in water indicates faecal pollution and possible contamination of the river with human enteric pathogens. Water containing high levels of faecal coliforms should thus not be used for irrigation purposes due to the risk of transfer of pathogen from the water to the fresh produce (Armon *et al.*, 2002).

Salmonella ser. Enteritidis, which was found in the Klip river, is a known human pathogen and has been the source of food-borne human infection outbreaks in the past. It is the most common serotype of *Salmonella* reported in the UK and most outbreaks have been associated with the use of raw eggs in food products. *Salmonella* ser. Gaminara which was isolated from the Moses River is mostly associated with poultry. It has however been the aetiological agent of a food-borne outbreak caused by infected orange

juice (Cook *et al.*, 1998). The presence of *Salmonella* spp. in irrigation water is a potential human health risk as pathogens can be transferred to fresh produce during irrigation. No *Salmonella* spp. was found in the Skeerpoort River. This could be because the pH of the Skeerpoort River was more alkaline than the pH of the Moses and Klip River. The optimum pH for the survival of S*almonella* being 7, *Salmonella* spp. might not have survived in the Skeerpoort river due to its higher pH. The temperatures recorded did not differ significantly between rivers and were within the growth range of enteric bacteria.

Table 23. Microbiological parameters of the Skeerpoort River.

Date	ASF (log cfu. mL^{-1})	AnSF log cfu. mL^{-1}	SA (log cfu. mL^{-1})	LM	Sal	FC (MPN.100 mL^{-1})	ACC	Crypto	Giar
May 2008	-	-	-	-	+	>1 600	3.6		
June 2008	-	-	-	-	-	>1 600	3.7		
July 2008	-	-	-	-	-	250	3.2		
June 2009					-	7.8	2 400	+	-
July 2009					-	33	2 200	+	-
August 2009					-	920	46 000	-	-
September 2009					-	490	255	-	-
October 2009					-	2 000 000	6 100	+	+
December 2009					-	16 000	1 545	-	-
January 2010					-	280	4 900	-	-
February 2010					-	3 500	2 150	+	-
March 2010					-	700	7 600	-	-
April 2010					-	2 100	8 200	+	+
February 2011	2.2	3.2	0.0			0.0	3.6		
March 2011	2.1	2.0	0.7			1.7			
April 2011	3.2	2.8	2.0			3.8	4.0		
May 2011	2.6	2.3	0.8			1.0	3.3		
June 2011	0.0	0.0	1.8			1.4	3.9		
July 2011	2.2	5.3	0.0			1.0	3.5		
August 2011	2.2	1.2	0.0			1.7	2.6		
September 2011	2.9	3.1	0.0			0.0	2.8		
October 2011	3.3	0.0	0.0			1.1	4.6		
November 2011	4.7	5.3	0.0			1.7	2.8		

The results of this study indicate the widespread presence of *Cryptosporidium* oocysts and *Giardia* cysts in surface water as the two protozoa were found in all three rivers. The incidence of *Cryptosporidium* and *Giardia* was the same in the Klip and Skeerpoort rivers despite much higher levels of faecal coliforms in the Klip River than in the Skeerpoort River. This suggests that the use of faecal coliforms as indicator for the presence of *Cryptosporidium* and *Giardia* is not reliable. The average incidence of *Cryptosporidium*

oocysts in the 30 water samples was higher than the incidence of *Giardia* cysts. A similar trend has been observed in Norway and Portugal (Robertson & Gjerde, 2001a; Lobo *et al.*, 2009). The opposite was however observed in a study on the prevalence of *Cryptosporidium* and *Giardia* in South African water by Kfir (Kfir *et al.*, 1995), where a higher incidence of *Giardia* cysts than *Cryptosporidium* oocysts was recorded in surface waters. The incidences of the two protozoa obtained in the present study are within the range of results obtained from other surveys undertaken in the UK (Kay *et al.*, 2008) and in Norway on the occurrence of *Cryptosporidium* and *Giardia* in raw water (Robertson & Gjerde, 2001b). The presence of both protozoa in the same sample was observed in 5 out of the 30 samples (17%) analysed. This contrasts with the results from Kfir *et al*. (1995) where only 2.9% of surface water sampled in South Africa was positive for both protozoa. However, Kfir (Kfir *et al.*, 1995) used the settling technique for concentrating cysts and oocysts while centrifugation was used in this study. The difference in methodology might have influenced the recovery rates.

On average, more *Cryptosporidium* and *Giardia* were isolated during spring and summer, the two parasites were found in the Klip and Moses rivers during both summer and winter which suggests lack of seasonality of the two protozoa. However, *Giardia* was only isolated from the Skeerpoort river during spring and summer which coincide with the rainy season. While lack of seasonality was reported in other studies (Robertson & Gjerde, 2000)**,** higher incidence of *Cryptosporidium* and *Giardia* in surface water has been observed after rainfall in some studies as heavy rainfall causes run off of top soil and contaminated debris into rivers (Atherholt *et al.*, 1998; Muchiri *et al.*, 2009). The sampling sites chosen for the Klip and Moses rivers happened to be watering points for cattle in the area. This means that direct contamination of the water with animal faeces was likely to happen throughout the year thus masking the possible effects of rainfall and seasons on the occurrence of *Cryptosporidium* and *Giardia*. On the other hand, no farm animals were seen in the vicinity of the Skeerpoort sampling site and *Giardia* was only isolated from the Skeerpoort river in summer. The effect of rainfall on the occurrence of *Cryptosporidium* and *Giardia* could thus be observed here. Determination of viability and infectivity of the oocysts would be important to assess the health risk associated with the presence of *Cryptosporidium* and *Giardia* protozoa in irrigation water.

There were no significant differences in the levels of indicator organisms and incidence of pathogens between the three rivers. This indicates widespread contamination of surface water with faecal matter and human pathogens independent of the location of the river.

The microbiological quality of the vegetables analysed was acceptable as the aerobic plate counts, faecal coliform counts and *E. coli* counts were low and no pathogens were isolated from the vegetables. No *Cryptosporidium* or *Giardia* was isolated from those samples despite the high incidence of these two protozoa in the water used for their irrigation. The low bacterial counts and absence of pathogens observed on the broccoli might be attributed to the fact that the broccoli had been irrigated only partially with water from the Loskop Dam Canal, which is supplied by the Moses and other rivers, and the rest of the irrigation water had come from a borehole. The absence of *Cryptosporidium* and *Giardia* from the vegetables might also be due to the low recovery rate of the method and the possibility of false negative cannot be ruled out.

The presence of high faecal coliform counts in the water sampled did not necessarily indicate the presence of high levels of *E. coli*. It was indeed observed that *E. coli* was absent of some samples which had high faecal coliform counts and was sometimes present in water samples with low faecal coliform

counts. The relationship between faecal coliform counts and presence of pathogen or *E. coli* counts and presence of pathogens was thus investigated.

No predictive relationship between levels of faecal coliform or *E. coli* and presence of *Cryptosporidium* or *Giardia* in surface water could be drawn from the results of this study. No correlation was found between temperature and pH and presence of *Cryptosporidium* or *Giardia* either. Similar poor correlation between faecal indicators and presence of pathogens has also been demonstrated in other studies. The absence of correlation between faecal indicators and *Cryptosporidium* and *Giardia* could be due to the different rates of survival of protozoa compared to those of bacterial faecal indicator and could also be due to the difference in recovery rate and detection limits (Scott *et al.*, 2002). The lower microbial density of *Cryptosporidium* and *Giardia* in surface water coupled with low recovery rate could have led to failure to detect them due to sampling volume that was too small. Higher sampling volumes have indeed been used in other studies leading to higher recovery (Lechevallier *et al.*, 1991).

The lack of correlation between indicator organisms and the presence of *Cryptosporidium* and *Giardia* suggests that the monitoring of environmental water samples with only indicator organisms is not sufficient to accurately predict the microbiological quality and safety of the water in terms of *Cryptosporidium* oocysts and *Giardia* cysts. *Cryptosporidium* and *Giardia* were indeed found in irrigation water that could have been classified as of acceptable microbiological quality if only faecal indicator counts were taken into account. The presence of these pathogens in irrigation water in South Africa indicates the potential for human infection acquisition through the consumption of fresh produce irrigated with those waters.

This study demonstrates the widespread presence of *Cryptosporidium* and *Giardia* in surface water used for irrigation of fruits and vegetables in three rivers from different provinces of South Africa. The presence of these pathogens in irrigation water has serious public health implication as these pathogens have been shown to attach and survive on the surface of fresh produce following irrigation with contaminated water. Commonly used faecal indicators failed to reliably predict the presence or absence of *Cryptosporidium* and *Giardia* in the water analysed. The use of only faecal indicator organisms for monitoring surface water quality is not sufficient to accurately predict the presence of *Cryptosporidium* and *Giardia* and assess the microbiological safety of irrigation water. Identification of the sources of water contamination and more understanding of the ecology of *Cryptosporidium* and *Giardia* and their distribution in the environment in comparison to those of indicator organisms is needed in order to identify good predictors for the presence of *Cryptosporidium* and *Giardia* in water.

3.7 VIROLOGY

3.7.1 Specific aims

The aim of this study was to determine which virus types were present in irrigation water from rivers in the Western Cape, Limpopo, Mpumalanga, and North West Provinces. This will include testing for NoV GI, NoV GII, HAV and Mengo viruses.

3.7.2 Irrigation water and fresh produce samples

From April 2008 to June 2011, 101 (10 l) irrigation water samples were collected from surface water sources (Table 24).

3.7.3 Viral detection from irrigation water

Among 101 water samples analysed, 12/67 (18%) of river water samples, 0/2 of tap water samples, and 3/32 (9%) of irrigation canal samples were positive for the presence of one or more enteric viruses.

Table 24. Summary of the number, source and province of origin of the irrigation water samples.

Geographical region	River *(n = 67)*	Tap *(n = 2)*	Irrigation canal *(n = 32)*
Limpopo	2	0	20
Mpumalanga	12	0	6
North West	1	0	0
Western Cape	52	2	6

Mpumalanga – From February 2008 to September 2008, 18 irrigation water samples, six from each site, were analysed on a monthly basis. Norovirus GII was detected in one or more samples from each site (Table 25). Of interest was the fact that NoV GII was detected at all three sites in the September (early spring) sampling period, while NoV GII was also detected in June (early winter). All four NoV GII strains could not be typed. Neither NoV GI nor HAV were detected in any of the samples analysed.

North West Province – No enteric viruses, NoV GI, NoV GII and HAV, were detected in the single irrigation water sample from the North West Province (results not shown).

Limpopo Province – From Table 26, it was evident that no viruses were detected in the river water sources. Hepatitis A virus (untypable) was, however, detected in the irrigation canal water sample collected in June 2009 (winter) and NoV GII (untypable) in the water sample collected in October 2008 (late spring). In total, HAV was found to be Present in 5% and NoV GII in 5% of the irrigation canal water samples, while NoV GI was not detected at all.

Table 25. Summary of results of viral analysis of irrigation water samples from Mpumalanga.

Site, name and location	Date	Real-time RT-PCR analysis			
		NoV GI	NoV GII	HAV	Mengovirus
Site A: Irrigation canal water (from Loskop Dam)	2008.02.20	Neg	Neg	Neg	Neg
	2008.03.?	Neg	Neg	Neg	Neg
	2008.04.02	Neg	Neg	Neg	Neg
	2008.04.28	Neg	Neg	Neg	Neg
	2008.07.09	Neg	Neg	Neg	**Pos**
	2008.09.09	Neg	**Pos**	Neg	Neg
Site B: River water (Olifants River)	2008.02.20	Neg	Neg	Neg	Neg
	2008.03.?	Neg	Neg	Neg	Neg
	2008.04.02	Neg	Neg	Neg	Neg
	2008.05.11	Neg	**Pos**	Neg	Neg
	2008.07.09	Neg	Neg	Neg	**Pos**
	2008.09.09	Neg	**Pos**	Neg	Neg

Site, name and location	Date	Real-time RT-PCR analysis			
		NoV GI	NoV GII	HAV	Mengovirus
Site C: River water	2008.02.20	Neg	Neg	Neg	Neg
(Wilge River)	2008.03.?	Neg	Neg	Neg	Neg
	2008.04.02	Neg	Neg	Neg	Neg
	2008.04.28	Neg	Neg	Neg	Neg
	2008.07.09	Neg	Neg	Neg	**Pos**
	2008.09.09	Neg	**Pos**	Neg	Neg

Table 26. Summary of results of viral analysis of irrigation water samples from Limpopo.

Site, name and location	Date	Real-time RT-PCR analysis			
		NoV GI	NoV GII	HAV	Mengovirus
Farmer 1: River Water	2008.09.23	Neg	Neg	Neg	NT
Farmer 2: River Water	2008.09.23	Neg	Neg	Neg	NT
Site 1: Irrigation canal water	2008.10.27	Neg	Pos	Neg	NT
(Phadzima community farm)	2008.11.17	Neg	Neg	Neg	NT
	2008.12.01	Neg	Neg	Neg	NT
	2009.01.26	Neg	Neg	Neg	NT
	2009.02.16	Neg	Neg	Neg	NT
	2009.03.16	Neg	Neg	Neg	NT
	2009.05.18	Neg	Neg	Neg	NT
	2009.06.24	Neg	Neg	**Pos**	NT
	2009.07.20	Neg	Neg	Neg	Neg
	2009.08.17	Neg	Neg	Neg	Neg
	2009.09.07	Neg	Neg	Neg	NT
	2009.10.13	Neg	Neg	Neg	NT
	2009.11.02	Neg	Neg	Neg	NT
	2010.12.06	Neg	Neg	Neg	Neg
	2011.01.03	Neg	Neg	Neg	Neg
	2011.02.14	Neg	Neg	Neg	Neg
	2011.03.01	Neg	Neg	Neg	Neg
	2011.04.04	Neg	Neg	Neg	Neg
	2011.05.03	Neg	Neg	Neg	Neg
	2011.06.14	Neg	Neg	Neg	Neg

NT: not tested

Western Cape Province – From May 2008 to December 2008, a total of 60 irrigation water samples were analysed for NoV GI, NoV GII and HAV. Norovirus GII was detected in ~12% (7/60) and HAV in 5% (3/60) of the samples, while NoV GI was not detected in any of the samples (Table 27). In the Mosselbank river sites (Sites 1 & 2) NoV GII.4 was detected in the river water (Site 1) in June (winter) 2008 and NoV

GII.2 and HAV in September 2008, while no viruses were detected in the irrigation water (Site 2: Table 27). The Plankenburg River (Site 4) showed a high level of viral contamination in the late spring and summer months with NoV GII and HAV being detected in one of the samples drawn in October 2008 and HAV being detected in the sample of December 2008. No viruses were detected in the surface water from Sites 5, 6, 10 and 11. Two of the three sampling sites (Site 16 and Site 18) from the Berg river showed virological contamination in June and September 2008 with NoV GII being detected at both sites in both months (Table 27a and b). Two NoV GII genotypes could be identified, i.e. NoV GII.4 and NoV GII.6, with NoV GII.6 being detected upstream at Site 16 and NoV GII.4 was being detected downstream at Site 18.

Table 27a. Summary of results of viral analysis of irrigation water samples from Western Cape.

Site, name and location	**Date**	**Real-time RT-PCR analysis**			
		NoV GI	**NoV GII**	**HAV**	**Mengovirus**
Site 1: River Water	2008.05.05	Neg	Neg	Neg	NT
(Mosselbank River)	2008.06.17	Neg	**Pos: GII.4**	Neg	Neg
	2008.09.08	Neg	**Pos: GII.2**	**Pos**	**Pos**
Site 2: Irrigation water	2008.06.17	Neg	Neg	Neg	NT
(Mosselbank River)	2008.08.08	Neg	Neg	Neg	NT
	2008.09.08	Neg	Neg	Neg	NT
Site 4: River water	2008.05.05	Neg	Neg	Neg	NT
(Plankenburg River)	2008.05.19	Neg	Neg	Neg	Neg
	2008.05.?	Neg	Neg	Neg	NT
	2008.06.17	Neg	Neg	Neg	NT
	2008.09.08	Neg	Neg	Neg	NT
	2008.10.06	Neg	Neg	Neg	Neg
	2008.10.?	Neg	Neg	Neg	Neg
	2008.10.?	Neg	**Pos**	**Pos**	Neg
	2008.12.?	Neg	Neg	**Pos**	Neg
Site 5: River water	2008.05.19	Neg	Neg	Neg	Neg
(Eerste River)	2008.06.17	Neg	Neg	Neg	NT
	2008.09.08	Neg	Neg	Neg	NT
	2008.10.06	Neg	Neg	Neg	Neg
	2008.10.?	Neg	Neg	Neg	Neg
	2008.10.?	Neg	Neg	Neg	Neg
	2008.12.?	Neg	Neg	Neg	Neg
Site 6: River water	2008.09.08	Neg	Neg	Neg	NT
(Eerste River)	2008.10.06	Neg	Neg	Neg	Neg
	2008.10.?	Neg	Neg	Neg	Neg
	2008.10.?	Neg	Neg	Neg	Neg
	2008.12.?	Neg	Neg	Neg	Neg
Site 10: Irrigation canal	2008.05.?	Neg	Neg	Neg	NT
(sloot) water	2008.05.?	Neg	Neg	Neg	NT
	2008.10.06	Neg	Neg	Neg	Neg
	2008.10.?	Neg	Neg	Neg	Neg
	2008.10.?	Neg	Neg	Neg	NT
	2008.12.?	Neg	Neg	Neg	Neg

NT: not tested

Table 27b. Summary of results of viral analysis of irrigation water samples from Western Cape.

Sample site, name and location	Sample date	Real-time RT-PCR analysis			
		NoV GI	NoV GII	HAV	Mengovirus
Site 11: Irrigation tap water	2008.05.19	Neg	Neg	Neg	Neg
	2008.12.?	Neg	Neg	Neg	Pos
Site 16: River water (Berg river – Berg-1)	2008.06.17	Neg	**Pos**	Neg	Neg
	2008.09.08	Neg	Neg	Neg	NT
	2008.09.?	Neg	**Pos:GII:6**	Neg	NT
	2008.09.22	Neg	Neg	Neg	Neg
	2008.11.?	Neg	Neg	Neg	NT
	2008.12.?	Neg	Neg	Neg	NT
	2008.12.?	Neg	Neg	Neg	Neg
	2008.12.?	Neg	Neg	Neg	Neg
Site 17: River water (Berg river – Berg-2)	2008.06.17	Neg	Neg	Neg	Neg
	2008.09.08	Neg	Neg	Neg	NT
	2008.09.?	Neg	Neg	Neg	NT
	2008.09.22	Neg	Neg	Neg	Neg
	2008.09.?	Neg	Neg	Neg	NT
	2008.11.?	Neg	Neg	Neg	NT
	2008.12.?	Neg	Neg	Neg	NT
	2008.12.?	Neg	Neg	Neg	Neg
Site 18: River water (Berg river – Berg-3)	2008.05.19	Neg	Neg	Neg	Neg
	2008.06.17	Neg	**Pos:GII.4**	Neg	Neg
	2008.09.08	Neg	Neg	Neg	NT
	2008.09.?	Neg	**Pos**	Neg	NT
	2008.09.22	Neg	Neg	Neg	NT
	2008.10.22	Neg	Neg	Neg	Neg
	2008.11.?	Neg	Neg	Neg	NT
	2008.12.?	Neg	Neg	Neg	NT
	2008.12.?	Neg	Neg	Neg	NT

NT: not tested

3.7.4 Discussion and Conclusions

In this study 12.9% (13/101) of irrigation water samples tested positive for one or more potentially pathogenic human viruses, namely NoV GII and HAV. Similar findings were reported for previous studies in SA where HRVs were detected in 16.7% of irrigation water samples analysed (Barnes & Taylor, 2004; Tshivhandekano, 2005; Van Zyl *et al.*, 2006) detected HAdVs, enteroviruses and HRVs in river water used by subsistence farmers for the irrigation of fresh produce. This level of viral contamination of irrigation water is not limited to SA as in South Korea, 17% of ground water used for irrigation of fresh produce was contaminated with enteric viruses (Cheong *et al.*, 2009). Norovirus GII was the most predominant virus detected in irrigation water samples in this study, being present in 12/101 (~12%) of samples with HAV being detected in 4/101 (~4%) of samples, respectively. Norovirus GI was not detected in any of the samples.

When the occurrence of viruses in the irrigation water from the different geographical areas was compared, NoV GII was detected in 4.5% (1/22) of samples from Limpopo, 0% from the North West, 11.7% (7/60) of samples from the Western Cape and 22.2% (4/18) of samples from Mpumalanga. Norovirus GII were detected at all three sites in Mpumalanga (Table 25) suggesting that the irrigation water in Mpumalanga had a high level of contamination. These NoV GII strains, were, however untypable and therefore could not be compared to other environmental or clinical strains. Norovirus GII was detected in three different rivers in the Western Cape, namely Mosselbank River, Plankenburg River and the Berg River. Genotypes II.2 and II.4 was identified in samples from the Mosselbank River while genotypes II.4

and II.6 were detected at different sampling sites, Berg 1 and Berg 3, on the Berg River, respectively (Table 27). For the past 15 years NoV GII has been shown to be the most prevalent NoVs associated with infection worldwide with NoV GII.4 being the predominant genotype (Atmar, 2010). In a South African study NoV GII.4 was the most frequently detected NoV in diarrhoeal stool specimens in hospitalised paediatric patients (Mans *et al.*, 2010). The detection of NoV GII.2 and GII.4 in water from the concrete furrow, which was a branch of the Mosselbank River, was not surprising as this sampling site was about 1 km downstream from the Kraaifontein sewage works and flows through a dilapidated area. The water is finally used for irrigation of a variety of vegetables mostly sold on the Cape fresh market. The detection of NoV GII.6 at the Berg 1 sampling site confirms bacteriological results indicating gross human faecal contamination of the river which could have emanated from the Groenendal sewage works. Norovirus GII.4 was detected at the Berg 3 sampling site, approximately 30 km downstream of Berg 1. The Berg 3 sampling site is downstream from a wastewater treatment plant which reportedly malfunctions at times and the bacteriological quality of the water exceeded safe levels for irrigation water (Britz TJ, Project leader WRC Project K5/1773/4, *personal communication*). There is only one other report of NoVs in surface water in SA (Mans *et al.*, 2010), where high levels of NoV GII contamination of river water in the Vaal Catchment Area, Gauteng was described, with NoV GII.4 and GII.6 being the predominant genotype identified. This indicates that the contamination of river water with NoVs is not limited to the Western Cape and Mpumalanga but is a more widespread problem. The occurrence of NoVs in surface water has been reported in other regions of the world, namely The Netherlands (Lodder & de Roda Husman, 2005); South Korea (Lee & Kim, 2008), Brazil (Victoria *et al.*, 2010) and Venezuela (Rodríguez-Díaz *et al.*, 2009) and have been detected in groundwater used for irrigation purposes in South Korea (Cheong *et al.*, 2009).

Hepatitis A virus was detected in 4.5% (1/22)of irrigation water samples from Limpopo, 0% from North West, ~5% from Western Cape and 0% from Mpumalanga. In the Western Cape, HAV (untypable) was detected in the Mosselbank River (Site 1) and the Plankenburg River (Site 4) (Table 27). The Plankenburg river sampling site is downstream of the Plankenburg industries and Kayamandi informal settlement. The source of contamination in this area might therefore be the untreated sewage from the informal settlement as previously reported (Barnes & Taylor, 2004). In SA, large numbers of people live in areas where surface water is the only available water for irrigation and washing of fresh produce. Since water availability is often critical, little attention is given to the microbial or virological quality of irrigation water. Detection of the viral pathogens in large volumes of water will depend upon the sensitivity of the recovery and detection procedures, the concentration of viral pathogens in the point sources. The data from these samples demonstrated that some contamination of irrigation water by enteric viruses had taken place. The Western Cape falls within the winter rainfall region while Mpumalanga, Gauteng and Limpopo fall within the summer rainfall region of SA. During the rainy season, it is anticipated that runoff water could pollute surface water sources and disseminate enteric viruses from contaminated point sources, e.g. from human defecation close to the rivers. In only one province, i.e. Mpumalanga was the occurrence of enteric viruses in the irrigation water more noticeable in the rainy season.

The detection of NoVs in the irrigation water samples from SA was not unexpected as a number of other enteric viruses, namely enteroviruses, HAdVs, HAstVs, RVs and HAV have been detected in surface water samples in SA (Taylor *et al.*, 2001; Nadan *et al.*, 2003; Barnes & Taylor, 2004; Van Heerden *et al.*, 2004; Vivier *et al.*, 2004; Ehlers *et al.*, 2005; Tshivhandekano, 2005; Van Heerden *et al.*, 2005; Van Zyl *et al.*, 2006; Taylor, 2007; Van Zyl, 2007). In addition, NoVs have been implicated in a number of waterborne

outbreaks of gastroenteritis (Maunula *et al.*, 2005; Hewitt *et al.*, 2007), Centers for Disease Control and Prevention (CDC, 2011) and have been detected in water sources worldwide, including The Netherlands (Lodder & de Roda Husman, 2005); Finland (Maunula *et al.*, 2005); New Zealand (Hewitt *et al.*, 2007), South Korea (Cheong *et al.*, 2009), Brazil (Victoria *et al.*, 2010); China (He *et al.*, 2010), and Ghana (Gibson *et al.*, 2011). The detection of genetically related NoVs in the Western Cape and Gauteng suggests that these strains have a widespread distribution in SA. From the results it is evident that the NoV GII.2, GII.4 and GII.6 strains detected in the irrigation water are of clinical importance as they showed a high genetic relatedness to strains associated with sporadic gastroenteritis in SA (Mans *et al.*, 2010) and other regions of the world such as the USA (Ando *et al.*, 1997); Korea (Han *et al.*, 2011); and Japan (Chan-it *et al.*, 2010) : *direct submission to GenBank*). Worldwide NoVs are the most common cause of epidemic gastroenteritis in all age groups (Patel *et al.*, 2009) and are becoming a prominent cause of travellers' diarrhoea (Apelt *et al.*, 2010; Koo *et al.*, 2010). The detection of common or closely related strains worldwide is of public health concern as they may be disseminated through a common vehicle such as the international food market (Glass *et al.*, 2009). From the results it was evident that in selected areas of SA, irrigation water was contaminated with potentially pathogenic human viruses, namely HAV and NoV GII.

CHAPTER 4

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CHAPTER 5

ARCHIVING OF DATA GENERATED DURING THE PROJECT

Large volumes of data were generated by the various research teams collaborating on this WRC research project. The raw data generated during this project is thus being archived by the respective research institutions. These include the:

Department of Food Science, University of Stellenbosch, Stellenbosch, Western Cape

Department of Food Science, University of Pretoria, Pretoria, Gauteng

Discipline of Microbiology, University of KwaZulu-Natal, Pietermaritzburg, KwaZulu-Natal

Department of Microbiology, University of Venda, Thohoyandou, Limpopo

Department of Medical Virology, University of Pretoria / National Health Laboratory Service