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

VOLUME IV: EXTENT OF CONTAMINATION IN IRRIGATION WATER AND LINKS TO IRRIGATED PRODUCE AT-HARVEST: WITH A FURTHER EXPLORATORY STUDY OF CONTAMINATION OF FRESH PRODUCE AT POINT-OF-RETAIL

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EXECUTIVE SUMMARY

1. BACKGROUND AND MOTIVATION

Agriculture is a key contributor to the stability of South Africa's economy, but agriculture is unavoidably dependant on the availability of clean irrigation water. The polluted state of many water resources led to the question of how this would impact the health of people who rely on the water resources on a daily basis. Other concerns refer to the possible carry-over of potential pathogens to fresh produce irrigated with polluted water and the impact there-of on the safety of fresh produce. The potential threat to human health from the likely transfer of pathogens from polluted rivers to fresh produce has consequently become a major concern. The potential of pathogenic organisms being transferred repeatedly onto the surface of fresh produce during multiple irrigation sessions, along with their ability to survive for several months in these unfavourable conditions presents the scenario where consumers unknowingly face a high risk of being infected with harmful organisms when consuming fresh produce.

As the irrigation water could be a possible source of contamination any link between contaminated irrigation water and contaminated fresh produce needs to be investigated to aid decision makers as to reducing the risk of foodborne pathogen transmission. Due to the socio-economic consequences the contamination of fresh produce could have, it is also crucial to determine the level of contamination of irrigation water with human pathogens.

2. OBJECTIVES

The purpose of this solicited Water Research Commission research project on "a quantitative investigation into the link between irrigation water quality and food safety" was to establish the link between water quality and safety of fresh produce by means of standard microbial methods for the detection of indicator and potential pathogenic index organisms (bacteria, protozoa and viruses).

3. FINDINGS AND CONCLUSIONS

3.1 Western Cape – Contamination on irrigated raw produce at harvest

As found for irrigation water, total coliform and faecal counts for produce at harvest were much higher than standards by the World Health Organisation (WHO) and Department of Water Affairs (DWA) and guidelines recommended by retailers. Typical growth of total coliforms (TC), faecal coliforms (FC) and *E. coli* was also observed in most samples. The data obtained also showed that the aerobic colony counts (ACC) varied from zero to unacceptably high. It was also found that both the aerobic and anaerobic spore formers were low to absent in the samples. Intestinal enterococci, *Salmonella, Listeria* and *Staphylococcus* were present in most produce samples indicating the presence of potential pathogens. The high counts clearly indicate that the produce studied may constitute a health risk.

3.2 Western Cape – Contamination of fresh produce purchased at-retail level

This was an exploratory study on produce available at selected Western Cape food markets and to get an indication of the microbial loads present on produce that is consumed raw or after minimal processing. When comparing the data obtained with that of the "microbial guidelines" as applied by retailers in South Africa some of the produce could be classed as microbiologically unsatisfactory.

3.3 Western Cape – Linking microbes in irrigation water to those on fresh produce

In this study it was also found that the microbial loads in irrigation water and on the raw produce at-harvest especially the TC and FC counts, were much higher than guidelines. The presence of *E. coli* was also confirmed in all samples monitored. The presence of intestinal enterococci, *Salmonella, Listeria* and *Staphylococcus* were also found in most produce samples indicating the presence of potential pathogens. Their presence in the river and irrigation water and then on the irrigated produce clearly indicates that the produce studied may constitute a health.

3.4 Western Cape – Linking coliforms in irrigation water to those on produce during field irrigation

Phenotypically and genotypically characterized faecal coliform isolates from irrigation water and irrigated produce were statistically clustered. More than 30% of the irrigation and produce isolates (105) were clustered together suggesting that isolates obtained from the produce are related to strains from irrigation water. It was therefore concluded that the *E. coli* strains from the surface of produce were present as a result of transfer from the contaminated irrigation water to the fresh produce.

It was important to note that no carry-over to produce was observed when the counts of irrigation water were in the 10^3 to 10^4 cfu.mL^{-1} range. This could be an indication that these are “safe” concentrations to irrigate fresh produce with and that no or a minimum number of *E. Coli* will be carried over. The presence of four *E. coli* strains was identified as enteropathogenic *E. coli* (EPEC). These strains were isolated during a 14 day irrigation period which suggests a upstream source continuously faecally polluting the river. Since the presence of EPEC strains have been shown, it is an indication that water from this river is unsafe for irrigation of fresh produce.

3.5 KwaZulu-Natal – establishing links between Baynespruit River water and fresh produce from a low cost housing in Sobantu

This study was conducted to establish potential links between Baynespruit River water quality and the microbiological quality of fresh produce from a low cost housing sub-urban area in Sobantu, Pietermaritzburg. Faecal coliform counts of up to 1.6×10^6.100 mL^{-1} of irrigation water and 1.6×10^5 g^{-1} of produce, were observed. Faecal coliform counts on most occasions exceeded the recommended maximum guidelines of the WHO and DWA for safe irrigation. It is generally stipulated by that fruit and vegetables that are likely to be eaten raw should not contain more than 200 total coliforms per gram and no *E. coli* is present per gram analysed to ensure its safe consumption. The produce samples were therefore, in many cases, not suitable for consumption regardless of the counts established for *E. coli*, as well as recommended maximum values for consumption of raw produce. The water of the Baynespruit River is thus not safe and suitable for produce irrigation.

3.6 Limpopo Province – Contamination of irrigation water from the Mutzhedzi River

The results from this study indicate that the irrigation water from the Mutzhedzi River was microbiologically unsafe and contained viruses, opportunistic and pathogenic bacteria. The presence of pathogenic *E. coli* strains were positively identified as enteropathogenic *E. coli* (EPEC), enterohaemoragic *E. coli* (EHEC), enterotoxigenic *E. coli* (ETEC), enteroaggregative *E. coli* (EAEC) and enteroinvasive *E. coli* (EIEC). The result further showed that a potential link exists that indicates that the irrigation water is contaminating the pre-harvest produce. This link was shown by the presence of *Staphylococcus*, Gram negative

Enterobacteriaceae spp. and diarrhoeagenic strains of *E. coli* present in both the irrigation water and on the produce at harvest. Retail produce from the local market, from the Phadzima farming field and from the Thohoyandou rural market showed the presence of the same bacterial species. It was thus concluded that the consumption of such produce might be a potential health risk to people in the community. The results indicated the need to follow the hygienic practices in handling procedures of vegetables in rural markets.

3.7 Limpopo Province – Contamination of fresh produce at-retail and post-harvest

It was found that there was an absence of potential pathogens and low coliform and ACC numbers on the retail and post-harvest produce examined. Leafy vegetables had higher microbial loads than tomatoes for both modified atmosphere packaging (MAP) and unpackaged vegetables. The data also shows that it is important that sanitation procedures used be effective because providing a modified atmosphere in the packages may have little effect in preventing microbial growth on some vegetables.

3.8 Mpumalanga, North West and Gauteng – Links between *Cryptosporidium* and *Giardia* in surface water and irrigated produce

This study demonstrated the widespread presence of *Cryptosporidium* and *Giardia* in surface waters used for produce irrigation. Their presence has serious public health implications as they can attach and survive on the surface of fresh produce after irrigation with contaminated water. Results showed that the use of faecal indicators for monitoring water quality is not sufficient to predict the presence of *Cryptosporidium* and *Giardia* and assess the microbiological safety of irrigation water. Identification of the sources of contamination and more understanding of the ecology of *Cryptosporidium* and *Giardia* is needed to help identify good predictors of the presence of *Cryptosporidium* and *Giardia*.

3.9 Mpumalanga – Linking water microbes to produce contamination in Loskopdam area

Heavy *E. coli* and faecal coliform contamination was found in three irrigation water sources and on irrigated produce. Contamination with other pathogens (*L. monocytogenes* and *Salmonella)* was also found and confirmed that the water sources are faecally polluted. It also indicated that the two rivers are sources of contamination of the Loskop canal. The levels of aerobic bacteria in water and vegetable samples were low, but a high prevalence of bacterial pathogens was observed. This shows that aerobic bacteria levels are not a good determinant of microbiological quality of irrigation water and produce.

It was found that the Loskop canal was the contamination source for broccoli and cauliflower, but the contamination level differed widely. The lower incidence of *S. aureus*, *Salmonella*, Intestinal Enterococci and absence of *L. monocytogenes* (LM) on cauliflower compared to broccoli show the attachment differences in surface characteristics. Broccoli was found to pose a higher risk of being associated with listeriosis because of enhanced *L. monocytogenes* attachment.

The prediction studies of LM in irrigation water signify that there is direct relationship between *L. monocytogenes* and the chemical oxygen demand (COD) in irrigation water. Higher COD values may result in high concentrations of LM in irrigation water. The result also signifies that there is direct relationship between Intestinal Enterococcus and turbidity. It was concluded that logistic regression analysis may be used as a tool for predictive microbiology modelling. This has an immediate practical application to help predict microbial produce safety and quality, and provide quantitative understanding of the microbial ecology of irrigation water and produce.

3.9 Virology – Linking viruses in river water to fresh produce

Human pathogenic viruses were detected in river water and included norovirus (NoV) types NoV GII.4 and NoV GII.2, and the hepatitis A virus (HAV). These results indicate that water sources are subject to continual human faecal pollution and would therefore pose a risk if used for irrigation. The findings showed that 3.8% of the pre-harvest produce had detectable enteric virus contamination, with norovirus type NoV GII.1 being detected on a cabbage sample. The identification of hepatitis A virus type HAV IB on lettuce and tomato samples confirms that the contamination on the fresh produce was of human origin as HAV 1B has only been described in association with human infection.

From this study it was evident that the fresh produce samples collected at-retail were more likely to be contaminated with enteric viruses, with either NoV or HAV or both viruses being detected on 19% samples tested. These results suggest that most of the viral contamination of the fresh produce occurred during harvesting, processing and packaging.

It was evident that in selected areas of South Africa, irrigation water and fresh produce was contaminated with potentially pathogenic human viruses, namely HAV and NoV GII. Irrigation water and food handling were identified as possible sources of contamination of the fresh produce. The detection of both NoV GII.9 and HAV on a tomato sample is of concern indicating the vulnerability of tomatoes to viral contamination. As this study represented an exploratory study to ascertain the extent of viral contamination in irrigation water and on fresh produce, further in-depth studies are required to determine what intervention strategies can be implemented to ensure the safety of the fresh produce.

4. GENERAL CONCLUSIONS

The purpose of the research reported in this Volume (IV) was to establish the link between water quality and safety of fresh produce by confirming the carry-over of specific indicator and index organisms (bacteria, protozoa and viruses) from the water to the produce.

The potential of pathogenic organisms being transferred from irrigation water to the surface of fresh produce plus their ability to survive in these unfavourable conditions presents the scenario where consumers unknowingly face a high risk of being infected with harmful organisms when consuming fresh produce.

In all the selected provinces where water and produce microbial loads were monitored it was found that the Indicator organisms counts (TC and FC) were in most cases much higher than guidelines recommended by the World Health Organisation (WHO) and DWA (previously known as Department of Water Affairs and Forestry) and local retailers. Index organisms including human viruses, protozoa, intestinal enterococci, *Salmonella, Listeria monocytogenes* and *Staphylococcus aureus* were present in most irrigation waters and on produce samples indicating the presence viruses, opportunistic and pathogenic bacteria. The study also showed the widespread presence of *Cryptosporidium* and *Giardia* in surface waters used for produce irrigation. The high microbial counts clearly indicate that the irrigation waters and different types of produce studied may constitute a health risk. It is generally stipulated by the WHO that fresh produce likely to be eaten raw should not contain more than 200 total coliforms per gram and no *E. coli* present per gram to ensure safe consumption.

The presence of pathogenic *E. coli* strains was also found. Since the presence of EPEC, EHEC, ETEC, EAEC and EIEC strains have been shown, it is an indication that water from rivers is unsafe for irrigation of fresh produce. Human pathogenic viruses were also detected in river water from selected

areas of South Africa (NoV GII.4 and NoV GII.2 and HAV) and 3.8% of pre-harvest produce evaluated had detectable enteric virus contamination. NoV GII.1 was detected on cabbage and HAV IB on lettuce and tomato samples. This confirms that the contamination on the fresh produce was of human origin as HAV 1B has only been described in association with human infection. These results indicate that water sources are subject to continual human faecal pollution and would therefore pose a risk if used for irrigation.

In the general linking studies where strain identification, phenotypic and genotypic characterisations was undertaken, at least 30% direct linkages could be made and it was concluded that species from the surface of produce were present as a result of transfer from the contaminated irrigation water. There can now be no doubt that specific carry-over does take place.

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ABBREVIATIONS

ACC	aerobic colony count
AnSF	anaerobic spore formers
API	analytical profile index
ARP	antibiotic resistance profiles
ASF	aerobic spore formers
ATCC	American Type Culture Collection
CDC	Centers for Disease Control and Prevention
cfu	colony forming units
COD	chemical oxygen demand
Crypto	*Cryptosporidium*
DAEC	diffusely adhering *E. coli*
DNA	deoxyribonucleic acid
DoA	Department of Agriculture
DoAFF	Department of Agriculture, Forestry and Fisheries(previously DoA)
DoH	Department of Health
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*
EC	*Escherichia coli*
EHEC	enterohaemoragic *E. coli*
EIEC	enteroinvasive E. coli
Entero	intestinal enterococci
EPA	Environmental Protection Agency
EPEC	enteropathogenic *E. coli*
ETEC	enterotoxigenic *E. coli*
EU	European Union
EXPEC	extraintestinal *E. coli*
FC	faecal coliforms
Giar	*Giardia*
HastV	human astroviruses
HAV	hepatitis A virus
HAV 1B	hepatitis A virus genotype 1B
HIV	human immunodeficiency virus
HRV	human rotavirus
HuCV	human caliciviruses
HUS	haemolytic uremic syndrome
LM	*Listeria monocytogenes*
MAP	modified atmosphere packaging
MAR	multi-antibiotic resistance profiling
MENEC	meningitis *E. coli*
m-PCR	multiplex PCR
MPF	minimally processed fruits and vegetables
MPN	Most Probable Number
ND	None detected
NoV	norovirus
NoV GII	norovirus genotype GII
NoV GII.4	norovirus genotype GII.4
N-PCR	nested polymerase chain reaction
NT	not tested
PCR	polymerase chain reaction
PSS	physiological saline solution
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
TNTC	Too numerous to count
t-PCR	triplex polymerase chain reaction
UK	United Kingdom
UPEC	urinary tract infection *E. coli*
USA	United States of America
VRBA	Violet Red Bile Agar
WHO	World Health Organisation
WRC	Water Research Commission

CHAPTER 1
INTRODUCTION

The weather conditions in South Africa play an important role in determining the amount of available fresh water resources. South Africa's meagre average annual rainfall of 500 mm is influenced predominantly by the cold Benguela and warm Mozambique ocean currents and subsequently rainfall is often scattered and erratic. Consequently, water is a scarce resource, with most parts of the country prone to droughts during seasons of low rainfall.

The continuously growing population, urbanisation, industrial effluent and out-dated, or in some cases, non-existent waste water treatment plants, all contribute to aggravate an already water-stressed situation (Barnes & Taylor, 2004). Precarious conditions in informal settlements due to the lack of adequate sanitation and waste disposal systems have forced many to use near-by rivers as means for disposing of both household and human waste. Ultimately, rivers are polluted with high concentrations of potentially pathogens and infectious viruses. Faecal pollution caused by surface run-off, flooding and sewerage treatment plants functioning well over capacity, have all contributed to severely polluted rivers and dams (Warner *et al.*, 2008).

Agriculture is a key contributor to the stability of South Africa's economy, with exports to the European Union estimated at R 27 billion per year. These exports not only generate much needed income through both international and local trade, but also create job opportunities and provide a livelihood for many subsistence farmers. Agriculture is unavoidably dependant on the availability of clean irrigation water from rivers and dams. Therefore, as the most important limiting factor to agriculture, it is not surprising that the restricted availability of clean water and extensive pollution is of great concern. With almost 50% of South Africa's water resources applied to irrigate an estimated 1.3 million hectares, and the scarcity of water regarded as a fundamental development constraint, managing and protecting our water resources in order to guarantee a sustainable supply of fresh water in future is of utmost importance.

Climate change has caused changes in weather patterns which have resulted in decreased rainfall patterns, thereby further aggravating the water-stressed problem. As a result, farmers have become even more dependent on irrigation water and have little control over the extent to which this water is polluted. The polluted state of many rivers and dams posed the question of how this would impact the health of the thousands of people who rely on these water resources on a daily basis. Other concerns refer to the possible carryover of potential pathogens to fresh produce irrigated with polluted water and the impact there-of on the safety of fresh fruit and vegetables.

The potential threat to the health of humans because of the likely transfer of pathogenic organisms from the polluted rivers to the produce has consequently become a major concern (Abadias *et al.*, 2008; Goss & Richards, 2008). The potential of pathogenic organisms being transferred repeatedly onto the surface of fresh produce during multiple irrigation sessions, along with their ability to survive for several months in these unfavourable conditions (Brackett, 1999; Goss & Richards, 2008) presents the scenario where consumers unknowingly face a high risk of being infected with harmful organisms when consuming fresh produce. Internationally, a sharp increase in foodborne outbreaks related to the consumption of fresh or minimally processed food (Abadias *et al.*, 2008; Warner *et al.*, 2008) has been observed and South Africa is most likely to follow.

Faecal contamination of water resources is a serious problem which many countries are facing (Lu *et al*., 2004). Waters contaminated with human faecal matter are generally regarded as a greater risk as they are more likely to contain human-specific enteric pathogens such as *E. coli*, *Salmonella spp.*, *Shigella* spp. and the hepatitis A virus (Scott *et al*., 2002). As a result, it is estimated that more than 3 million people, mostly children, die each year from water-related diseases (Wilkes *et al*., 2009). It is therefore critical that a clear understanding of the various contamination sources of faecal pollution be achieved for the successful risk assessment and development of corrective management techniques.

Fresh produce, leafy greens, tomatoes, herbs and cantaloupes are the leading source of *E. coli* outbreaks and the second highest source of food borne disease outbreaks (Franz & Van Bruggen, 2008; Mandrell, 2009). *E. coli* O157:H7 (EHEC) outbreaks are also common, especially as the result of consuming leafy greens (Mandrell, 2009). The largest outbreak of bacterial enteric disease occurred in 1996 when *E. coli* O157:H7 contaminated radish sprouts resulting in 9 451 cases, 7 900 hospitalisations and 12 deaths in Japan (Michino *et al.*, 1999). The biggest outbreak regarding *E. coli* O157:H7 in the USA occurred in 2006, when contaminated, packaged baby spinach resulted in 206 infections and three deaths (Buchanan, 2006). The most recent *E. coli* outbreak occurred in Germany, with >4 000 cases, 852 patients with haemorrhagic uremic syndrome and 32 deaths due to *E. coli* O104:H4 contamination on raw sprouts (CDC, 2011). *Listeria monocytogenes, Salmonella* and *E. coli* have been isolated from raw vegetables which have become contaminated while growing or during harvesting, postharvest handling or distribution (Oliviera *et al.*, 2010). Although *L. monocytogenes, Salmonella* and *E. coli* get much of the attention, many other bacterial pathogens cause outbreaks too, including certain species of *Bacillus, Campylobacter, Clostridium, Shigella, Staphylococcus, Vibrio* and *Yersinia* (Xia *et al.*, 2009).

It has also been shown that 40% of norovirus (NoV) and 5% of hepatitis A virus (HAV) infections are due to the consumption of contaminated foods (Le Guyader & Atmar, 2008). In addition the viruses are stable in the environment and in food matrices and survive post-contamination food production processes (Duizer & Koopmans, 2008).

Cryptosporidium and *Giardia* are another group of potential pathogens and have been shown to be of the most common and resistant human waterborne parasitic protozoa worldwide (Chaidez *et al.*, 2005). They have been isolated from raw water, treated effluent and drinking water in South Africa (Kfir *et al.*, 1995). *Cryptosporidium* has been shown to be able to attach to the surface of vegetables following irrigation and become internalized making its removal difficult to achieve. *Cryptosporidium* oocysts have also been found on the surface of raw vegetables in both developing and developed countries (Robertson & Gjerde, 2001b).

Many rural households in South Africa depend on Minimal Processed Food (MPF) for their daily needs and some people sell it to the local rural market for additional household income. In most rural communities in the Vhembe district of Limpopo province cabbages and tomatoes are also grown in community farm fields by subsistent farmers generally to grow enough food to feed their families and planting decisions are made with an eye towards what the family will need during the coming year before selling to markets. These farming fields are usually situated next to surface waters which are surrounded by rural settlements and which are used by animals for drinking. In addition the community also uses the bushes close to these rivers as toilet areas and when it rains these waste are washed into these rivers. The same water from the rivers is used for the irrigation by the farmers to irrigate the farming fields (Thomas *et al.*, 2009).

As the irrigation water could be a possible source of contamination any link between contaminated irrigation water and contaminated fresh produce needs to be investigated to aid decision makers as to reducing the risk of foodborne pathogen transmission. Due to the socio-economic consequences the contamination of fresh produce could have, it is also crucial to determine the level of contamination of irrigation water with human pathogens.

To enable a microbial risk assessment, this research intends to verify whether there is evidence for a transfer of hygiene indicators or potential pathogens present in some South African river water used for irrigation onto the surface of fresh produce via the irrigation route. However very little information is available on the microbiological quality of fresh produce especially that grown by small-scale commercial farmers and that grown by subsistent farmers and sold in rural markets.

This WRC research project was therefore initiated with the main objective of establishing the link between the water quality and safety of fresh produce by means of standard microbial methods for the detection of indicator and potential pathogenic organisms. The aim was thus to link carry-over of faecal organisms present in the water from different water sources across the country to fresh produce irrigated with this water. Faecal indicators present in the irrigation water and on the surface of fresh produce will be isolated and phenotypically and genotypically characterised and the data compared using numeric and statistical analysis to confirm degrees of similarity.

CHAPTER 2
EXPERIMENTAL PROCEDURES

2.1 MICROBIOLOGICAL ANALYSIS

The Aerobic Colony Counts (ACC) were carried out according to the SANS procedure 4833 (SANS, 2007) while aerobic and anaerobic spore formers (ASF and AnSF) were quantified according to Health Canada method MFLP-44 (Austin, 1998). The total (TC), faecal coliforms (FC) and *E. coli* (EC) numbers were established according to Health Canada method MFHPB-19 (Cristensen *et al.*, 2002). The presence of *E. coli* was confirmed via PCR of the *gadA* gene (Kim *et al.*, 2006). *Salmonella* spp. (Sal) was detected according to the ISO procedure 6579 (SANS, 2003). The presence of *Staphylococcus aureus* (SA) was according to ISO procedure 6888-1 (SANS, 2000) and confirmation was done via PCR (Morot-Bizot *et al.*, 2004). Intestinal enterococci (IE) were according to the ISO guideline 7899-2 (ISO, 2000) while the presence of *Listeria monocytogenes* (LM) was verified according to the SANS procedure 11290-1 (SANS, 2001). All methods applied were according to the standard SANS and ISO methods and all recommended quality control procedures were followed.

2.2 PHYSICO-CHEMICAL ANALYSIS

The temperature of the samples was measured with a portable device (Hanna Instrument, Inc. Woonsocket, USA**)** when collecting the samples. The pH was measured using a 211 Microprocessor pH meter (Hanna Instruments, Inc. Woonsocket, USA).

2.3 WESTERN CAPE – IRRIGATION UNDER FIELD CONDITIONS

2.3.1 Selected river site and sampling

The Plank-3 sampling site on the Plankenburg River was selected because it is an irrigation source point both upstream and downstream by nearby farmers. For the baseline studies the river was sampled every second week for eleven weeks according to the SANS 5667-6 (SANS, 2006) guideline and analysed within 60 min after sampling.

2.3.2 Plot design and sampling procedure

A 16 m^2 plot, as part of a larger field of beans (*Phaseolus vulgaris*), was irrigated daily for nine consecutive days using irrigation water collected from the Plank-3 site. The beans were irrigated using 6 L irrigation water per session, simulating the over-head irrigation technique used by most small-scale farmers.

2.3.3 Base-line values of irrigation water and produce

Samples were analysed prior to the first irrigation session to establish base-line values. Thereafter, duplicate sets of both beans (400 g) and irrigation water (1 L) were analysed daily for nine consecutive days using the Multiple Tube Fermentation technique – MFHPB 19 (Cristensen *et al.*, 2002) for the detection of coliforms, faecal coliforms and *E. coli*. The presence of *E. coli* was confirmed on LEMB agar and positive colonies randomly selected for further studies. The green bean samples were obtained daily, prior to each irrigation session, by randomly selecting beans across the 16 m^2 plot in order to obtain a representative sample of that area.

2.3.4 Isolate characterisation and clustering

Phenotypic characterisation – 92 Gram negative isolates and reference strains were characterised using API 20E kits (Biomerieux). For numerical clustering the characteristics of 105 strains composed of 54 isolates from the irrigation water, 38 from the irrigated beans, 14 reference strains were included. Twenty-seven characters were included in the data set and analysed using the Jaccard (SJ) and Sokal & Michener (SM) coefficients and the unsorted similarity matrix was rearranged into groups by average linkage cluster analysis (Lockhart & Liston, 1970). Dendrogramme distances were calculated based on the phenotypic characteristics as calculation concept.

Multi-Antibiotic Resistance (MAR) profiling – MAR was additionally used to differentiate *E. coli* from different sources by isolating and culturing the target organism and then replica plating the isolates on media containing the different antibiotics at varying concentrations (Scott *et al.*, 2002). Organisms were then scored according to their resistance to the antibiotics (Table 1) resulting in Antibiotic Resistance Profiles (ARP). Reaction to 23 different antibiotics (Davies Diagnostics, Cape Town) was determined.

Table 1. Antibiotics used in this study (Davies Diagnostics).

Antibiotic	Concentration
Cefotaxime	3 and 5 µg
Vancomycin	5 and 30 µg
Trimethoprim	2.5 µg
Tetracycline	10 and 25 µg
Gentamicin	10 µg
Cefuroxime	30 µg
Cotrimoxazole	25 µg
Ciprofloxacin	1 and 5 µg
Amoxycillin	25 µg
Amikacin	30 µg
Cephazolin	30 µg
Ofloxacin	5 µg
Chloramphenicol	10 and 30 µg
Neomycin	30 µg
Ceftriaxone	30 µg
Ampicillin	2 and 25 µg
Erythromycin	15 µg

2.4 WESTERN CAPE – IRRIGATION UNDER ENVIRONMENTALLY CONTROLLED CONDITIONS

2.4.1 Site selection and experimental design

A suitable site for environmentally controlled plot studies was identified on a farm in the Devon Valley Stellenbosch, South Africa (Carinus, 2012, Champagne, Devon Valley, Stellenbosch, personal communication). A vegetable tunnel (Rhino Plastics, Cape Town) was set up to minimise direct UV-rays, excessive winds and rain and contaminants from making contact with the beans. Star 2000 beans (Starke Ayres) were planted in four sub-plots. Spaces were left open between the sub-plots to minimise cross-contamination.

For the first plot study (Study I) two irrigation treatments were applied daily for 14 days including one with a “pure” *E. Coli* culture and the second with Plankenburg River (Plank-3) irrigation water. In each case Day zero of the trial served as the control. Beans (400 g) were harvested daily from each sub-plot, 24 h after each irrigation session. The beans harvested on the first day served as the control sample as no

irrigation treatment had been applied. The beans were hygienically and randomly harvested from various plants in each plot and placed in sterile stomacher bags and then washed with sterile physiological saline solution (PSS). In the second study "once-off" irrigation was applied. Two of the sub-plots were irrigated only once with the "pure" *E. Coli* irrigation water at cell concentrations of 10^3 cfu.mL^{-1} and 10^4 cfu.mL^{-1}, respectively. The *E. Coli* numbers in the irrigation waters and on the beans of the plots were determined and isolates selected for further clustering and molecular analysis.

2.4.2 Coliform and *E. Coli* enumeration, characterisation and clustering

Enumeration was done according to the APHA Standard Method (APHA, 1998) using Violet Red Bile Agar (VRBA) (Merck) as medium. All red colonies were taken as numbers of *E. Coli* present (Merck, 2007). For further analysis colonies (red and pink) were selected using the Harrison's disc method (Harrigan & McCance, 1974) and transferred to Brilliance™ *E. Coli*/Coliform selective agar (Oxoid). Isolates were then characterized and identified using the API Web data base (Biomerieux).

For the numerical clustering, the characteristics of the 162 strains composed of 72 isolates from the irrigation water (river water), 67 from the irrigated beans, 19 isolates from the "pure" culture studies (irrigation water and beans) and four ATCC reference strains (ATCC 13135(=404), 10799(=158), 11775(=58) and 4350(=157)) were included. Twenty-seven characters were included in the data set and analysed using the Sokal & Michener (SM) coefficient and the unsorted similarity matrix was rearranged into groups by average linkage cluster analysis (Lockhart & Liston, 1970). Dendrogramme distances were calculated based on the phenotypic characteristics as calculation concept.

2.4.3 Molecular methods

uidA PCR – All isolates that tested positive for *E. Coli* with API 20E were subjected to *uidA* gene PCR using the method described by Heijnen and Medema (Heijnen & Medema, 2009). Pathotype multiplex PCR (m-PCR) was done on *uidA* PCR confirmed *E. Coli* strains using the method of Omar and Barnard (Omar & Barnard, 2010). Triplex PCR method used was as described by Clermont and co-workers (Clermont *et al.*, 2000). Strains were selected for sequencing based on the API clustering data, specifically where strains from the irrigation river water and beans were found to be biochemically identical. The PCR method was based on the method described by Nhung and co-workers (Nhung *et al.*, 2007). The PCR products were sequenced at the Central Analytical Facility at the University of Stellenbosch.

oriC-locus sequencing – *E. Coli* strains were randomly selected for *oriC-locus* sequencing based on the API groups. These were strains from the river water and were biochemically identical to the strains on the beans a few days after. The PCR method was as described by Roggenkamp (Roggenkamp, 2007).

Sequencing analysis – The BLAST-tool was used to compare the generated sequences to sequences available in the GenBank library. The phylogenetic tree analysis was done with phylogenetic analysis using parsimony (PAUP) software with 1 000 bootstraps and the neighbour-joining algorithm (Roggenkamp, 2007) and the sequences of each gene (*dnaJ* and *oriC*) were used to construct phylograms.

2.5 KWAZULU-NATAL

2.5.1 Site selection

The Sobantu site is a suburban community in Pietermaritzburg which relies on the Baynespruit River water for agricultural irrigation. The river flows through a number of informal settlements and is used to irrigate

agricultural land including commercial and small scale market gardens. Fresh produce irrigated via bucket based overhead irrigation using the Baynespruit River water was also collected from a market garden and analysed microbiologically.

2.5.2 Produce sampling

Over a period of 13 months, monthly produce samples (the choice governed by seasonal availability) were collected at the study site by randomly removing not less than 20 g of produce material (leaves in the case of spinach and parsley and florets in the case of cauliflower) from at least three different plants. Produce samples were prepared by adding 90 mL quarter strength Ringers solution to 10 g of uncut leaf material (or florets in the case of cauliflower) present followed by 10 min treatment (200 rpm at ambient temperature on an orbital shaker). Decimal dilutions were prepared.

2.6 VENDA

2.6.1 Molecular identification of commensal and diarrhoeagenic *E. coli strains*

Samples were removed from positive identified *E. coli* MPN tubes and DNA extracted as reported by Borodina (Borodina *et al.*, 2003). The DNA was used as template in all PCR reactions (Omar & Barnard, 2010).

2.6.2 Site selection and sampling

The subsistent farming field used was situated in the Phadzima region of the Vhembe region of the Limpopo Province. This farm used the Mutzhedzi River as a source of irrigation water, drinking water, waters their livestock as well as for bathing and to wash clothes.

Sampling was according to the SANS 5667-6 (SANS, 2006) method. Cabbages and tomato samples were collected once a month together with the irrigation water sample. At least 3 cabbages of each variant (Conquest and Tennessee) were collected in 3 different areas on the field and 5 tomato samples were picked at different sites on the field. Once a month cabbage and tomato samples were also collected from the Thohoyandou local market. A total of 2 cabbages and 5 tomato samples were used in this study per month to assess the microbiological quality.

2.7 MPUMALANGA, NORTH WEST AND GAUTENG – EXPLORATORY STUDY

2.7.1 Sampling of vegetables

The vegetables collected included spinach (packaged and unpackaged), PVC packaged broccoli, ready-to-cook broccoli and cauliflower florets, PVC and modified atmosphere (MA) packaged lettuce, strawberries and baby tomatoes. The history and source of irrigation water for cultivation of these vegetables are unknown.

2.7.2 Gas analysis

The gas composition for the MAP samples was analysed using a Gas Headspace Analyser (Systech Gaspace 2 Instruments). The needle was inserted into the package after calibration to measure the content of oxygen, carbon dioxide and nitrogen in the package.

2.7.3 Treatment of samples

Different leaves and different sections of leaves do not have a homogenous distribution of bacteria. To have a representative sample, different sections of the Swiss chard and endive lettuce leaves were broken off to make a 25 g sample. For the MAP crisp lettuce, different leaves were taken to make 25 g. A 25 g sample was homogenized in 225 mL of 0.1% sterile buffered peptone water (bpw) for 2 minutes in the stomacher 400. For the tomato samples homogenization was done by manually shaking the tomatoes in a sterile stomacher bag containing 225 mL of 0.1% sterile buffered peptone water for 2 minutes. Enumeration was done for *Staphylococcus aureus,* coliforms and aerobic microbes. Detection was done for *E. coli, Listeria monocytogenes* and *Salmonella.*

2.7.4 Scanning electron microscopy

A fixative to preserve the sample was made by using 1 mL glutaldehyde, 5 mL of buffer and 4 mL distilled water. A sample of the lettuce leaf, spinach leaf and tomato skin (5 mm X 5 mm) was cut while in the fixative and transferred to a tube containing the fixative. This was left overnight. The sample was rinsed for 15 min three times in 0.075 M buffered phosphate to remove the aldehyde. The samples were dehydrated in ethanol for 15 min three times. A scanning electron microscope was used to view the inner and outer surface structure of lettuce and Swiss chard but only the outer surface structure for tomatoes.

2.7.5 Statistical analysis

A two way analysis of variance using the Statistica programme was used. The Turkeys' LSD at 5% significance level was used to determine whether a significant difference existed between the two types of packaging and among the vegetables.

2.8 MPUMALANGA, NORTH WEST AND GAUTENG – LINKING STUDIES

2.8.1 Extent of contamination in irrigation water and links to irrigated produce at-harvest and at point-of-retail

The selected sampling sites monitored are all used as irrigation water sources for the irrigation of fruits and vegetables (Table 2).

2.8.2 Linking *Cryptosporidium* and *Giardia* in Skeerpoort river and that found on produce

Water and fresh produce sampling – 30 samples (1 L) were collected monthly from the three sampling sites over a period of 10 months from June 2009 to April 2010. Samples (50 L) were collected for *Cryptosporidium* and *Giardia* analysis at the Department of Food Science, University of Pretoria.

Broccoli irrigated by the Loskop Dam irrigation scheme was sampled over a period of three weeks prior to harvest. Lettuce from Skeerpoort farm was sampled over a period of three months at the same time as the water samples. Three lettuce and broccoli heads were selected based on accessibility and analysed within 12 h.

Isolation of Cryptosporidium oocysts and Giardia cysts – *Cryptosporidium* and *Giardia* were isolated using filtration, immunomagnetic separation and staining with (O'Donoghue, 1995) as previously described. Positive controls were performed to verify the efficiency of the method using ColorSeed™ and

EasySeed™ (Biomerieux, Sydney, United Kingdom). Each ColorSeed or EasySeed capsule contains an exact amount of *Cryptosporidium* oocysts as per certificate of analysis.

Table 2. Sampling sites and products grown under irrigation in the provinces included in this study.

Province	Site	River	Products under irrigation
Mpumalanga	Groblersdal	Moses River	Wheat, vegetables, peanuts, cotton and citrus fruit)
North West	Skeerpoort	Skeerpoort River	Lettuces, potatoes, cabbage, peppers, beans and tomatoes
Gauteng	Vereeniging	Klip River	Maize, fodder crops, carrots, spinach, cabbage, onions, potatoes and salad greens

Nested PCR – DNA was extracted and amplification of *Cryptosporidium* DNA was performed (Nichols *et al.*, 2003).

Fresh produce analysis – *Cryptosporidium* oocysts and *Giardia* cysts were isolated from fruit and vegetable samples according to the method of Robertson and Gjerde (Robertson & Gjerde, 2000). The wash water was then analysed for *Cryptosporidium* and *Giardia* according to EPA method 1623 described above.

Statistical analysis – Analyses of variance performed using Statistica software for windows version 7 (Tulsa, Oklahoma, USA, 2003) were used to test for significant differences in physico-chemical and microbiological quality at 95 % confidence interval between the different water sites and produce. Percentages prevalence was calculated for each pathogen analysed, arithmetic means and standard deviations were calculated for MPN of faecal coliforms and *E. Coli*, temperature and pH for each sampling site. A binary logistic regression model analysis was performed to determine whether indicator parameters, faecal coliforms, aerobic plate counts, pH and temperature predicted the probability of the occurrence of pathogen in water samples. The dependant variable, the pathogen, was treated as a binary variable. The independent variables, the indicator parameters, were continuous.

2.9 MPUMALANGA – LINKING *LISTERIA, SALMONELLA* AND INTESTINAL ENTEROCOCCUS IN IRRIGATION WATER AND ON PRODUCE USING REGRESSION ANALYSIS

2.9.1 River, site and produce selection

The Loskop dam irrigation scheme in the Mpumalanga Province was selected as the sampling area. Surface water samples were collected from three points 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 subsequently released to Loskop canal system which is used to irrigate the vegetables. Surface water from the three points was aseptically collected during 12 intervals (November 2007 to October 2008). Three farms cultivating vegetables irrigated with water from the Loskop dam irrigation scheme were also visited three times over a period of 3 months for collection of vegetables, cauliflower and broccoli.

2.9.2 Statistical analysis

Analysis of variance (ANOVA), 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 river, Wilge river 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, 1984-2009) was used for the statistical analysis.

The associations of the occurrence of *Listeria monocytogenes, Salmonella* spp. and Intestinal Enterococcus in irrigation water and vegetables were explored using binary logistic regression analysis. For this analysis, we dichotomised the dependent variables, *Listeria monocytogenes, Salmonella* spp and Intestinal Enterococcus where values for absence were coded as '0' while values for presence were coded as '1'. For prediction of the three bacterial pathogens in irrigation water, four predictor variables (i.e. faecal coliform, location, COD and turbidity) were taken into the model. On the other hand, ACC, *S. aureus*, location, AnSF and faecal coliform were used as predictor variables in the model for prediction of the bacterial pathogens in vegetables. The resulting regression coefficients quantified the type of association between the predictor variable and the respective dependent variable. A *p*-value of ≤0.05 was considered statistically significant and all reported *p*-values were two-tailed.

2.10 VIRUS DETECTION IN IRRIGATION WATER AND ON PRODUCE

2.10.1 Irrigation water sites

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.

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 the Western Cape, the irrigation water sites included: i) a concrete furrow, which was a branch of the Mosselbank river, about 1 km downstream from a sewage works (Site 1); and ii) tap water drawn from a dam filled from the Plankenburg River (Site 11 = Site 10a)

2.10.2 Produce samples

To ensure that each fresh produce sample was a representative sample, a sample comprised of three individual items of the fruit/vegetable collected from different parts or areas of the field, crate, box or punnet, and each item was analysed individually. Samples were collected in separate sterile plastic bags, transported to the laboratory in cooler bags and stored at 4°C until further processing and analysis.

Samples collected pre-harvest or at harvest – Samples included fruit/vegetables with a rough or convoluted surface, namely spinach, lettuce, cabbage and beans and fruit/vegetables with a smooth surface, namely tomatoes were collected in Limpopo and the Western Cape. A subset of these samples included fruit/vegetable samples paired or linked to irrigation water samples, i.e. these were samples of vegetable/fruit for which a sample of irrigation water used to irrigate the produce could be collected at the same time.

Samples collected post-harvest – Fresh produce samples from Gauteng were purchased from a variety of formal and informal outlets in the Tshwane Metropolitan area. Samples included fruit/vegetables with a rough or convoluted surface, namely lettuce, cabbage, spinach and rocket and fruit/vegetables with a smooth surface, namely tomatoes. Fresh produce samples from Limpopo were purchased from formal and informal outlets (Fig. 4.1) in Thohoyandou. These samples included vegetables with a rough or convoluted surface, namely lettuce and cabbage, and fruit/vegetables with a smooth surface, namely tomatoes and onions. Fresh produce samples from the Western Cape, namely lettuce (X1) and cabbage (X1) were obtained from a farm stall which had been irrigated with water from Plankenburg river.

2.10.3 Viral recovery from irrigation water and fresh produce

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 from the irrigation water by means of a glass wool adsorption-elution technique based on the method of Vilaginès (Vilaginès *et al.*, 1997) as described previously (Wolfaardt *et al.*, 1995; Grabow *et al.*, 1996; Vivier *et al.*, 2004), and further modified and optimised by Venter (Venter, 2004).

For the fresh produce each item of non-leafy fruit/vegetable was weighed and seeded with 10 µl (1 x 10^5 $TCID_{50}$) mengovirus as a process control. The outer leaves of the cabbage or lettuce were selected and cut to fit the area of a 140 mm Sterilin Petri dish (Bibby Sterilin Ltd, Stone, UK) and then seeded with mengovirus. Viruses were recovered using tris-glycine-beef extract buffer pH 9.5 with elution for 20 min at room temperature. Thereafter, the pH of the eluate was adjusted to pH 7.2 and the viruses were further concentrated to a final volume of 500 µl in phosphate buffered saline using a polyethylene glycol 6000/sodium chloride (Merck) precipitation method and the suspension stored at -20ºC.

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 was aliquoted and stored at -70°C before use. Five microliters of the eluted nucleic acid was used for virus nucleic acid 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. *HAV*: The primers and probes by Costafreda (Costafreda *et al.*, 2006) were used. *NoV GII*: The primers and probes as described by Loisy (Loisy *et al.*, 2005) and da Silva (Da Silva *et al.*, 2007) were used. *NoV GI:* The primers and probes as described by Svraka (Svraka *et al.*, 2007) and da Silva (Da Silva *et al.*, 2007) were used.

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 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.

Viral characterisation – Norovirus: Norovirus strains from samples that were NoV positive by real-time RT-PCR were genetically characterised (Mans *et al.*, 2010). Nucleotide sequences were analysed using Sequencer™ 4.9 (Gene CODES Corporation, Ann Arbor, MI), BioEdit Sequence Alignment Editor (V.7.09.0) and BLAST-N. Sequences were aligned with reference sequences from NoV GI and GII using MAFFT Version 6. After manual adjustment of the alignment, phylogenetic analysis was performed with

MEGA 4 using neighbour-joining methods. For determining the reliability of tree topology, bootstrap analysis was carried out on 1 000 replicates.

Hepatitis A virus – The HAV strains were characterised by nucleotide sequence analysis of the VP1/P2A junction region of the genome (Robertson *et al.*, 1992). The nucleotide sequences were verified using the BioEdit Sequence Alignment Editor V6.0.5 (Isis Pharmaceuticals, Inc.). Nucleotide sequences were analysed using Sequencer™ 4.9 (Gene CODES Corporation), BioEdit Sequence Alignment Editor (V.7.09.0) and BLAST-N. Sequences were aligned with reference sequences of HAV using MAFFT Version 6. After manual adjustment of the alignment, phylogenetic analysis was performed with MEGA 4 using neighbour-joining methods. For determining the reliability of tree topology, bootstrap analysis was carried out on 1 000 replicates.

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 – CONTAMINATION ON IRRIGATED PRODUCE AT HARVEST

3.1.1 Specific aim

The aim of this study was to do a study on the extent of contamination found on irrigated raw produce at harvest in the Western Cape.

3.1.2 Sampling sites and produce

Mosselbank site – The farm at this site produces lettuce and cabbage using large scale overhead-irrigation methods with water directly sourced from the Mosselbank River. With the exception of rainy days, irrigation was done daily.

Plankenburg River site – In this case farm workers grow mainly beans and also utilised an overhead-irrigation method to irrigate their crops. Their water is drawn from a holding dam filled from the Plankenburg/Eerste River, and then piped through a tap system to daily irrigate the beans. No irrigation was applied during rainy weather.

Eerste River site – Two different sites were selected to study the contamination on grapes produced for the local and international markets. In both cases the produce was only be sampled at the end of the growing season. One site made use of overhead-irrigation and the other used drip-irrigation. Water was drawn from holding dams filled from the Eerste River, and then pumped directly to the irrigation systems. Irrigation was done on a daily bases.

3.1.3 Microbiological evaluations

ACC – the counts (Table 3) on the lettuce and the cabbage samples were found to be on average, unacceptably high, varying from low to extremely high. During several of the sampling of both the lettuce and cabbages, values of above 1 000 000 cfus were recorded. These high values found on both products cannot be taken as splash-up contamination as during sampling the outer two layers of both the lettuce and the cabbage were aseptically removed.

The ACC for the beans (Table 3) were lower but in two cases still unacceptably high. For this type of produce it must be remembered that the beans are sampled as is and the data shows what is present on the beans. Continuous irrigation might have led to a build-up of the microbial load. However, what must be taken into account is the latest consumer trend to consume products raw without any further processing steps.

In the case of the grapes (Table 3) only two samples could be taken at harvest but based on the data obtained the ACC values for the grapes that had been irrigated by the overhead system were higher.

Spore formers – The counts for both the ASF and AnSF (Table 3) were either absent, very low, or with values <3 000.

Coliforms (TC) – The TC counts (Table 3) were found to vary from very low on the cabbage and some of the lettuce samples to extremely and unacceptably high values (>500 000 cfus).

Faecal coliforms – The thermotolerant FC counts (Table 3) were found to vary from undetectable, low (10-500 MPN.100 mL^{-1}) to unacceptably high value (1 600 000 MPN.100 mL^{-1}).

Salmonella – *Salmonella* was found in the samples from all the lettuce, cabbage, beans and grapes.

Listeria – Typical growth of LM was found on the lettuce, grapes and bean samples as well as on some of the cabbage samples.

Staphylococci – *Staphylococcus aureus* were found on most of the lettuce, cabbage and bean samples examined with values from not-detectable to one high value of 5 600 $cfu.ml^{-1}$ on the cabbage.

Intestinal Enterococci – The IE counts were found to low and varied from not-detectable to <300 cfus.

3.1.4 Bacterial species identification

The following species were found to be present in the produce samples:

Grape profile – Enterobacter sp., Serratia sp., Listeria innocua, Shigella sp., Spore formers – several species*, Bacillus* – several species*, Staphylococcus aureus, Enterococci sp., Enterobacter aerogenes, Staphylococcus lentus.*

Bean profile – Spore formers – several species*, Bacillus* – several species*, Klebsiella pneumoniae, Listeria monocytogenes, Listeria innocua, Enterococci sp., Enterobacter aerogenes,* Coliforms*, Listeria grayi,* faecal coliforms*, Serratia sp., Escherichia coli,* and *Aerococcus viridans.*

Lettuce profile – Staphylococcus saprophyticus, Listeria seeligeri, Listeria grayi, Citrobacter freundii, Enterobacter cloacae, Enterobacter aerogenes, Coliforms*, Escherichia coli,* faecal coliforms*.*

Cabbage profile – Enterobacter cloacae, Proteus mirabilis, Listeria grayi, Staphylococcus aureus, Enterobacter aerogenes, Listeria seeligeri, Coliforms, Escherichia coli, faecal coliforms*.*

3.1.5 Conclusions

As found for the irrigation water, coliform and faecal counts for the produce at harvest were much higher than standards by the WHO, EPA, DWAF and guidelines recommended by SA retailers. Typical growth of *E. coli* was also observed in most samples. The data obtained also showed that the ACC varied from zero to unacceptably high. It was also found that both the aerobic and anaerobic spore formers were low to absent in the samples. Intestinal enterococci, *Salmonella, Listeria* and *Staphylococcus* were present in most produce samples indicating the presence of potential pathogens. The high counts of coliforms, faecal coliforms and *E. coli* and the presence of *Salmonella, Staphylococcus*, *Listeria,* anaerobic spore formers and *Enterococci* clearly indicates that the vegetables and fruit studied may constitute a health risk to consumers.

3.2 WESTERN CAPE – CONTAMINATION OF FRESHLY PURCHASED PRODUCE AT-RETAIL

3.2.1 Specific aim

The aim of this phase of the investigation was to do an exploratory study to determine the extent of contamination found on raw produce at the retail level in the Western Cape.

Table 3. Microbiological data obtained from lettuce and cabbage from the sites that had been irrigated with water from the Plankenburg/Eerste and Mosselbank Rivers.

Produce	ACC (cfu.mL^{-1})		ASF (cfu.mL^{-1})		AnSF (cfu.mL^{-1})		TC (MPN.100 mL^{-1})		FC (MPN.100 mL^{-1})		*E. coli* (MPN.100 mL^{-1})	
	Min	Max	Min	Max	Min	Max	Min	Max	Min	Max	Min	Max
Lettuce Mosselbank (n=8)	57000	970000000	10	3000	0	2600	4	1600000	0	1600000	TG	TG
Cabbage Mosselbank (n=8)	75000	3600000	ND	3200	ND	190	33	1600000	ND	2600	TG	TG
Grapes Plankenburg Drip-irrigat (n=2)	14600	32000	ND	910	ND	ND	ND	1400	ND	1400	ND	TG
Grapes Plankenburg Overhead (n=6)	1320	550000	ND	1030	ND	1830	ND	21000	ND	2300	ND	TG
Beans Plankenburg (n=20)	ND	258000	ND	570	ND	1830	ND	133000	ND	7900	ND	TG

Produce	Sal		LM		SA (cfu.mL^{-1})		IE (cfu.100 mL^{-1})	
	Min	Max	Min	Max	Min	Max	Min	Max
Lettuce Mosselbank (n=8)	TG	TG	TG	TG	ND	2300	ND	55
Cabbage Mosselbank (n=8)	TG	TG	ND	TG	50	96000	ND	ND
Grapes Plankenburg Drip-irrigat (n=2)	TG	TG	TG	TG	ND	ND	ND	60
Grapes Plankenburg Overhead (n=6)	TG	TG	ND	TG	ND	11100	ND	116
Beans Plankenburg (n=20)	ND	TG	ND	TG	ND	TG	ND	3410

3.2.2 Microbiological evaluations

The microbiological data obtained for each of the different sites is summarised in Table 4.

Coliforms – The TC counts were found to vary from very low on the cabbage and lettuce samples to unacceptably high values (>500 000 MPN.100 mL^{-1}). From the limited data it is not possible to reach any conclusion in terms of where the produce was obtained. According to guidelines applied by retailers in South Africa some of the produce would be considered “unsatisfactory” and even “potentially hazardous”.

Faecal coliforms and E. coli – The thermotolerant FC counts were on average low and varied from not-detectable to unacceptably high. The microbial guidelines applied by retailers in South Africa recommend for satisfactory produce the *E. coli* load should not exceed 20 cfu and the *E. coli O157* must be zero in 25 gram of produce. A fair amount of the samples examined in this study would be considered “unsatisfactory” and “potentially hazardous”.

Aerobic colony counts – The ACC counts on the lettuces and the cabbage samples were found to be on average, varying from fairly low (around 12 000 per ml) too fairly high (>200 000 cfus) to extremely high (11 300 000 cfus) (Table 4). The ACC on grapes were found to be low but the data consists of only two samples and before any conclusions can be reached this will have to be expanded.

Spore formers – The counts for both the ASF and AnSF were found to be either absent, very low, or with values <4 000 especially for the ASF. If one takes into account the "Microbial guidelines" applied by retailers in South Africa some of the produce would not be considered "satisfactory" especially if *Bacillus cereus* is present.

Salmonella – Typical growth of *Salmonella* was found in the samples from all the lettuce, cabbage, and beans and even from both grape samples. Again if one uses the "microbial guidelines" most, if not all the samples examined in this study would be considered "potentially hazardous". The guidelines recommended for satisfactory produce are that no *Salmonella* spp. should be present in 25 gram of produce.

Listeria – Typical growth of LM was found on all the lettuce, cabbage, grapes and bean samples. Again the data shows produce quality below "acceptable" standards.

Staphylococci – *Staphylococcus* were found on most of the lettuce, cabbage and bean samples examined with values from not-detectable to high values of >30 000 cfu.mL^{-1} on the beans. The "retail microbial guidelines" recommend a Staphylococci load of less than 20 for a "satisfactory" product. Thus many of the products examined would not be classified as microbiologically satisfactory but rather as potentially hazardous.

Intestinal Enterococci – The IE counts were found to be low and varied from not-detectable to <3 000 cfu.100 mL^{-1}.

3.2.3 Conclusions

The purpose of this study was to do an exploratory study on produce available in the food market and to get an indication of the microbial loads on produce that is consumed raw or after minimal processing. When comparing the data obtained with that of the "microbial guidelines" as applied by retailers in South Africa most of the produce samples could be classed as unsatisfactory to potentially dangerous to the consumer. However, it must also be taken into consideration that these are general indicators of microbial loads and since all the produce examined would probably be washed before being consumed, the final loads will be at least 2-3 log values lower than reported here. For this type of produce it must also be remembered that they are sampled as is and the data shows what is present. It is also possible that continuous irrigation might have led to a build-up of the microbial load and this is an aspect that must be examined in future studies. It is important to take into account the latest consumer trend to consume products raw without any further processing steps.

3.3 WESTERN CAPE – LINKING MICROBES IN IRRIGATION WATER TO THOSE ON PRODUCE

3.3.1 Specific aim

The aim of this phase was to monitor and later to follow the "presence" of a specific indicator organisms as occurred at the river sampling point and on the surface of fresh produce.

Table 4. Microbiological data from freshly purchased produce (TG = typical growth; ND = none detected).

Produce	ACC (cfu.mL^{-1})		ASF (cfu.mL^{-1})		AnSF (cfu.mL^{-1})		TC (MPN.100 mL^{-1})		FC (MPN.100 mL^{-1})		*E. coli* (MPN.100 mL^{-1})	
	Min	Max	Min	Max	Min	Max	Min	Max	Min	Max	Min	Max
Lettuce (n=27)	2379	680000	ND	19700	ND	2600	ND	540000	ND	4300	ND	TG
Cabbage (n=14)	76000	580000	ND	4600	ND	1560	11	1600	4	1600	TG	TG
Beans (n=22)	2420	1100000	160	4600	ND	1190	2300	790000	ND	220000	ND	TG
Grapes (n=5)	2420	7800	ND	720	ND	ND	1300	22000	28	180	TG	TG
Snap peas (n=12)	3500	1960000	ND	4510	ND	3650	ND	1800000	ND	46000	ND	TG
Tomatoes (Romanita) (n=12)	1750	1030000	ND	1300	ND	ND	230	2300000	ND	23000	ND	ND

Produce	Sal		LM		SA (cfu.mL^{-1})		IE (cfu.100 mL^{-1})	
	Min	Max	Min	Max	Min	Max	Min	Max
Lettuce (n=27)	ND	TG	ND	TG	ND	2000	ND	600
Cabbage (n=14)	ND	TG	ND	TG	ND	35000	ND	ND
Beans (n=22)	TG	TG	ND	TG	ND	39600	ND	2880
Grapes (n=5)	TG	TG	TG	TG	ND	ND	220	6006
Snap peas (n=12)	ND	TG	ND	TG	51	420000	ND	715
Tomatoes (Romanita) (n=12)	TG	TG	ND	TG	ND	75	ND	30

3.3.2 Background

In the first phase sampling was done to monitor the normal daily transfer of microbes from irrigation water to produce during normal farm-level overhead irrigation. For this phase water from the Plankenburg River as used by small-scale farmers to grow beans using a daily overhead irrigation procedure. The irrigation water and beans were sampled and analysed using standard methods.

In the second phase the microbial types and loads on the surface of fresh produce (beans) were investigated by irrigating a plot of beans (same farm as used in the first phase) on a daily basis for ten days. For control purposes bean samples were analysed prior to this trial in order to obtain a base-line value of the fresh produce from the same cultivation area. In this case municipal tap water was used as irrigation source and then irrigation water from river site Plank-3 was used. Units of 6 L were used per irrigation session. The beans were irrigated by simulating the over-head irrigation technique. After 24 h, during which the irrigation water was allowed to dry, bean samples were aseptically harvested. This process was repeated for 10 days and included controlled sampling. The faecal coliform load present on the beans was analysed 24 h after each irrigation (prior to the next) and the consequent increase in microbial load was assumed to be because of the transfer of organisms from the irrigation water to the produce during the repeated irrigations. The faecal coliform load and faecal species types of each batch of

the irrigation water was also determined if there is a correlation between the microbial load and specific species of the irrigation water and the extent to which the transfer occurred.

3.3.3 Microbial levels and transfer from irrigation water to produce during normal farm-level overhead irrigation

In the first phase study, the microbial load for the water used for irrigating the beans (Plank-3) was found to be low. Even though the faecal coliform concentration for this set of irrigation water did not, with one exception, exceed the DWAF's guidelines in during the specific study, it did in most cases fail to comply with WHO regulations (DWAF 1996; WHO 1998). The frequent presence of other potential pathogens, which were able to survive the severe environmental conditions, also suggests that their presence may indicate potential health hazards.

Microbial levels – Microbial analyses conducted on the beans sampled during this phase (Table 5), shows higher microbial loads when compared to the irrigation water. At these levels the safety qualities of the beans have to be considered as of unacceptable standard. According to guidelines formulated by the DoH (2006) for environmental health officers on the interpretation of microbiological data analysis of food, food samples should not contain an ACC count higher than 1 000 cfu.g^{-1} of product. In this study aerobic plate counts up to 258 000 cfu.g^{-1} were recorded. The DoH guidelines also stipulate that no *Salmonella* should be present in at least 25 g of product, total coliforms should not exceed 10 cfu.g^{-1} and no *E. coli* should be present in 1 g of product. In contrast to these guidelines the data indicated the presence of *Salmonella* in all the bean samples, consistently high total coliform concentrations, and *E. coli* to be present in at least 60% of the samples.

Table 5. Microbial loads present on beans that had been irrigated with water from the Plank-3 site.

Sampling session	ACC	ASF	AnSF	SA	TC	FC	*E. coli*	IE	Sal	LM
1	211000	ND	ND	2800	ND	ND	ND	8	TG	TG
2	10900	ND	ND	330	23	ND	ND	ND	TG	TG
3	7100	ND	ND	ND	240	230	TG	5	TG	TG
4	258000	160	330	ND	1300000	2300	TG	TNTC	TG	TG
5	TNTC	570	ND	ND	33	17	TG	64	TG	TG
6	123000	420	ND	900	33000	ND	ND	TNTC	TG	TG
7	2300	310	ND	ND	ND	ND	ND	30	TG	TG
8	970	520	460	ND	790	490	TG	TNTC	TG	TG
9	48000	110	ND	4500	7900	7900	TG	3410	TG	TG
10	7600	460	1830	ND	1100	1100	TG	17	TG	ND

TNTC – Too numerous to count; TG – Typical growth; ND – None detected

Possible carry-over – All colonies obtained during the microbial analyses of samples for the first phase study, even those resembling atypical growth, were, after purification, identified to at least the genus level with the API system. The organisms isolated from the river water, water used to irrigate the produce and the organisms isolated from the irrigated beans (first phase study) are presented in Table 6. The link between the quality of the irrigation water and the safety of fresh produce can be illustrated by comparing

the type of organisms which had been isolated from the respective sampling sites, irrigation water and produce.

3.3.4 Conclusions

As was reported in previous studies it was again found that the microbial loads in the irrigation water and on the raw produce at harvest especially the TC and FC counts, were much higher than DWAF and WHO guidelines and those recommended by South African retailers. The presence of *E. coli* was also confirmed all samples monitored. The presence of intestinal enterococci, *Salmonella, Listeria* and *Staphylococcus* were also found in most produce samples indicating the presence of potential pathogens. Their presence in the river and irrigation water and then on the irrigated beans clearly indicates that the produce studied may constitute a health risk to consumers.

The assessment of health risks is to establish a verifiable link between the potential pathogens present in irrigation water and those present on irrigated produce. When comparing the presence of potential pathogens present in the Plankenburg/Eerste River water with those present on the farmers beans it is clear that most the species present in the river water are present on the beans. Again the data clearly indicates that if the microbial species is present in the irrigation water the chances are more than excellent that it will be present on irrigated produce. This suggests a *"direct link"* between the organisms present in irrigation water and those found on the irrigated produce and confirms the first step in this assessment of health risks that there is a verifiable link between the potential pathogens present in the irrigation water and those present on the produce.

As stated before, certain assumptions have to be assumed before the term *"direct link"* can be used because of the wide range of uncertainties associated with many of the crucial variables needed to arrive at this judgement which makes this a hugely complex task. Given the margin of uncertainty, the only reliable resolution to this problem to establish whether the microbe in the water and the one on the produce are beyond doubt the same genotype. However, we cannot ignore the fact the data clearly identifies potential pathogens of the same species in both water and fresh produce and at times the loads are far higher than are acceptable in terms of food safety.

3.4. WESTERN CAPE – LINKING MICROBES IN PLANKENBURG RIVER TO CARRY-OVER TO PRODUCE UNDER "UNCONTROLLED" FIELD IRRIGATION CONDITIONS

3.4.1 Specific aim

The aim of this study was to link the suspected carry-over of faecal organisms present in the water from the Plankenburg River to fresh produce irrigated with this water. This study will be conducted on the basis that a high numerical similarity indicates isolates that originate from the same source.

3.4.2 Impact of multiple irrigations on the microbial quality of fresh produce

The frequency of irrigation sessions could also play an important role in influencing the microbial quality of fresh produce as it creates the opportunity for organisms to be transferred to the surface of fresh produce repeatedly. If true, this could also suggest that the microbial quality of irrigation water does not necessarily have to exceed the recommended guidelines in order to pose a possible health risk to anyone who consumes these fruit and vegetables raw or minimally processed.

Table 6. Organisms isolated from river sampling site (Plank-3), the irrigation water (at sprayer) and the irrigated beans (the X indicates presence of organism).

Organisms	**Plank-3**	**Irrigation H_2O**	**Beans**
ASF	X	X	X
AnSF	X	X	X
Aerococcus viridans	X	X	X
Bacillus spp.	X		X
TC	X	X	X
FC	X	X	X
E. coli	X	X	X
Enterobacter	X	X	X
Enterobacter aerogenes	X	X	X
Enterobacter cloacae	X		
Enterococcus spp.	X	X	X
Klebsiella	X	X	X
Klebsiella pneumoniae	X		X
Listeria innocua	X	X	X
L. grayi	X	X	X
L. welshimeri	X		
L. monocytogenes	X	X	X
Salmonella enteritidis	X		
S. typhimurium	X		X
Serratia marcescens	X	X	
Shigella spp.	X	X	
Staphylococcus aureus	X	X	X
S. lentus	X		

It can be seen from the data in Fig. 1 that the *E. coli* loads ranged between 4 600 and 790 000 MPN.100 mL^{-1} P in the irrigation water from the Plank-3 site. This exceeded the 1 000 cfu.100 mL^{-1} faecal coliform WHO and DWAF guideline during all nine irrigation sessions (WHO, 1989; DWAF, 1996b). According to these guidelines a high health risk is associated with irrigation water of which the faecal coliform concentration exceeds these stipulated requirements. The microbial quality was therefore concluded to be of unacceptable quality.

The microbial levels from the surface of the irrigated beans increased from the zero at the start of the irrigation sessions to irrigation session 6 with a maximum coli concentration of 44 x 10^3 cfu.100 mL^{-1}. On day 5 a slight decrease in concentration on the beans was observed. This was possibly as a result of rainfall the night before which could have "washed" some of the microbial load off the surface of the beans. The microbial concentration of the irrigation water was also lower at irrigation session 7 (49 000 cfu.100 mL^{-1}) than the microbial concentration detected for water used during irrigation session 6 (92 000 cfu.100 mL^{-1}). This decreased microbial concentration could also have been as a result of a dilution effect in the River caused by heavy rainfall. The microbial load then once again increased following the next irrigation session to 130 000 cfu.100 mL^{-1}. It is interesting to note that in total the data shows, with the exception of day 5, from day 1 to day 6 a nearly logarithmic increase in numbers on the beans followed by a stabilisation of numbers for the rest of the study.

Based on these results it was concluded that multiple irrigations sessions contribute to the build-up of organisms on the surface of fresh produce and thus promote the carryover of potential pathogenic organisms to fresh produce.

3.4.3 Water and produce isolates

A total of 92 *E. coli* isolates were obtained from the irrigation water from the Plankenburg River and the beans which had been irrigated using water from site Plank-3 (Fig. 1). These isolates and the reference strains were all phenotypically characterised. Additionally their growth on three indicator media: MacConkey agar (Oxoid), Chromogenic *E. coli* agar (Oxoid) and Brilliance *E. coli*/coliform agar (Oxoid) was determined to compare the colony characteristics. Typical colony characteristics on these media varied between reddish pink and dark purple colonies. Although the majority exhibited the typical colony characteristics of *E. coli*, some strains did however, show unexpected colony characteristics. At first, it was thought that these isolates might not be *E. coli* strains but rather contaminants which were able to pass through the hurdles posed by the MTF method. This could possibly be ascribed to variances that exist between different isolates of the same species, a phenomenon known to occur in nature as organisms are required to constantly adapt to a particular host or environment in order to survive (Scott *et al.*, 2002).

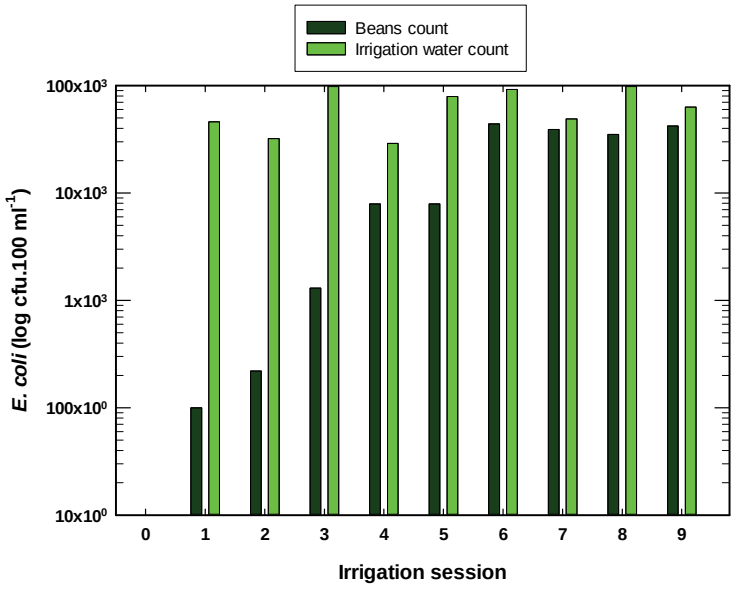


Figure 1. The *E. Coli* loads in the irrigation water (Plank-3) and the *E. coli* accumulation on the surface of beans after multiple irrigations under uncontrolled conditions. The *E. coli* counts are given as log MPN.100 mL^{-1}.

3.4.4 Identification and clustering

The API Web based data system was used to identify the isolates and identification of >95% were regarded as a positive identification. Based on this 53 of the 105 isolates were identified as *E. coli*, 4 as *Enterobacter*, 2 as *Chromobacterium*, 3 as *Citrobacter*, 3 as *Raoultella*, 7 as *Pseudomonas*, 29 isolates showed only a low identification discrimination between *Klebsiella pneumoniae* and *E. coli,* and the remaining 5 could not be identified using the API 20E. Phenotypic characteristics of each isolate were then used to determine similarities and grouping clusters as in the dendrogramme in Fig. 2. The dendrogramme

illustrates the spatial distribution of the isolates based on the degree of similarity and were grouped into 15 clusters (clusters A to O) of which the three main groups (Groups D, E and M) collectively contained 82% of the isolates.

In total, 39 of the 53 isolates which had been identified as *E. coli* were clustered in group D. Group E consisted of 39 isolates, of which 11 were identified as *E. coli* and the remaining 28 were identified as *Klebsiella*. The clustering of the reference strains not only indicated a high degree of variance between isolates of the *E. coli* species, but also indicated similar phenotypic characteristics between some of the reference strains and the water and bean isolates (92).

3.4.5 Linking by phenotypic characterisation and identification

Isolates originating from a specific environment often acclimatise or adapt to environmental conditions in order to survive and should therefore tend to share similar characteristics (Scott *et al.*, 2002). Organisms grouped together based on similar phenotypic characteristics can therefore be considered to potentially have originated from the same source. Strains isolated from beans (highlighted in green, Fig. 1) and irrigation water (highlighted in blue, Fig. 2) that were grouped together can thus be considered to be of the specific species/strain present in both these sources and therefore be taken, based on the phenotypic clustering, as an indication of direct transfer from irrigation water to fresh produce during irrigation.

3.4.6 Linking by multi-antibiotic resistance (MAR) profiling

MAR profiling is based on the resistance or sensitivity of isolates to specific antibiotics to generate profiles for every individual isolate (Carroll *et al.*, 2009). For this study the resistance that an isolate displayed to a specific antibiotic was determined by grouping them based on the radius of each inhibition zone as either resistant (0-3 mm), intermediate (4-8 mm) or sensitive (≥9 mm).

The resistance of a specific isolate to the 23 antibiotics tested was combined to create a resistance profile using the SJ and SSM coefficient clustering similarities (Fig. 3). The isolates were grouped into 27 clusters based on their MAR profiles with isolates within each group exhibiting similar MAR traits. The five main clusters, clusters O, P, Q, X and Y collectively comprised 72% of the isolates. Isolates in cluster O (5% of the isolates) were all resistant to Vancomycin and sensitive to Ofloxacin, Ceftriaxone, Ciprofloxacin, Cefuroxime and Cefotaxime. Isolates in cluster P (7%) were also resistant to Vancomycin, but were inhibited by Ampicillin, Ofloxacin, Ceftriaxone, Cefuroxime and Cefotaxime. The largest cluster (Q), comprising 33% of the isolates, was resistant to Vancomycin, and inhibited by Ofloxacin, Ceftriaxone, Ciprofloxacin and Cefotaxime. The majority of isolates in this cluster also showed sensitivity to Cefuroxime and Chloramphenicol. Clusters X (16%) and Y (11%), showed resistance towards Vancomycin, Ampicillin, and Amoxicillin. However, cluster X was resistant to Tetracycline and Cotrimoxazole and cluster Y resistant to Trimethoprim. The majority of cluster X were inhibited by Ofloxacin, Ceftriaxone, Ciprofloxacin and Cefotaxime whilst cluster Y were only sensitive to Ceftriaxone and Ciprofloxacin.

Isolates were grouped together based on similar resistance profiles and therefore suggesting similar genotypic characteristics. These genotypic abilities could have been acquired through the repeated exposure to specific antibiotics. Similar MAR profiles could indicate exposure to the same environmental conditions and consequently, that isolates clustered together probably originated from the same environment and therefore from the same species. The above MAR results therefore suggest that irrigation water and green bean isolates which clustered together were exposed to similar antibiotics, therefore

possibly originating from the same environment. This grouping consequently confirms the possible transfer of harmful faecal organisms from irrigation water to fresh produce via the irrigation system.

3.4.7 Comparison of the API and MAR clustering data

In theory isolates clustering together when using combined data should therefore share similar phenotypic and genotypic (MAR) characteristics which could in turn suggest that these isolates originate from the same source of pollution. This information could then be used to positively confirm the transfer of potentially pathogenic organisms from polluted irrigation water to fresh produce.

In this study both the API characteristics and MAR profiling were used to link organisms P in irrigation water to those isolated from beans irrigated with this water. If one considers the three main clusters (clusters Q, X and Y) in the MAR dendrogramme (Fig. 3) and retrace the grouping of the isolates to the API data (Fig. 2) it becomes evident that a significant portion of these isolates were grouped together based on both genotypic and phenotypic characteristics. Almost half (47%) of cluster Q (Fig. 3) were grouped in cluster E (Fig. 2), 76% of cluster X (Fig. 3) were grouped together in cluster D (Fig. 2) and 50% of cluster Y (Fig. 3) was grouped together in cluster M of the API dendrogramme (Fig. 2). In total, 34% of the isolates were grouped together based on both their genotypic and phenotypic characteristics.

Equally important, was that approximately a third (26%) of the isolates based on both genotypic and phenotypic characteristics were isolated from irrigation water (site Plank-3), 54% obtained from beans irrigated with water from site Plank-3, whilst the remaining 20% were the reference strains. These results therefore suggest that strains isolated from both the irrigation water and beans originate from the same source of pollution. Potential pathogens P in the Rivers because of faecal pollution could therefore be transferred to fresh produce when water from this River was used as irrigation source.

3.4.8 Conclusions

The data showed the colony character variance that exists between the *E. coli* isolates within the faecal coliforms. The phenotypic characters data also confirmed the large phenotypic variation between isolates which could possibly be because of adaptations from changing environmental conditions. Results of the MAR profiles also indicated variation between isolates of the same species based on the different responses to the 23 different antibiotics. In spite of this >30% of the isolates from irrigation water and the fresh produce were clustered together based on both genotypic and phenotypic characteristics.

Similar traits obtained suggest that bean isolates are related to those from irrigation water. It was therefore concluded that the *E. coli* strains from the beans were present due to their transfer from the contaminated Plank-3 irrigation water. This clearly indicates the transfer of potential pathogens from polluted irrigation water to fresh produce. The consumption of such fresh produce could therefore be seen as health risk when contaminated water is applied during the cultivation of fresh produce.

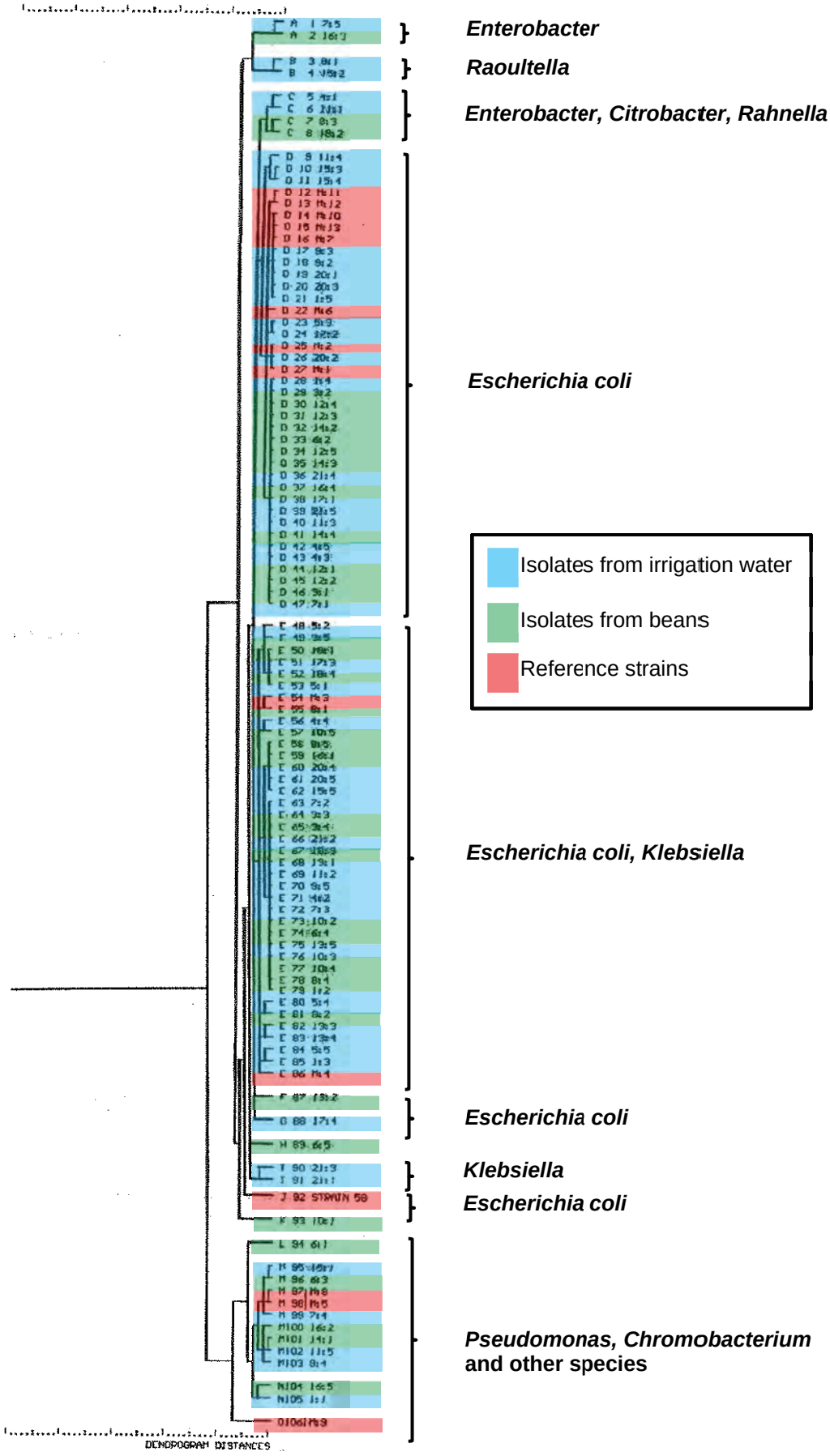


Figure 2. Dendrogramme grouping of isolates based on API characterisation data.

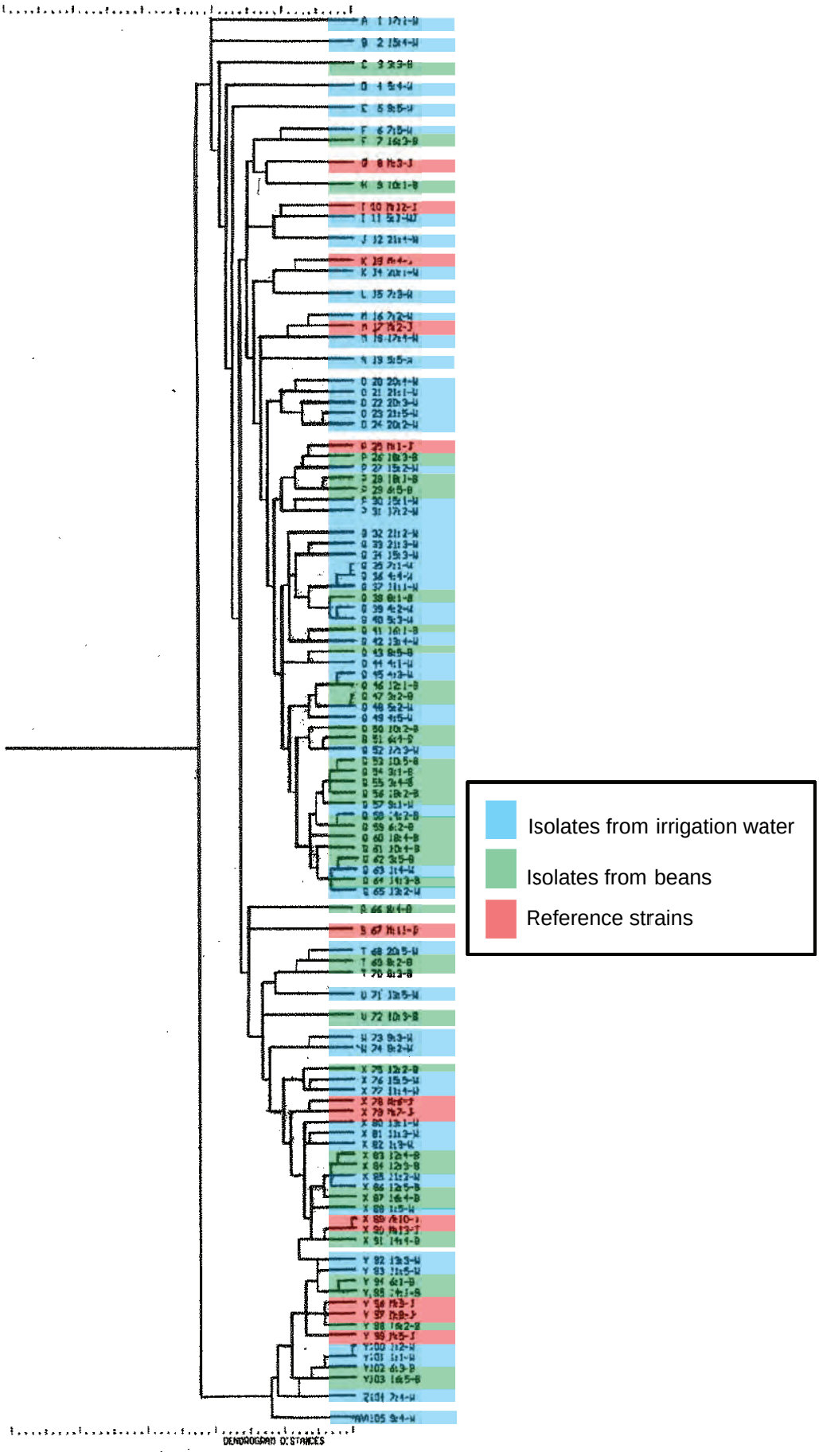


Figure 3. Dendrogramme clustering of isolates based on their MAR profiles.

3.5 LINKING FAECAL COLIFORMS IN THE PLANKENBURG RIVER TO THOSE PRESENT ON PRODUCE UNDER "CONTROLLED" FIELD IRRIGATION CONDITIONS

3.5.1 Specific aim

The objective is to determine the carry-over and linking of *E. Coli* from contaminated irrigation water to fresh produce. The study will focus on: effect of daily irrigation on carry-over; effect of "once-off" irrigation on the survival of *E. Coli* on produce; identifying different types of *E. Coli* in the irrigation water and those present on the irrigated fresh produce; linking different *E. Coli* types in the irrigation water to those present on the irrigated produce; and using the above information, the faecal pollution source of the irrigation water will be determined.

3.5.2 Carry-over of *E. Coli* from irrigation water to beans

The *E. Coli* loads in the Plankenburg irrigation water as well as the effect of the irrigation water 24 h after the daily application onto the beans is shown in Fig. 4. The *E. Coli* counts in the River water varied between 3 900-118 500 cfu.mL^{-1} during the study period. All the counts obtained in this study were above the WHO and DWAF recommended guideline of <1 000 cfu.100 mL^{-1} (WHO, 1989; DWAF), making the River unsuitable for irrigation purposes.

Beans from the plots were sampled on day zero before irrigation with Plank-3 water began, and these served as the control. On day 1, the results showed no *E. coli* on the beans 24 h after irrigation for the first time (Fig. 4). Previous research reported that when a surface is uncolonised it is difficult for an organism to survive when compared to when it is colonised. Ackerman (2010) also found that when beans were exposed to an inoculum of 10^2 cfu.mL^{-1}, there were no *E. Coli* survivors on the beans, which indicated that a concentration of 10^2 cfu.mL^{-1} could be considered as "safe" for *E. Coli* in irrigation water.

The counts for days 2 to 4 show that the longer the beans were exposed to the contaminated irrigation water, the more the *E. Coli* counts increased on the beans. One explanation for this can be that the hairy surface of beans could help entrap the *E. Coli* (Ackermann, 2010). The *E. Coli* also has various attachment mechanisms it could have used to attach to the green bean surfaces and hairs (Doyle & Erickson, 2008b).

The first major *E. Coli* impact on the beans was found on day 5, when the irrigation water from the previous day had a higher count (50 500 cfu.mL^{-1}) and subsequently the beans showed an increase in load to 15 250 cfu.mL^{-1}. A small decrease in *E. Coli* count in the irrigation water on day 6 was followed by a similar decrease on the beans. On day 7 the highest *E. Coli* count was observed in the irrigation water (118 500 cfu.mL^{-1}) followed on day 7 to an increased count of 87 000 cfu.mL^{-1} on the beans. Overall, the subsequent profile of the *E. Coli* numbers on the beans was similar to the pattern found in the irrigation water. On days 8 and 9 it was however observed that the *E. Coli* counts on the beans were higher than the counts of the irrigation water on days 8 and 9. On day 8 the *E. Coli* counts reached 87 000 cfu.mL^{-1} on the beans, compared to the irrigation water on day 7 which was only 44 000 cfu.mL^{-1}. The reason for this was probably the impact of the high count in the irrigation water on day 7. Another reason could be due to the formation of biomass clumps. Ackermann (Ackermann, 2010) reported that *E. Coli* aggregates to form clumps and even biofilms. Although hard to break apart, once a clump is broken apart it could contain millions of cells, which will lead to a large increase in the number of enumerated cells.

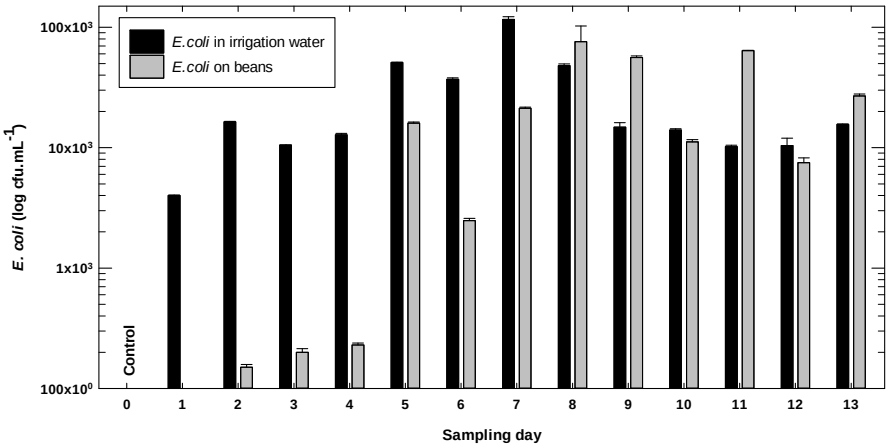


Figure 4. The 13 day plot study where beans were irrigated daily for 13 days with water from the Plankenburg River (Plank-3) and harvested 24 h after irrigation. Day 0 served as the control as those beans had not been exposed to the River water before harvest. The *E. Coli* values are averages of counts from the two sub-plots and each done in duplicate.

Although the counts on the beans were still higher on day 9, the loads started to decline. A reasons for this variation in numbers may be the impact of natural microbial inhabitants on the beans competing for nutrients and space. It is known that in the River water as well as on beans there are many natural inhabitants P that can adapt well to the environment on the beans, leading to the organisms growing better and being able to compete for the nutrients more successfully when compared to *E. coli*. Cooley and co-workers (Cooley *et al.*, 2006) found that *Enterobacter asburiae*, a plant endophytic bacterium, can limit the growth and survival of *E. Coli* on produce. In this study *E. asburiae* strains were isolated on days 4, 8, 9, 11, and 12 (see isolation section) from the beans, which may be a reason why the *E. coli* counts on the beans were low and started declining on those days. Another reason might also be due to quorum sensing, where an organism has the ability to regulate its own numbers if the population density is too high (Easton *et al.*, 2005).

Another factor that must be considered in plot studies is that in this study the outside temperature varied between 13° and 36°C over the 14 day study period (Fig. 4). Inside the tunnel the temperatures were found to vary 6-8°C higher than the outside temperature. This at times resulted in temperatures reaching more than 40°C inside the tunnel. Higher temperatures usually lead to an increase in the growth rates, resulting in the microbes being able to utilise more nutrients, which in turn results in larger growth increases.

3.5.3 Carry-over of "pure" culture *E. coli in the* irrigation water to beans

In this study beans were irrigated with the "pure" *E. coli* culture at a constant concentration of 10^6 cfu.mL^{-1} and harvested 24 h after irrigation. The aim of this plot study was to determine if *E. coli* is transferred to the beans without any "natural" competitors present in the irrigation water.

The general daily profile of the "pure" *E. coli* loads on the beans was similar to the profile of the beans when river water was applied (Fig. 4) but in this case the inoculation concentration of 10^6 cfu.mL^{-1} was kept constant. Overall, the population profile of the *E. coli* on the beans again gradually increased to day 7 reaching 445 000 cfu.mL^{-1}. In this case it was speculated that the similar profiles found in both studies could possibly be ascribed to the numbers reaching a maximum load on the beans.

The data shows that when the applied irrigation water was at a constant cell concentration of 10^6 cfu.mL^{-1}, then 50% or less of the cells attached to the beans. Overall, it was found that when the *E. coli* concentration (10^6 cfu.mL^{-1}) in the irrigation water is high, it could be carried-over to the beans and could result in a health hazard for the consumer.

3.5.4 Survival of the "pure" *E. coli* culture on beans after a "once-off" irrigation

Beans in this study were irrigated with the "pure" *E. Coli* culture at 10^3 and 10^4 cfu.mL^{-1} cell concentrations, respectively. The aim was to determine what the lowest *E. Coli* concentration is that would be transferred from irrigation water to produce and how long the cells will survive on the beans. It was found that no *E. coli* could be isolated the beans 24 h after having been irrigated with the 10^3 cfu.mL^{-1} *E. coli* concentration in the irrigation water. The data from this study supports the results reported by Ackermann (Ackermann, 2010) who found under strict laboratory conditions that there were no survivors on beans after exposure to a 10^3 cfu.mL^{-1} cell concentration.

When the concentration of *E. coli* in the irrigation water was increased to 10^4 cfu.mL^{-1}, a similar survival profile on the beans was found with a count of <10 cfu.mL^{-1} day 1 and by day 3 onwards no *E. Coli* were detected on the beans. Ackermann (Ackermann, 2010) also found that 10^4 cfu.mL^{-1} is the lowest *E. Coli* concentration that beans could be exposed to for carry-over still to occur.

The reason why only a few *E. coli* survived on the beans during this study could be that the population was too small to colonise and compete for nutrients with the organisms "naturally" present on the beans (Cooley *et al.*, 2006)

3.5.5 Identification of isolates from Plank-3 irrigation water and the irrigated beans

Irrigation water and beans – During the 14 day irrigation period a total of 72 organisms were isolated from the River water. Of the 72 isolates, 30 (41.7%) were identified as *E. coli* with the API 20E system.

In this study three species of *Citrobacter* were isolated from the River: *Citrobacter koseri/farmeri* (2 strains); *C. braakii* (1 strain) *and C. freundii* (3 strains).

Although *E. coli* was the most frequently isolated organism, there were other species also present and isolated more than once: *Klebsiella pneumoniae* (4 strains)*; Enterobacter cloacae* (10 strains); *Enterobacter aerogenes* (5 strains)*; Kluyvera* spp. (7 strains); *Serratia odorifera 1* (2 strains); *Enterobacter asburiae* (3 strains); and *Pantoea* spp 1 (2 strains). *Pantoea* spp 2, *Serratia liquefaciens, Serratia fonticola* and *Citrobacter braakii* were only isolated once from the river water.

A total of 67 organisms were isolated from the beans that were irrigated with the Plank-3 water. Twenty of the 67 strains were positively identified as *E. coli.* Other organisms that were isolated from the beans included *Klebsiella pneumoniae* (4 strains)*, Enterobacter cloacae* (21 strains)*, Enterobacter asburiae* (7 strains)*, Enterobacter amnigenus 2* (2 strains)*, Klebsiella oxytoca* (2 strains)*, Pantoea* spp 1 (2 strains)*, Pantoea* spp 2 (2 strains), *Kluyvera* spp. (2 strains) a*nd Enterobacter aerogenes* (3 strains). Strains of *Pantoea* spp 3 and *Enterobacter sakazakii* were only isolated once from the beans.

All the species from the river water that were not present on the beans (*Serratia* and *Citrobacter* isolates) were isolated at a low frequency from the river water (three isolates or less). This might suggest that these organisms were not present in high numbers in the river water, which could have resulted in the low number of isolates being isolated from the River water samples. This low concentration of these species in the irrigation water could have resulted none being carried-over to the beans as the levels were too low to be carried over. The "pure" *E. Coli* culture used to irrigate the beans at a concentration of 10^6 cfu.mL^{-1} was recovered from both the irrigation water and the beans. Based on their phenotypical characters, the *E. Coli* strains from the beans were identical to the irrigated "pure" *E. Coli* strain.

3.5.6 Linking studies

Phase-1 linking: Phenotypical distribution of isolates based on species identification – According to the clustering data, eight species present in the river water were also present on the beans (Table 7). *E. coli*, *E. cloacae* and *E. asburiae* were the prevalent isolates. This was taken as an indication of first phase linking as these strains from the irrigation water were phenotypically identical to the strains on the beans based on species identification.

Table 7. Species present in both irrigation water and on the beans (phase-1 linking).

Species	Number of isolates
Escherichia coli	50
Enterobacter cloacae	31
Enterobacter asburiae	10
Kluyvera spp.	9
Klebsiella pneumoniae	8
Enterobacter aerogenes	8
Pantoea spp. 1	4
Pantoea spp. 2	3

Phase-2 linking: Dendrogramme clustering based on the phenotypic characters – The data in Fig. 5 shows the dendrogramme based on the statistical clustering of the phenotypic data using the simple matching coefficient. Cluster A was taken as representative of the *K. pneumoniae* group; B = *E. aerogenes*; C, D, E = *E. Coli*; F = *E. asburiae* and G = *E. cloacae*. To do the phase-2 linking, 13 strains were selected from the five largest clusters (A, C, D, E and G) to determine whether a strain from the irrigation water could be linked to a strain from the beans.

In the above clusters, strains that were phenotypically identical were from both the river water and a day or more after irrigation, from the beans. In the first example (Cluster A), *K.pneumoniae* was isolated frequently throughout the 14 day period from both the irrigation water and beans. Specifically, this species was also isolated on day 8 from the water and on day 9 from the beans. Both these incidences were thus taken as an indication that *K.pneumoniae* was carried-over from the river water to the beans. Thus the two strains (W839 and B943) isolated on day 8 and 9 were selected as they indicated direct carry-over after irrigation. Further analyses will be necessary to determine whether these strains (from the irrigation water and the beans) are genotypically similar based on sequencing analysis.

The second largest cluster (G) consisted of 27 *E.cloacae* strains (Fig. 5). Some of the *E.cloacae* strains were isolated from the river water on days 3 and 13. Some of the *E.cloacae* strains in this cluster were also isolated from the beans on days 4, 7, 14 and 15. The fact that *E.cloacae* strains were isolated on

days 7 and 15, suggests that they were probably the same strain that survived on the beans for a period of at least a day or more.

The four *E. Coli* clusters (C, D1, D2 and E) (Fig. 10) only differed in their ability to utilise three carbon sources (Table 7). Cluster E was the largest cluster and contained the reference cultures and the "pure" *E. Coli* isolates. A phenotypic link can also be shown between the *E. Coli* isolates in this cluster on days 4 and 10 isolated from the River water, and on days 6 and 12 from the beans.

Cluster C was a smaller *E. Coli* cluster. It did, however, also confirm carry-over of phenotypically similar *E. Coli* strains isolated on day 6 from the River water to those isolated on day 8 from the beans.

Cluster D (Fig. 5) was further grouped into two smaller sub-clusters (D1 and D2). These two sub-clusters differed as cluster D2 strains were able to utilise LDC (Table 8). However, no D2 strains were isolated from the beans. Only cluster D1 showed a carry-over of *E. Coli* with phenotypically identical strains isolated on days 3 and 7 from the River water and on day 14 from the beans. This was taken as confirmation that the related strain survived for longer periods on beans.

Table 8. The phenotypic differences (LDC, ODC and SAC) between the four *E. Coli* clusters.

Cluster	**L-lysine (LDC)**	**L-ornithine (ODC)**	**D-sucrose (SAC)**
Cluster C	+	+	+
Cluster D1	-	-	-
Cluster D2	+	-	-
Cluster E	+	+	-

From the data showed in this section and the clustering of phenotypically similar isolates, the carry-over of several bacterial species including *E. Coli* was shown to take place from the river water to the beans. This was thus taken as proof of phase-2 linking. It was also found that some strains isolated from the beans on different days show that the *E. Coli* can survive on the beans for 24 h or longer. In other cases, when a strain was present in the river water a few days before it was isolated from the beans, it could be assumed that the strain was in the river water and after irrigation it was able to survive for longer periods on the beans. On a phenotypical level there were different clusters of *E. Coli* and each could be used to confirm linking based on the days irrigated and the day when isolated from the beans.

Phase-3 linking: Confirmation based on uidA gene PCR – In this study the *uidA* gene was used for further identity confirmation (Martins *et al.*, 1993). All 50 strains that were confirmed as *E. Coli* with the API system were subsequently analysed and tested positive for the *uidA* gene. As these strains from both the irrigation water and beans are *E. Coli* on a molecular level, it supports linking in terms that species are carried over from the water to the produce.

Phase-4 linking: Confirmation based on Multiplex-PCR – The 52 water and bean *uidA* positive isolates were further subjected to multiplex PCR to determine if pathotypes were present. All the strains tested positive for the *mdh* gene, again confirming that all the strains were *E. Coli* (Tarr *et al.*, 2002). The multiplex PCR data revealed that four strains (W630, W1371, W733A and W1052) from river water were EPEC types, as they were positive for the *mdh* and *eaeA* genes. EPEC can be taken as a pathogenic *E. Coli* pathotype which can cause diarrhoea (Donnenberg & Kaper, 1992). In most countries, EPEC strains are frequently responsible for diarrhoeal diseases, especially among children (Matar *et al.*, 2002).

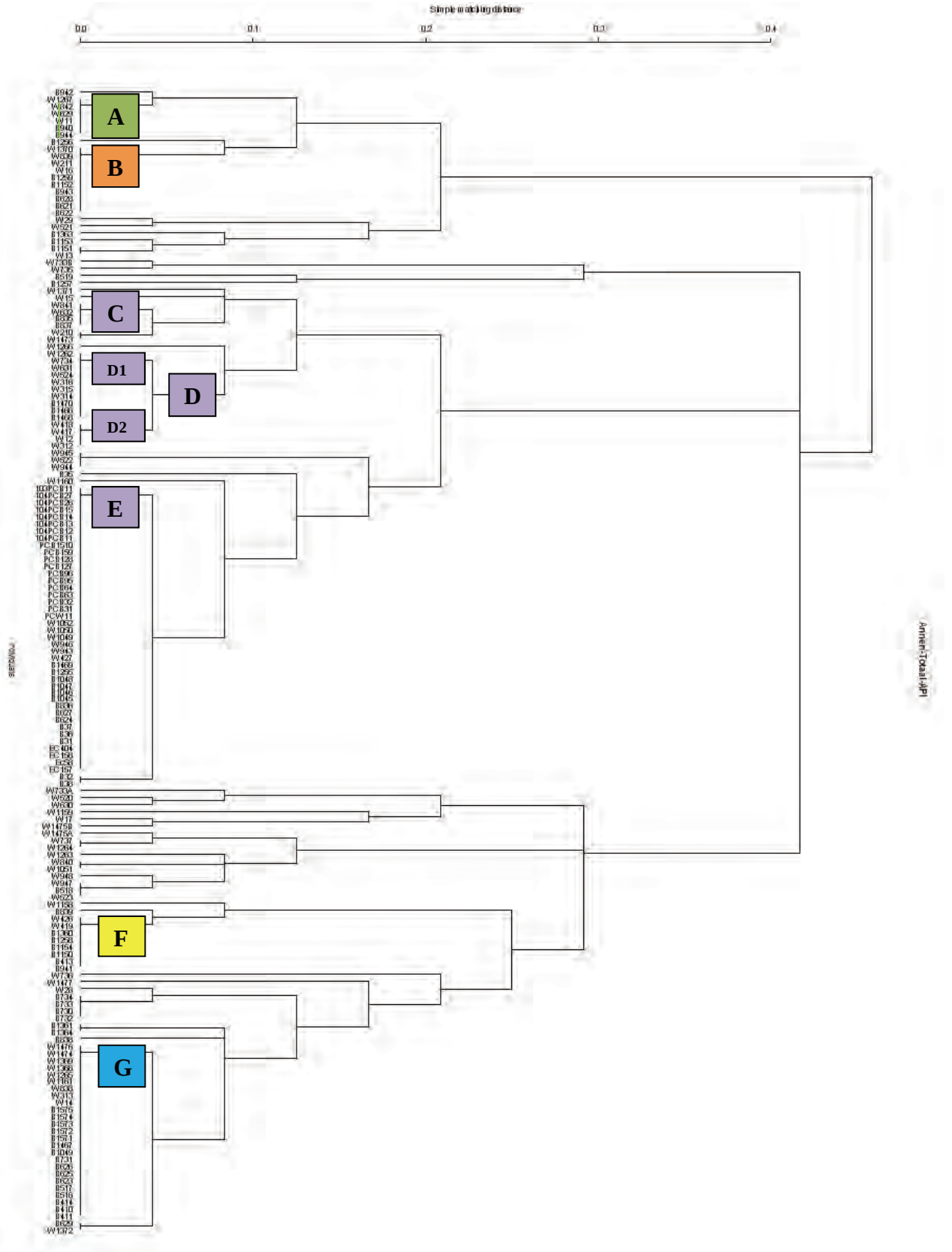


Figure 5. Dendrogramme illustrating the strain clustering based on the phenotypic characters. The Sj coefficient was used to cluster the isolates. Seven clusters were present (A-G). A = *K. pneumoniae*; B = *E. aerogenes*; C,D,E = *E. coli*; F = *E. asburiae* and G = *E. cloacae.*

Sequencing analysis using the Basic Local Alignment Search Tool (BLAST) of the *dnaJ* and *oriC*-locus revealed that all four EPEC strains were identical to specific reference *E. Coli* strains (99%). The four EPEC strains were isolated on days 6, 7, 10 and 13 and since the Plankenburg is a constant flowing river, their presence suggests there was a source continuously faecally polluting the River. Based on the multiplex PCR data no linking could be confirmed based on carry-over of pathotypes (EPEC).

Phase-5 linking: E. Coli phylogenetic group determination (Triplex PCR) – E. Coli can be divided into four main phylogenetic groups (A, B1, B2 and D) (Clermont *et al.*, 2000). From Fig. 5, 13 *E. Coli* isolates were selected and analysed using the triplex PCR method. These strains were chosen based on strain links between the river water and the beans on a phenotypic level. The data obtained showed phylogenetically one *E. Coli* isolate classified in group A_0 (*chuA*-, *yjaA*-, TSPE4.C2-), three in group B1 (*chuA*-, *yjaA*-, TSPE4.C2+) and nine in $B2_3$ (*chuA*+, *yjaA*+, TSPE4.C2+), respectively. Of the selected strains none showed a link on a phylogenetic level. Overall, the phylo-groups did not facilitate linking except for the "pure" culture *E. Coli.* The "pure" culture *E. Coli* from the irrigation water was in the same phylogroup ($B2_3$) as the *E. Coli* on from beans.

Phase-6 linking: Linking based on oriC-locus and dnaJ sequence analysis – Strains, based on the phenotypic clustering data and those that indicated a carry-over from the river water to the beans were selected for further sequencing analysis. These strains were found to be identical at the phenotypical level, but sequencing analysis was needed to confirm the identification. The selected strains included two of the EPEC strains that did not belong to any of the API 20E clusters and the reference strains.

The chromosomal replication origin, *oriC*-locus and the housekeeping gene, *dnaJ* were chosen as sequencing base since they are highly conserved genes unlike the 16S rRNA (Nhung *et al.*, 2007; Roggenkamp, 2007). It is known that the 16s rRNA cannot be used to distinguish between species that are closely related, since there are high similarities in this gene region between species (Spröer *et al.*, 1999; Roggenkamp, 2007). The primers selected for the *oriC*-locus sequencing (EcOriC-73-f and EcOriC-292-r) were specific for *E. Coli*, therefore only the *E. Coli* strains (Table 9) were sequenced. The *E. Coli* sequences were highly similar and had no bootstrap support. This indicated that when based on the sequences of the *oriC*-locus, no significant differences could be found between the *E. Coli* strains from the different API clusters, even though the primers used were *E. Coli* specific. The *E. Coli*, *K.pneumoniae* and *E.cloacae* strains in Table 9 were also subjected to *dnaJ* sequence analysis but showed no more differences between the *E. Coli* strains from the different API clusters.

Generally bootstrap values of >85% are considered significant and can be used to identify different clades between the *E. Coli*, *K.pneumoniae* and *E.cloacae* (Reid *et al.*, 2000). In this study (Fig. 6) the *K.pneumoniae* clade had a significant bootstrap value of 100%. The *Klebsiella* strains were found to be identical. Therefore, based on the *dnaJ* sequence, the *K.pneumoniae* strain in the River water (W839) was taken to be identical to the strain found on the beans (B943) a day after irrigation, confirming direct carry-over and linking at a different level.

Two *E.cloacae* clades were identified with a bootstrap value of 99% but differed significantly. Two major *E. Coli* clades, with a high bootstrap value of 100% were found. This again can be used to confirm that *E. Coli* is indeed carried over from irrigation water to beans, proving that although no linking was found for *E. Coli* from the River water to beans, it does take place. If more strains were isolated it could possibly have increased the possibility of linking *E. Coli* in River water to *E. Coli* on beans after irrigation.

Table 9. Species selected for sequencing analyses based on API clustering data.

Strain	Source	Day of Isolation	Species	Phenotypic cluster
W839	River water	8	*K.pneumoniae*	A
B943	Beans	9	*K.pneumoniae*	A
W632	River water	6	*E. Coli*	C
B835	Beans	8	*E. Coli*	C
W315	River water	3	*E. Coli*	D1
W734	River water	7	*E. Coli*	D1
B1466	Beans	14	*E. Coli*	D1
W427	River water	4	*E. Coli*	E
B627	Beans	6	*E. Coli*	E
W1052	River water	10	*E. Coli*	E
B1255	Beans	12	*E. Coli*	E
PCW11	"Pure" culture water	1	*E. Coli*	E
PCB95	"Pure" culture beans	9	*E. Coli*	E
W313	River water	3	*E.cloacae*	G
B411	Beans	4	*E.cloacae*	G
B731	Beans	7	*E.cloacae*	G
B1467	Beans	14	*E.cloacae*	G
B1573	Beans	15	*E.cloacae*	G
W630*	River water	7	*E. Coli*	-
W733A*	River water	7	*E. Coli*	-

The next *E. Coli* clade had a bootstrap value of 100% and contained two strains, W632 and W1052 both isolated from the river water and both were classified in phylogroup B1. The strain W1052 was identified as an EPEC strain in the previous analysis and placed in cluster E based on API 20E data. Strain W632 was placed in a different API cluster C, indicating that phenotypical analysis are not always completely accurate in identifying different types of *E. Coli*. No strains isolated from beans were classified in this phylogroup, therefore carry-over was not observed based on the strains in this clade.

The last *E. Coli* clade had a bootstrap value of 88% and also had two strains isolated from River water, W427 and W315. The two strains were however classified in two different phylo-groups, B1 and A_0 respectively. Again both strains are from different API clusters (E and D1), indicating the importance of sequencing to confirm linking. It could be that these strains were carried-over to the beans, but were not enumerated or sequenced. If more strains were isolated it might further confirm carry-over in each clade.

3.5.7 Conclusions

During the 14 day plot study the *E. Coli* counts in the irrigation water were found to vary greatly. The counts were always above the WHO irrigation guideline of <1 000 faecal coliforms per 100 mL (WHO, 1989) and reached a maximum value of 118 500 cfu.mL^{-1}. This indicated that the Plankenburg River (Plank-3 site) is not suitable for irrigation. It was, however, found that no impact regarding the carry-over to beans was observed when the counts of the irrigation water were in the 10^3 cfu.mL^{-1} range. It appears that *E. Coli* does not survive for long on beans when present at low concentrations (10^3 and 10^4 cfu.mL^{-1}) in the irrigation water. This could be an indication that it is possibly a "safe" concentration to irrigate fresh produce with and that no or a minimum number of *E. Coli* will be carried over.

The counts on the beans varied depending on the counts in the irrigation water. It was found that large *E. Coli* increases on the beans occurred even though the *E. Coli* in the irrigation water was lower. This was also previously found by Ackermann (Ackermann, 2010). The reason for this was possibly due to "clumping" as temperatures did not vary much across the 14 days. This indicates the importance that plate counts of the beans could be lower than the actual count on the beans due to the formation of clumps.

The data found in this study clearly indicated that there is indeed a direct carry-over of *E. Coli* and other organisms from irrigation water to the beans with daily irrigation. The effect of "once-off" irrigation at low concentrations lead to minimal impact and decreased survival of the *E. Coli* on the beans, possibly due to minimal moisture and nutrients.

The first phase of linking was shown using API clustering where identical strains were found in the irrigation water and on the beans a few days after being irrigated. Phase 2 linking statistically identified the linking of organisms based on the data (carbon source utilisation) of the API 20E. *E. Coli*, *E. asburiae* and *E. cloacae* were the species mostly isolated from the beans as well as from the Plankenburg irrigation water. It was therefore concluded that these organisms are the most abundant organisms in the Plankenburg River.

The importance of combining phenotypical methods in combination with molecular methods was confirmed in this study. It was also shown that molecular methods such as PCR (phase-3 linking), m-PCR (phase-4 linking) and sequencing (phase-6 linking) were necessary for correct identification and direct carry-over.

With the Multiplex-PCR four strains in the River water were identified as EPEC strains. These strains were isolated over 14 days and showed that there was a continuous source faecally polluting the River water. Since the presence of EPEC strains have been shown, it is an indication that the Plankenburg River could possibly be a source of foodborne diseases making the River unsafe for irrigation of fresh produce. Even though these strains were not found on the beans, it might be that if more colonies were enumerated from the beans EPEC strains would also have been found on the beans.

Based on the genotyping data (phase-5 linking) the strains of groups A_0 and B1 and the majority of selected strains belonging to phylo-group $B2_3$, were identified. It has previously been found that strains belonging to phylo-group $B2_3$ are only found in humans (Carlos *et al.*, 2010). Therefore it is a strong indication the faecal pollution in the Plankenburg River is from human origin. It can be speculated that the informal settlement, Kayamandi, might be the source as it has previously been found that the faecal pollution increased after the Plankenburg River flowed past Kayamandi (Barnes & Taylor, 2004). Further source-tracking methods would be of great importance to confirm whether Kayamandi is the only source contributing to the faecal pollution of the Plankenburg River.

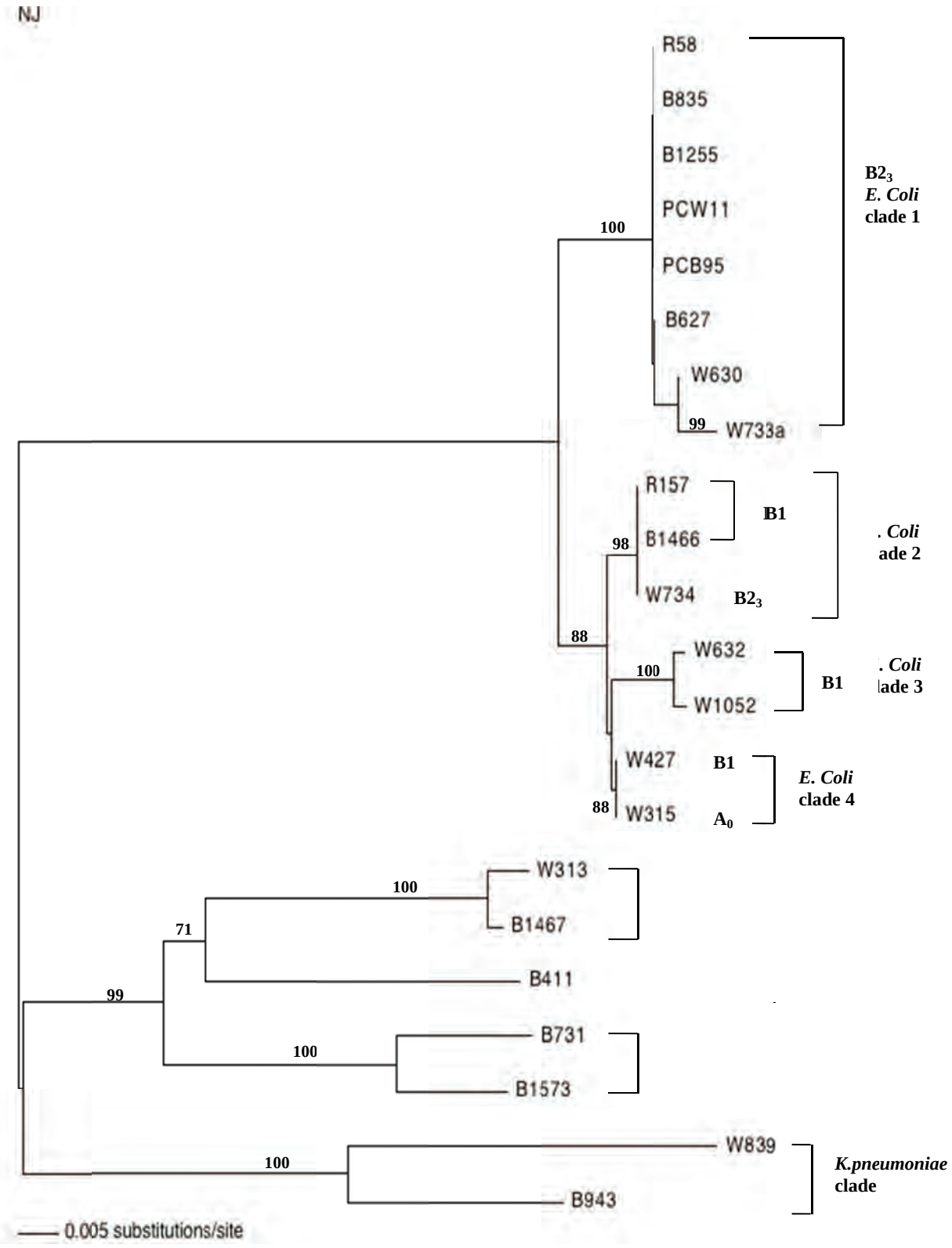


Figure 6. Phylogenetic tree of *E. Coli*, *K.pneumoniae* and *E.cloacae* strains based on the *dnaJ* sequences with neighbour-joining and bootstrap values with included *E. Coli* phylogenetic groups.

Overall, several diverse types of *E. Coli* were found to be present in both the irrigation water and on the beans indicating carry-over and linking. The API data gave the first indication of different *E. Coli* types as clusters with different phenotypical characters were found. Further confirmation of linking was confirmed with PCR methods and sequencing, which is necessary for accurate confirmation. The linking confirmation of *E. Coli* from the "pure" culture irrigation water to the beans shows that *E. Coli* is indeed carried over. The carry-over of *E. Coli* via irrigation water to fresh produce is therefore very likely to occur and should be seen as a potential health risk.

3.6 KWAZULU-NATAL – TRANSFER OF HYGIENE INDICATORS IN IRRIGATION WATER TO PRODUCE

3.6.1 Specific aim

To enable a microbial risk assessment, this research intended to verify whether there is evidence for a transfer of hygiene indicators or potential pathogens present in river water used for irrigation onto the surface of fresh produce via the irrigation route.

3.6.2 Results and Discussions

The analyses of fresh produce samples (Tables 10 and 11) irrigated with River water established that generally the highest overall counts occurred on produce in the rainy season (January 2010 to March 2010 and January 2011). Hence, total coliform counts per gram of produce analysed were highest in March 2010 at a level of 350 000 MPN.g^{-1} while the highest faecal coliform counts occurred in January 2011 (Table 10).

Table 10. Microbiological data obtained from produce samples irrigated with water from the Baynespruit River (Sobantu, Pietermaritzburg, KwaZulu-Natal) over a period of 13 months.

Date 2010-2011	*E. coli* (MPN.g^{-1})	ASF/AnSF (cfu.g^{-1})	IE	SA	LM	Sal
January	TG**	TNTC/200	TG	TG	ND	TG
February	790	TFTC/TFTC	TG	ND	ND	TG
March	<18	150/500	TG	TG	ND	TG
April	130	TFTC/220	TG	ND	ND	ND
May	<18	900/1200	TG	ND	ND	ND
June	<18	200/100	ND	ND	ND	ND
July	18	TFTC/TFTC	TG	ND	ND	ND
August	130	3 100/3 600	TG	TG	ND	TG
September	<18	2 400/3 900	TG	ND	ND	TG
October	<18	1 500/TFTC	TG	ND	ND	ND
November	<18	1 950/2 300	ND	ND	ND	ND
December	<18	2 600/2 500	ND	ND	ND	ND
January	84 000	4 400/4 400	TG	TG	ND	TG

January 2010, September 2010 and January 2011 = Spinach; February 2010 to July 2010 and October 2010 to December 2010 = Parsley; August 2010 = Cauliflower; ***E. coli* present but not quantified; TFTC = <10; TNTC = >300; TG = typical growth ; ND = not detected

For *E. coli* the highest count per gram of produce was detected in January 2011 with 84 000 MPN.g^{-1} (Table 11). In the dry season (April 2010 to July 2010) counts were generally lower than in the rainy season even with an increased irrigation frequency due to low rainfall. However, an unexpected increase in counts took place in the dry season. In August 2010 a total coliform count of 49 000, a faecal

coliform count of 230 and an *E. coli* count of 130 MPN.g^{-1} of produce were established. At the same time high counts of aerobic heterotrophs were P on the produce at 4.8 x 10^{6} cfu.g^{-1}. The increased counts for aerobic heterotrophs and total coliforms on produce in a month not receiving any rain might be due to sewage overflow, pasture runoff, or illegal disposal of faecal matter into the River upstream of the sampling site. This would also explain the presence of *Salmonella* spp. on produce in August 2010 and September 2010 as in the previous months (April 2010-July 2010) *Salmonella* spp. was not detected. Similarly, the count of aerobic and anaerobic spore formers had increased from below limit of detection in July 2010 to >3 000 cfu.g^{-1} in August 2010 (Table 11). These data indicate the presence of faecal matter in River water in the dry season which could have resulted in a somewhat higher microbial burden of the River water used for produce irrigation, thus leading to higher counts accumulated on produce samples over time.

The South African Department of Health stipulates that fruit and vegetables that are likely to be eaten raw should not contain more than 200 total coliforms per gram and *E. coli* must not be P per gram analysed to ensure its safe consumption (DoH, 2000). The produce samples were therefore, in many cases, not suitable for consumption regardless of the counts established for *E. coli*. In addition to the presence of the hygiene indicator *E. coli,* which might have included pathogenic, toxin producing strains such as STEC that are known to exhibit a low infective dose and have recently caused a severe outbreak in Europe due to contaminated fenugreek seeds imported from Egypt, and high counts of total and faecal coliforms, the frequent detection of potentially pathogenic *Salmonella* spp., *Staphylococcus aureus* and intestinal enterococci on the produce samples illustrates that a transfer of potential pathogens from contaminated irrigation water from the Baynespruit River might take place.

Table 11. Microbiological data obtained from produce samples irrigated with water from the Baynespruit River (Sobantu, Pietermaritzburg, KwaZulu-Natal) over 13 months.

Date 2010-2011	ACC (cfu.g^{-1})	TC (MPN.g^{-1})	FC (MPN.g^{-1})
January	TNTC	>160 000	160 000
February	51 000	92 000	3 300
March	TNTC	350 000	41 000
April	32 000	1 100	130
May	43 000	230	<18
June	18 000	330	78
July	95 000	130	18
August	4 800 000	49 000	230
September	79 000	45	<18
October	913 000	130	<18
November	6 800 000	33 000	<18
December	2 300 000	78	<18
January	2 400 000	84 000	84 000
* January 2010, September 2010 and January 2011 = Spinach; February 2010 to July 2010 and October 2010 to December 2010 = Parsley; August 2010 = Cauliflower; TNTC = >300 at highest dilution			

The presence of potential bacterial pathogens on the surface of produce is a reason for concern, as it has been established that *Salmonella enterica* when present on produce surfaces are able to actively enter the plant tissue via stomata and thus escape post-harvest surface treatment processes (Kroupitski *et al.*, 2009). In addition, Islam (Islam *et al.*, 2004) demonstrated the long term survival of pathogenic *E. coli* cells on parsley after irrigation. More recently, GFP labelled *E. coli* O157:H7 isolates were detected at

levels exceeding 2 log cfu.g^{-1} even 14 days after lettuce and spinach leaves had been contaminated by spraying (Doyle & Erickson, 2008a). Given that regular irrigation using water of poor quality might lead to a pathogen build up on the produce surface (possible even in the form of biofilm like structures), pathogen elimination due to environmental factors or processes such as irradiation of unshaded surface areas might become insignificant. This indicates that using River water unsuitable for safe irrigation is a practice that can contribute to the increasing number of foodborne outbreaks caused by contaminated produce.

3.6.3 Conclusion

It appears that a transfer of potential pathogens onto produce irrigated with water taken from the Baynespruit River can take place. Counts detected on irrigated produce were sometimes exceeding counts present in the irrigation water used, therefore indicating the involvement of build-up, microbial growth, and survival for extended periods of time. The water of the Baynespruit River is not safe and suitable for produce irrigation.

3.7 VENDA – QUALITY OF PRODUCE GROWN BY SUBSISTENT FARMERS

3.7.1 Specific aim

Very little information is available on the microbiological quality of fresh produce grown by subsistent farmers and sold in rural markets. This study will investigated the microbiological quality of these types of products from a rural subsistent farming project as well as those purchased from a local rural market in the Limpopo Province.

3.7.2 Results and Discussions

Irrigation water and at-harvest produce – The minimum ACC counts in water were 1.0x10^{2} cfu.mL^{-1}, the maximum ACC counts were 3.0x10^{7} cfu.mL^{-1} (Table 12). The minimum aerobic/heterotrophic plate counts in Conquest cabbages were 3.0x10^{2} cfu.mL^{-1}, the maximum aerobic/heterotrophic plate counts were 3.0x10^{7} cfu.mL^{-1} (Table 13). The minimum aerobic/heterotrophic plate counts in Tennessee cabbages were 2.0x10^{3} cfu.mL^{-1}, the maximum aerobic/heterotrophic plate counts were 3.0x10^{7} cfu.mL^{-1} (Table 14). The minimum aerobic/heterotrophic plate counts in the Tomato samples were 10 cfu.mL^{-1}, the maximum aerobic/heterotrophic plate counts were 3.0x10^{7} cfu.mL^{-1} (Table 15). The API identification of presumptive positive Gram negative isolates obtained from irrigation water and at-harvest produce from the Phadzima farming field in the Limpopo Province is given in Table 16. According to DWAF and FAO guidelines the maximum limits of aerobic/heterotrophic plate counts in irrigation water is 1.0x10^{2} cfu.mL^{-1}, which means that the values of aerobic/heterotrophic plate counts in the P study for water were much higher than the acceptable maximum limits prescribed by DWAF (DWAF, 1996b) and WHO (WHO, 1989). 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 to be harmless. The predominant heterotrophic bacterial strains that have been shown by a number of studies are *Acinetobacter* spp, *Aeromonas* spp, *Alcaligenes* spp, *Comamonas* spp, *Enterobacter* spp, *Flavobacterium* spp, *Klebsiella* spp, Legionella spp, *Moraxella* spp, *Pseudomonas* spp, *Sphingomonas* spp, *Stenotrophomonas* spp, atypical

Mycobacterium spp, *Bacillus* spp and *Nocardia* (WHO, 2003). However studies have showed that some aerobic/heterotrophic plate counts are opportunistic pathogens (Payment *et al.*, 1991).

Opportunistic pathogens such as *Flavobacterium* spp, *Klebsiella pneumonia*, *Bacillus* spp, and *Enterobacter* spp have been associated with diseases in immunocompromised individuals, infants, and elderly during exposure to or consumption of contaminated water (Payment *et al.*, 1991). *Legionella* spp can cause a potential fatal disease characterized by more severe form of the infection and produces high fever and pneumonia. *Mycobacteria spp* 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* spp 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* spp, the *Pseudomonas aeruginosa* species is the most important species for public health considerations that 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 spp* has been associated with diarrhoeal disease in many cases (WHO, 2003). *Aeromonas* spp 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* spp 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 also been isolated from infants in association with nerotising enterocolitis.

Pantoea spp 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 spp* 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* spp have been implicated in more than 95% of all outbreaks and sporadic cases of nosocomial bloodstream infections related to contaminated parenteral admixtures (Habsah *et al.*, 2005). *Serratia spp* 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 *Enterobacter 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 (Obi *et al.*, 2002) on the Luvubu, Mutale, Ngwedi, Tshinane, Makonde, Mutshindudi and Mudaswali Rivers in rural Venda communities, showed that the range of counts with regard to all the water sources investigated for HPC were between $1.8x10^{2}$ and $1.3x10^{6}$ cfu.mL^{-1}. Comparing the results of the present study with the results of Obi (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 13).

In a study done by Bukar (Bukar *et al.*, 2010) at different market in the Kano metropolis in Nigeria with the aim of analysing the presence of enteropathogens from minimally processed food; 5 tomato samples were examined and found to have the bacterial range of 6.00×10^{3}-1.32×10^{7} cfu.g^{-1} with the highest mean bacterial count of 1.60×10^{7} cfu.g^{-1}. The mean count exceeded the standard limit that should be less than 10^{5} cfu.g-1/mL set according to the Food and Agricultural Organization (FAO) for aerobic mesophilic bacterial count and this makes them to be of food safety concern (Bukar *et al.*, 2010). Fresh (tomatoes) vegetables sold in the local markets of Dhaka city samples were found to be heavily contaminated with bacteria in a study that was carried out (Akter *et al.*, 2011) to evaluate the total bacterial load and to isolate and identify antibiotic resistant bacteria in fresh vegetables sold in the local markets. The highest ACC of 5.17 log (cfu.g^{-1}) was found in tomato samples of local market (Annanda Bazar Market) and the lowest ACC of 4.90 log cfu.g^{-1} was found from another local market (Malibagh Bazar). It was concluded in this studies that the fresh vegetable samples collected from local markets were heavily contaminated with bacteria and are of special concern for human consumption (Akter *et al.*, 2011). In another study that was conducted to examine microbiological quality of raw salad vegetables and their role as a source of antibiotic resistant bacteria in Chittagong City, Bangladesh; In this study eight types of vegetables which were commonly used for salad including tomato were collected from two open markets and two super

shops of Chittagong City, Bangladesh. The microbial load of tomato count per gram was found to be 9.0×10^4 up to 3.8×10^5 cfu.g^{-1} which indicates the high levels of bacterial contamination on tomatoes at the market place.

In this study 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 test kits, no *Salmonella* spp. were identified in the irrigation water or the two variants of the cabbages. However *Salmonella* were identified on one tomato sample during March 2011 indicating that it is prevalent. Several opportunistic Gram negative *Enterobacteriaceae* were also identified (Table 16). The Gram negative bacteria identified on the samples did show that on several occasions (e.g. *Aeromonas* spp on October 2009) were identified in irrigation water samples as well as on the food produce which could indicate a potential link between the irrigation water and the pre-harvest produce.

The most dominant organism isolated in this study was *Enterobacter* spp (21.80%), followed by *Pseudomonas* spp (19.17%), *Vibrio* spp. (16.92%), *Lactobacillus* spp. (15.04%), *Staphylococcus* spp (10.15%), *Klebsiella* (9.04%), *E. coli* (4.89%), *Citrobacter* spp (2.26%), *Serratia* spp (0.37%) and *Salmonella* spp. (0.37%). However in this study no *Salmonella* spp isolated from the tomatoes samples.

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. The minimum and maximum counts of aerobic spore formers on Conquest cabbages ranged between zero and 10 000 000 cfu.mL^{-1} respectively. The minimum and maximum counts for anaerobic spore formers in the water were zero and 2 000 000 cfu.mL^{-1} respectively. The minimum and maximum counts of aerobic spore formers on Tennessee cabbages ranged between zero and 8 000 000 cfu.mL^{-1}, respectively. The minimum and maximum counts for anaerobic spore formers on Tennessee cabbages ranged between zero and 20 000 000 cfu.mL^{-1} respectively. The minimum and maximum counts of aerobic spore formers on tomato samples were zero and 9 000 000 cfu.mL^{-1} respectively. The minimum and maximum counts for anaerobic spore formers on tomato samples were zero and 2 000 000 cfu.mL^{-1} respectively. The high count of spore formers raises a cause for concern because a large number of spores could be produced. Endospore formers such as *Bacillus cereus* are often found in soil and are likely to be detected in River water if they are P 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 (Tables 9 to 11).

High microbial counts of intestinal *Enterococcus* were obtained frequently throughout the period of the study in the irrigation water. The minimum Enterococci counts in water, Conquest cabbages and Tennessee cabbages were zero and the maximum Enterococci counts was >3 000 cfu.100 mL^{-1}. The minimum Enterococci counts on tomato samples were 3 cfu.100 mL^{-1} and the maximum Enterococci counts was >3 000 cfu.100 mL^{-1}. The results in this study are very similar to other studies which investigated irrigation water and minimally processed food (MPF) products. In a study done Ackermann (2010), for the assessment of microbial load of the Plankenburg and Berg Rivers in the Western Cape Province and the survival of *Escherichia coli* on raw vegetables under laboratory condition, showed that intestinal *Enterococci* were detected in all four of the samples of the Berg 3 River with the loads ranging from 1 to >1 000 organisms.100 mL^{-1}. Intestinal *Enterococci* are typically excreted in the faeces of humans and other warm blooded animals. The presence of intestinal *Enterococci* provides evidence of recent faecal contamination. In a study done by Johnston (Johnston *et al.*, 2005), for the antimicrobial resistance of

Enterococcus species isolated from fresh produce (parsley, cabbage, cantaloupe, spinach, mustard green and celery) harvested in the south western United States, 97 (52%) were *E. faecium*, 38 (21%) were *E. faecalis* and 50 (27%) were other *Enterococcus* species. High microbial loads of intestinal *Enterococcus* were obtained frequently throughout the period of the study in the irrigation water and the minimal processed produce from the subsistent farm. Therefore it can be concluded that irrigation water serve as a potential source of contamination and irrigation of produce with this water may pose great health risk to the consumers since the produce may be consumed without lethal processing.

No *Listeria monocytogenes* were isolated from any of the samples tested during the study period.

Typical growth of *Staphylococcus* isolates were seen on petri plates. These isolates were identified further with Staphylococcus Latex Agglutination test kits and two of the months tested positive for *Staphylococcus* (Table 17). A total of 12 samples from all the samples which showed typical growth on the Petri plates were confirmed by the Latex agglutination test to be *Staphylococcus* bacteria. A total of 42% of the Conquest cabbage samples, 17% of the Tennessee cabbage samples, 33% of the tomato samples and 8% of the irrigation water samples contained *Staphylococcus* bacteria. November 2010 and January 2011 showed a potential linked from the irrigation water to one or more of the produce (Table 17). The results in this study are very similar to other studies which investigated irrigation water and minimally processed food (MPF) products. In a study by (Halablab *et al.*, 2011), *Staphylococcus aureus* was detected in the majority (80%) of the tested fresh-cut carrots samples and were present at 100 to 2 300 cfu.g^{-1}. Only 3 (20%) samples of 15 samples contained *Staphylococcus aureus* less than 100 cfu.g^{-1}, indicating good microbiological quality. Higher counts (>100 cfu.g^{-1}) of *Staphylococcus aureus* occurring as contaminant in minimally processed vegetable is to be viewed as a health hazard. The study concluded that contamination of fresh-cut carrot samples with *Staphylococcus aureus* might be due to handlers and cross-contamination during processing steps. In a study done by Halablab (Halablab *et al.*, 2011), the results for vegetable (lettuce, parsley and malva) sample collected from different locations (Barelias, Rawda, Jib-Janine and Kaaroun) irrigated by Litani River in Bekaa valley, Lebanon, showed that out of the total of 33 vegetable samples collected from Barelias, 51.5% samples were positive for *S. aureus* and 30% samples from Rawda were positive for *S. aureus*, 26, 66% samples from Jib-Janine were positive for *S. aureus*.

The minimum Total coliform counts in water was 14 cfu.100 mL^{-1}, the maximum Total coliform counts was >2 400 cfu.100 mL^{-1}. The minimum Faecal coliform counts in water was 14 cfu.100 mL^{-1}, the maximum Faecal coliform counts was >2 400 cfu.100 mL^{-1}. The minimum Total coliform counts on Conquest cabbages were 0 cfu.100 mL^{-1}, the maximum Total coliform counts was >2 400 cfu.100 mL^{-1}. The minimum Faecal coliform counts on Conquest cabbages were zero cfu. 100 mL^{-1}, the maximum Faecal coliform counts was >2 400 cfu.100 mL^{-1}. The minimum Total coliform counts on Tennessee cabbages were 9 cfu.100 mL^{-1}, the maximum Total coliform counts was 920 cfu.100 mL^{-1}. The minimum Faecal coliform counts on Tennessee cabbages were 9 cfu.100 mL^{-1}, the maximum Faecal coliform counts was 920 cfu.100 mL^{-1}. The minimum Total coliform counts on tomato samples were 0 cfu.100 mL^{-1}, the maximum Total coliform counts was >2 400 cfu.100 mL^{-1}. The minimum Faecal coliform counts on tomato samples were 0 cfu.100 mL^{-1}, the maximum Faecal coliform counts was >2 400 cfu.100 mL^{-1}. The presence of Faecal coliform bacteria in water is the indication of faecal contamination and the molecular identification of diarrhoeagenic *E. coli* strains in these samples indicated a potential health risk to the people using these water for domestic purposes (Table 18) (Nataro & Kaper, 1998). The results showed that many of the samples contained more than one diarrhoeagenic *E. Coli* strain.

A total of 27 water samples, 32 Conquest cabbage samples, 17 Tennessee cabbage samples and 19 Tomato samples showed possible *E. coli* presence over the study period from the MPN isolation. A total of 37% of the water samples, 28% Conquest cabbage samples, 24% Tennessee cabbage samples and 58% Tomato samples were positive for the commensal *E. coli* gene. In this study a total of 48% of the water samples, 16% Conquest cabbage samples, 24% Tennessee cabbage samples and 5% Tomato samples showed the presence of pathogenic atypical EPEC strains and a total of 22% water samples, 3% Conquest cabbage samples, 6% Tennessee cabbage samples and 11% Tomato samples tested positive for the prevalence of typical EPEC strains. Enteropathogenic *E. Coli* (EPEC) is 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). According to studies done by Trabulsi (Trabulsi *et al.*, 2002) humans are the only reservoir for typical EPEC, whereas both animals and humans are the reservoirs for atypical EPEC. And in the studies done by Nataro (Nataro & Kaper, 1998) typical EPEC is well recognized as a cause of gastroenteritis in infants in developing countries. In this study the presence of typical EPEC is of health risk to humans as they are able to cause infection to human In addition, 56% of the water samples, 16% Conquest cabbage samples, 18% Tennessee cabbage samples and 5% Tomato samples had Enteroaggregative *E. coli*, 11% of the water samples, 0% Conquest cabbage samples, 0% Tennessee cabbage samples and 3% Tomato samples contained Enteroinvasive *E. coli* and 30% of the water samples, 16% Conquest cabbage samples, 6% Tennessee cabbage samples and 11% Tomato samples tested positive for Enterohaemorrhagic *E. coli* and 30% of the water samples, 25% Conquest cabbage samples, 24% Tennessee cabbage samples and 11% Tomato samples Enterotoxigenic *E. coli* bacteria (Table 18).

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 (Harris *et al.*, 2003; Ahmed *et al.*, 2007a; Ahmed *et al.*, 2007b). Enteroinvasive *E. coli* are transmitted through the faecal-oral route. Even minimal contact is adequate for transmission. EIEC are rare causes of diarrhoea and dysentery. Enteroaggregative *E. coli* have been associated with diarrhoeal illness in infants and travellers and have been linked to outbreaks and are 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). A potential linked between the irrigation water and the different food produce can be concluded using the prevalence of commensal and diarrhoeagenic strains of *E. coli* bacteria which is shown in Table 14.

The result of this study compared to other studies shows that tomatoes were highly contaminated with faecal coliforms *(E. coli)*. In a study done by Enabulele (Enabulele & Uraih, 2009) 72 tomatoes samples were among other vegetable samples from abattoir and open traditional market collected in Benin City, Nigeria and were screened to determine the presence of *E. coli* O157:H7. Out of 72 tomatoes samples

64 (88-89%) were found with *E. coli* but with no *E. coli* O157:H7 were found (Enabulele, 2009). In addition out of 5 tomato samples analysed in a study done by Bukar and co-workers only 2 (40%) of *E. coli* bacteria were found with no pathogenic *E. coli* identified (Bukar *et al.*, 2010).

In this study virus isolation showed for three of the months (Table 19) the prevalence of Norovirus GI, Norovirus GII and Hepatitis A virus, respectively. No viruses were isolated from any of the food produce. From the abovementioned table it is evident that irrigation canal water available to, and used by, the Phadzima community farm for the production of fresh produce was contaminated with potentially pathogenic viruses.

Table 12. Bacterial counts for irrigation water from the Phadzima irrigation canal in the Limpopo Province.

Date	ASF (cfu.mL^{-1})	AnSF (cfu.mL^{-1})	IE (cfu.100 mL^{-1})	SA	LM	Sal
October 2008	400	30000	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	A
October 2009	10	1	192	P	ND	P
November 2009	2000	1	1400	P	ND	P
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	P	A	A
May 2010	ND	ND	ND	ND	ND	ND
June 2010	2 000	500	>3 000	A	A	P
July 2010	40 000	4 000	229	A	A	P
August 2010	300 000	800	>3 000	P	A	A
Sept. 2010	300 000	10 000	>3 000	P	A	P
October 2010	800 000	2 000	>3 000	P	A	P
November 2010	400 000	30 000	>3 000	P	A	P
December 2010	7 000 000	3 000 000	>3 000	P	A	P
January 2011	8 000 000	4 000	>3 000	P	A	P
February 2011	90 000	500	183	P	A	P
March 2011	1 000 000	200 000	>3 000	P	A	P
April 2011	90 000	1 000	>3 000	P	A	P
May 2011	700 000	40 000	>3 000	P	A	P
June 2011	7 000 000	ND	>3 000	A	A	P

ND = none detected; P = typical growth; A = no growth

Table 13. Bacterial counts for at harvest produce (Tennessee Cabbages) from the Phadzima Farming field in the Limpopo Province.

Date	ASF (cfu.mL^{-1})	AnSF (cfu.mL^{-1})	IE (cfu.100 mL^{-1})	SA	LM	Sal
October 2008	0	2	ND	ND	ND	ND
November 2008	100	27	ND	ND	ND	ND
December 2008	40	9	ND	ND	ND	ND
January 2009	4 000	1 000	ND	ND	ND	ND
February 2009	60	60	ND	ND	ND	ND
March 2009	2	1	ND	ND	ND	ND
April 2009	5	2	ND	ND	ND	ND
May 2009	ND	ND	ND	ND	ND	ND
June 2009	ND	ND	ND	ND	ND	ND
July 2009	ND	ND	ND	ND	ND	ND
August 2009	ND	ND	ND	ND	ND	ND
September 2009	ND	ND	ND	ND	ND	ND
October 2009	ND	ND	ND	ND	ND	ND
November 2009	20	0	>3 000	P	ND	A
December 2009	5 000 000	20 000 000	54	P	ND	A
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	60 000	30 000	0	P	A	A
May 2010	ND	ND	ND	ND	ND	ND
June 2010	10 000	2 000	123	P	A	P
July 2010	ND	ND	ND	ND	ND	ND
August 2010	ND	ND	ND	ND	ND	ND
September 2010	ND	ND	ND	ND	ND	ND
October 2010	ND	ND	ND	ND	ND	ND
November 2010	ND	ND	ND	ND	ND	ND
December 2010	ND	ND	ND	ND	ND	ND
January 2011	ND	ND	ND	ND	ND	ND
February 2011	ND	ND	ND	ND	ND	ND
March 2011	ND	ND	ND	ND	ND	ND
April 2011	8 000 000	500 000	>3 000	P	A	P
May 2011	1 000 000	200 000	>3 000	P	A	P
June 2011	800 000	ND	>3 000	P	A	A

ND = none detected; P = typical growth; A = no growth

Table 14. Bacterial counts for at harvest produce (Conquest cabbages) from the Phadzima Farming field in the Limpopo Province.

Date	ASF (cfu.mL^{-1})	AnSF (cfu.mL^{-1})	IE (cfu.100 mL^{-1})	SA	LM	Sal
October 2008	0	0	ND	ND	ND	ND
November 2008	90	25	ND	ND	ND	ND
December 2008	20	7	ND	ND	ND	ND
January 2009	600	4 000	ND	ND	ND	ND
February 2009	30	6	ND	ND	ND	ND
March 2009	30	10	ND	ND	ND	ND
April 2009	20	1	ND	ND	ND	ND
May 2009	6	6	ND	ND	ND	ND
June 2009	2	1	ND	ND	ND	ND
July 2009	2	2	ND	ND	ND	ND
August 2009	10	1	27	ND	ND	ND

Date	ASF (cfu.mL^{-1})	AnSF (cfu.mL^{-1})	IE (cfu.100 mL^{-1})	SA	LM	Sal
Sept. 2009	200 000	2 000	300	ND	ND	A
October 2009	200	1	0	P	ND	P
November 2009	200	0	231	P	ND	A
December 2009	300	1 000	23	P	ND	A
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	P	A	A
May 2010	ND	ND	ND	ND	ND	ND
June 2010	4 000	100	166	P	A	P
July 2010	200 000	2 000	26	A	A	P
August 2010	1 000 000	2 000 000	113	P	A	A
Sept. 2010	10 000	30 000	87	P	A	P
October 2010	1 000 000	20 000	239	P	A	A
November 2010	300 000	30 000	>3000	P	A	P
December 2010	1 000 000	2 000 000	122	P	A	P
January 2011	4 000 000	50 000	173	P	A	P
February 2011	4 000 000	7 000	>3000	P	A	P
March 2011	800 000	4 000	>3000	P	A	P
April 2011	10 000 000	2 000	226	P	A	P
May 2011	ND	ND	ND	ND	ND	ND
June 2011	ND	ND	ND	ND	ND	ND

ND = none detected; P = typical growth; A = no growth

Table 15. Bacterial counts for at harvest produce (Tomatoes) from the Phadzima Farming field in the Limpopo Province.

Date	ASF (cfu.mL^{-1})	AnSF (cfu.mL^{-1})	IE (cfu.100 mL^{-1})	SA	LM	Sal
October 2008	3	10	ND	ND	ND	ND
November 2008	50	20	ND	ND	ND	ND
December 2008	70	50	ND	ND	ND	ND
January 2009	80	40	ND	ND	ND	ND
February 2009	60	33	ND	ND	ND	ND
March 2009	200	10	ND	ND	ND	ND
April 2009	300	100	ND	ND	ND	ND
May 2009	4	1	ND	ND	ND	ND
June 2009	100	50	ND	ND	ND	ND
July 2009	10	5	ND	ND	ND	ND
August 2009	2	2	35	ND	ND	ND
Sept. 2009	ND	ND	ND	ND	ND	ND
October 2009	0	0	3	P	ND	P
November 2009	10 000	4 000	112	P	ND	A
December 2009	2 000	5 000	197	P	ND	A
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	ND	ND	ND	ND	ND	ND
May 2010	ND	ND	ND	ND	ND	ND
June 2010	20 000	10 000	>3 000	P	A	P
July 2010	300 000	7 000	>3 000	P	A	A
August 2010	10 000	5 000	163	P	A	A
Sept. 2010	ND	ND	ND	ND	ND	ND
October 2010	1 000 000	400 000	186	P	A	P

Date	ASF (cfu.mL^{-1})	AnSF (cfu.mL^{-1})	IE (cfu.100 mL^{-1})	SA	LM	Sal
November 2010	2 000 000	30 000	>3 000	P	A	P
December 2010	3 000 000	1 000 000	>3 000	P	A	P
January 2011	400 000	3 000	>3 000	P	A	P
February 2011	1 000 000	500 000	>3 000	P	A	P
March 2011	900 0000	3 000	121	P	A	P
April 2011	3 000 000	5 000	>3 000	P	A	P
May 2011	1 000 000	2 000 000	>3 000	P	A	P
June 2011	1 000	ND	>3 000	P	A	P

ND = none detected; P = typical growth; A = no growth

Table 16. API identification of presumptive positive Gram negative isolates obtained from irrigation water and at-harvest produce from the Phadzima farming field in the Limpopo Province.

Date	Sample	API Identification	Possible link
October 2009	Water	*Pseudomonas spp.* *Aeromonas spp*	*Enterobacter spp.* *Aeromonas spp.*
	Tomato	*Enterobacter spp.* *Aeromonas spp.*	
	Cabbage Conquest	*Enterobacter spp.*	
November 2009	Water	*Enterobacter spp.*	
June 2010	Water	*Enterobacter spp.* *Citrobacter spp*	*Enterobacter spp.* *Citrobacter spp*
	Tomato	*Enterobacter spp.*	
	Cabbage Conquest	*Klebsiella spp.*	
	Cabbage Tennessee	*Aeromonas spp.* *Pseudomonas spp.* *Citrobacter spp.*	
July 2010	Water	*Pantoea spp.* *Enterobacter spp.* *Proteus spp.*	*Enterobacter spp.*
	Cabbage Conquest	*Enterobacter spp.*	
September 2010	Water	*Aeromonas spp.* *Providencia alcalifaciens*	
	Cabbage Conquest	*Enterobacter spp.*	
October 2010	Water	*Enterobacter spp.* *Citrobacter spp.*	*Enterobacter spp.*
	Tomato	*Enterobacter spp.*	
November 2010	Water	*Aeromonas spp.* *Pseudomonas spp.*	*Aeromonas spp.* *Pseudomonas spp.*
	Tomato	*Pseudomonas spp.*	
	Cabbage Conquest	*Aeromonas spp.* *Pseudomonas spp.*	
December 2010	Water	*Pantoea spp.* *Aeromonas spp.*	*Pantoea spp.* *Aeromonas spp.*
	Tomato	*Pantoea spp.* *Aeromonas spp.*	
	Cabbage Conquest	*Aeromonas spp.* *Enterobacter spp.*	
January 2011	Water	*Enterobacter spp.* *Citrobacter spp.*	*Enterobacter spp.* *Citrobacter spp*
	Tomato	*Enterobacter spp.*	
	Cabbage Conquest	*Pantoea spp.* *Providencia*	

Date	Sample	API Identification	Possible link
		alcalifaciens *Aeromonas spp.* *Pseudomonas spp.*	
February 2011	Water	*Pseudomonas spp.* *Aeromonas spp.*	*Aeromonas spp.*
	Tomato	*Enterobacter spp.* *Aeromonas spp.*	
	Cabbage Conquest	*Aeromonas spp.*	
March 2011	Water	*Enterobacter spp* *Pseudomonas luteda*	*Enterobacter spp* *Pseudomonas spp*
	Tomato	*Non-fermenter spp.* *Salmonella*	
	Cabbage Conquest	*Enterobacter spp.* *Pseudomonas spp.* *Klebsiella spp.*	
April 2011	Water	*Serratia marcescens* *Pantoea spp.*	*Enterobacter spp.*
	Tomato	*Enterobacter spp.*	
	Cabbage Conquest	*Klebsiella spp.* *Aeromonas spp.*	
	Cabbage Tennessee	*Enterobacter spp.*	
May 2011	Water	*Aeromonas spp.* *Enterobacter spp.* *Serratia marcescens*	*Enterobacter spp.*
	Tomato	*Enterobacter spp.*	
	Cabbage Tennessee	*Enterobacter sakazakii*	
June 2011	Water	*Enterobacter spp* *Klebsiella spp*	*Enterobacter spp.*
	Tomato	*Enterobacter spp.*	

Table 17. Latex agglutination identification of presumptive positive *Staphylococcus* isolates obtained from irrigation water and at-harvest produce from the Phadzima farming field in the Limpopo Province.

Date	Sample	Staphylococcus Latex Agglutination test	Potential link
October 2009	Water	Neg	
	Tomato	Neg	
	Cabbage Conquest	Pos	
November 2009	Water	Neg	
	Tomato	Neg	
	Cabbage Conquest	Neg	
	Cabbage Tennessee	Neg	
December 2009	Tomato	Neg	
	Cabbage Conquest	Neg	
	Cabbage Tennessee	Neg	
April 2010	Water	Neg	
	Cabbage Conquest	Neg	
	Cabbage Tennessee	Neg	
June 2010	Tomato	Neg	
	Cabbage Conquest	Neg	
	Cabbage Tennessee	Neg	
July 2010	Tomato	Neg	
August 2010	Water	Neg	
	Tomato	Pos	
	Cabbage Conquest	Neg	

Date	Sample	Staphylococcus Latex Agglutination test	Potential link
September 2010	Water	Neg	
	Cabbage Conquest	Neg	
October 2010	Water	Neg	
	Tomato	Neg	
	Cabbage Conquest	Neg	
November 2010	Water	Pos	*S. aureus*
	Tomato	Pos	
	Cabbage Conquest	Pos	
December 2010	Water	Neg	
	Tomato	Neg	
	Cabbage Conquest	Neg	
January 2011	Water	Pos	*S. aureus*
	Tomato	Neg	
	Cabbage Conquest	Pos	
February 2011	Water	Neg	
	Tomato	Neg	
	Cabbage Conquest	Pos	
March 2011	Water	Neg	
	Tomato	Neg	
	Cabbage Conquest	Neg	
April 2011	Water	Neg	
	Tomato	Pos	
	Cabbage Conquest	Neg	
	Cabbage Tennessee	Neg	
May 2011	Water	Neg	
	Tomato	Neg	
	Cabbage Tennessee	Pos	
June 2011	Tomato	Pos	
	Cabbage Tennessee	Pos	

Neg = negative; Pos = positive

Table 18. Prevalence of commensal and diarrhoeagenic *E. coli* bacteria from irrigation water and post-harvest produce from the Phadzima farming field in the Limpopo Province.

Date	Produce type	*E. coli*	Potential link
October 2008	Water	-	
	Tomato	-	
	Cabbage Conquest	-	
	Cabbage Tennessee	-	
November 2008	Water	AP / I	
	Tomato	-	
	Cabbage Conquest	-	
	Cabbage Tennessee	-	
December 2008	Water	A / AP / I	AP
	Tomato	-	
	Cabbage Conquest	-	
	Cabbage Tennessee	AP	
January 2009	Water	-	
	Tomato	-	
	Cabbage Conquest	-	
	Cabbage Tennessee	-	
February 2009	Water	AP / T / I	
	Tomato	-	
	Cabbage Conquest	-	

Date	Produce type	*E. coli*	Potential link
	Cabbage Tennessee	-	
March 2009	Water	AP	
	Tomato	-	
	Cabbage Conquest	-	
	Cabbage Tennessee	-	
April 2009	Water	AP / T / A	AP / T
	Tomato	-	
	Cabbage Conquest	-	
	Cabbage Tennessee	AP/ T	
May 2009	Water	AP	
	Tomato	T	
	Cabbage Conquest	-	
June 2009	Water	A / H	
	Tomato	-	
	Cabbage Conquest	T	
July 2009	Water	AP / A	
	Tomato	-	
	Cabbage Conquest	H	
August 2009	Water	AP / H / A	AP / H / A
	Tomato	AP / H / A	
	Cabbage Conquest	-	
October 2009	Water	H / A / AP	AP / A
	Tomato	A	
	Cabbage Conquest	A / AP	
November 2009	Water	AP / A	H / T
	Tomato	H	
	Cabbage Conquest	H / T	
	Cabbage Tennessee	T	
December 2009	Tomato	TP	TP / H / A
	Cabbage Conquest	A / TP / H / T	
	Cabbage Tennessee	C / TP / T / H / A	
April 2010	Water	TP / H / T / A	AP / H / T / A
	Cabbage Conquest	AP / H / T / A	
	Cabbage Tennessee	T / A / AP	
June 2010	Water	AP / T / A / H	C / AP / A
	Tomato	C	
	Cabbage Conquest	C / T	
	Cabbage Tennessee	AP / A	
July 2010	Water	C	C
	Tomato	C	
	Cabbage Conquest	C	
Augustus 2010	Water	TP / C	C
	Tomato	C	
	Cabbage Conquest	AP / H / I / T / A	
September 2010	Water	TP / T / A / AP / H	
	Cabbage Conquest	C	
October 2010	Water	C / AP	C
	Tomato	C	
	Cabbage Conquest	C	
November 2010	Water	C / T / A / H / TP	C / T
	Tomato	C	
	Cabbage Conquest	C / T	
December 2010	Water	C / TP / T / A	C / T
	Tomato	C	
	Cabbage Conquest	C / T	
January 2011	Tomato	C	C
	Cabbage Conquest	C	
February 2011	Water	C / A	C / A

Date	Produce type	*E. coli*	Potential link
	Tomato	C / TP	
	Cabbage Conquest	C / A	
March 2011	Water	C / A	
April 2011	Water	C / T / H	C
	Tomato	C	
	Cabbage Conquest	C	
	Cabbage Tennessee	C	
May 2011	Water	C / A	C
	Tomato	C	
	Cabbage Tennessee	C	
June 2011	Water	C	C
	Tomato	C	
	Cabbage Tennessee	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*

Table 19. Prevalence of specific viruses from irrigation water and post-harvest produce from the Phadzima farming field in the Limpopo Province.

Date	Sample/ Produce type	Real-time RT-PCR analysis		
		NoV GI	**NoV GII**	**HAV**
October 2008	Water	Neg	Pos	Neg
	Tomato	Neg	Neg	Neg
	Cabbage Conquest	Neg	Neg	Neg
	Cabbage Tennessee	Neg	Neg	Neg
November 2008	Water	Neg	Neg	Neg
	Tomato	Neg	Neg	Neg
	Cabbage Conquest	Neg	Neg	Neg
	Cabbage Tennessee	Neg	Neg	Neg
December 2008	Water	Neg	Neg	Neg
	Tomato	Neg	Neg	Neg
	Cabbage Conquest	Neg	Neg	Neg
	Cabbage Tennessee	Neg	Neg	Neg
January 2009	Water	Neg	Neg	Neg
	Tomato	Neg	Neg	Neg
	Cabbage Conquest	Neg	Neg	Neg
	Cabbage Tennessee	Neg	Neg	Neg
February 2009	Water	Neg	Neg	Neg
	Tomato	Neg	Neg	Neg
	Cabbage Conquest	Neg	Neg	Neg
	Cabbage Tennessee	Neg	Neg	Neg
March 2009	Water	Neg	Neg	Neg
	Tomato	Neg	Neg	Neg
	Cabbage Conquest	Neg	Neg	Neg
	Cabbage Tennessee	Neg	Neg	Neg
April 2009	Water	Neg	Neg	Neg
	Tomato	Neg	Neg	Neg
	Cabbage Conquest	Neg	Neg	Neg
	Cabbage Tennessee	Neg	Neg	Neg
May 2009	Water	Neg	Neg	Neg
	Tomato	Neg	Neg	Neg
	Cabbage Conquest	Neg	Neg	Neg
June 2009	Water	Neg	Neg	Pos
	Tomato	Neg	Neg	Neg
	Cabbage Conquest	Neg	Neg	Neg

Date	Sample/ Produce type	Real-time RT-PCR analysis		
		NoV GI	NoV GII	HAV
July 2009	Water	Neg	Neg	Neg
	Tomato	Neg	Neg	Neg
	Cabbage Conquest	Neg	Neg	Neg
August 2009	Water	Neg	Neg	Neg
	Tomato	Neg	Neg	Neg
	Cabbage Conquest	Neg	Neg	Neg
September 2009	Water	Neg	Neg	Neg
	Cabbage Conquest	Neg	Neg	Neg
October 2009	Water	Neg	Neg	Neg
	Tomato	Neg	Neg	Neg
	Cabbage Conquest	Neg	Neg	Neg
November 2009	Water	Neg	Neg	Neg
	Tomato	Neg	Neg	Neg
	Cabbage Conquest	Neg	Neg	Neg
	Cabbage Tennessee	Neg	Neg	Neg
December 2009	Tomato	Neg	Neg	Neg
	Cabbage Conquest	Neg	Neg	Neg
	Cabbage Tennessee	Neg	Neg	Neg
April 2010	Water	Neg	Neg	Neg
	Cabbage Conquest	Neg	Neg	Neg
	Cabbage Tennessee	Neg	Neg	Neg
June 2010	Water	Neg	Neg	Neg
	Tomato	Neg	Neg	Neg
	Cabbage Tennessee	Neg	Neg	Neg
July 2010	Water	Pos	Neg	Neg
	Tomato	Neg	Neg	Neg
	Cabbage Conquest	Neg	Neg	Neg
December 2010	Water	Neg	Neg	Neg
	Tomato	Neg	Neg	Neg
	Cabbage Conquest	Neg	Neg	Neg
January 2011	Water	Neg	Neg	Neg
	Tomato	Neg	Neg	Neg
	Cabbage Conquest	Neg	Neg	Neg
February 2011	Water	Neg	Neg	Neg
	Tomato	Neg	Neg	Neg
March 2011	Water	Neg	Neg	Neg
April 2011	Water	Neg	Neg	Neg
May 2011	Water	Neg	Neg	Neg
	Tomato	Neg	Neg	Neg
June 2011	Water	Neg	Neg	Neg
	Tomato	Neg	Neg	Neg
	Cabbage Tennessee	Neg	Neg	Neg

Neg = negative; Pos = positive

Post-harvest produce – The minimum aerobic/heterotrophic plate counts on the cabbage samples from the market were 1.0 x 10^2 cfu.mL^{-1}, the maximum aerobic/heterotrophic plate counts were 3.0 x 10^7 cfu.mL^{-1} (Table 20). The minimum aerobic/heterotrophic plate counts on the tomato samples from the market were 20 cfu.mL^{-1}, the maximum aerobic/heterotrophic plate counts were 3.0 x 10^7 cfu.mL^{-1} (Table 21).

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 test kits and no *Salmonella* spp. were identified, although several opportunistic Gram negative *Enterobacteriaceae* were identified (Table 22).

Although *Salmonella* species were not present in this study, the species that were found as shown in Table 22 were of a potential health risk.

The minimum and maximum counts of aerobic spore formers in cabbages were 100 cfu.mL^{-1} and 200 000 cfu.mL^{-1} respectively. The minimum and maximum counts for anaerobic spore formers in cabbages were 300 cfu.mL^{-1} and 500 000 cfu.mL^{-1} respectively. The minimum and maximum counts of aerobic spore formers in tomato samples were 20 cfu.mL^{-1} and 30 000 000 cfu.mL^{-1} respectively. The minimum and maximum counts for anaerobic spore formers in tomato samples were 14 cfu.mL^{-1} and 70 000 cfu.mL^{-1} respectively (Tables 16 and 17).

The minimum Enterococci counts in cabbages were 36 cfu.100 mL^{-1} and the maximum Enterococci counts was >3 000 cfu.100 mL^{-1}. The minimum Enterococci counts in tomato samples were 54 cfu.100 mL^{-1} and the maximum Enterococci counts was >3 000 cfu.100 mL^{-1}.

No *Listeria monocytogenes* were isolated from any of the water samples tested during the study period.

Typical growth of *Staphylococcus* isolates were seen on plates. These isolates were identified further with Staphylococcus Latex Agglutination test kits and all found to be negative (Table 23).

The minimum Total coliform counts on cabbages were 0 cfu.100 mL^{-1}, the maximum Total coliform counts was >2 400 cfu.100 mL^{-1}. The minimum Faecal coliform counts on cabbages were 0 cfu.100 mL^{-1}, the maximum Faecal coliform counts was >2 400 cfu.100 mL^{-1}. The minimum Total coliform counts on tomato samples were 9 cfu.100 mL^{-1}, the maximum Total coliform counts was >2 400 cfu.100 mL^{-1}. The minimum Faecal coliform counts on tomato samples were 9 cfu.100 mL^{-1}, the maximum Faecal coliform counts was >2 400 cfu.100 mL^{-1}. The presence of Faecal coliform bacteria in water is the indication of faecal contamination and the molecular identification of diarrhoeagenic *E. coli* strains in these samples indicated a potential health risk to the people using these water for domestic purposes (Table 24) (Nataro & Kaper, 1998). The results showed that many of the samples contained more than one diarrhoeagenic *E. Coli* strain. A total of 27 cabbage samples and 30 tomato samples over the study period from the MPN isolation showed possible *E. coli* presence. A total of 41% of the cabbage samples and 23% of the tomato samples were positive for the commensal *E. coli* gene. In this study a total of 11% of the cabbage samples and 3% of the tomato samples showed the presence of pathogenic atypical EPEC strains and a total of 19% of the cabbage samples and 23% of the tomato samples tested positive for the prevalence of typical EPEC strains. In addition, 11% of the cabbage samples and 20% of the tomato samples had Enteroaggregative *E. coli*, 4% of the cabbage samples and 3% of the tomato samples contained Enteroinvasive *E. coli,* 8% of the cabbage samples and 7% of the tomato samples tested positive for Enterohaemorrhagic *E. coli* and 7% of the cabbage samples and 20% of the tomato samples tested positive for Enterotoxigenic *E. coli* bacteria (Table 24).

Virus isolation (Table 25) indicated that no viruses were present on any of the produce from the market.

Table 20. Bacterial counts for post-harvest (Cabbages) produce from the Thohoyandou rural market in the Limpopo Province.

Date	ASF (cfu.mL^{-1})	AnSF (cfu.mL^{-1})	IE (cfu.100 mL^{-1})	SA (P/A)	LM (P/A)	Sal (P/A)
May 2010	200 000	20 000	>3 000	P	A	A
June 2010	2 000	60 000	152	P	A	A
July 2010	ND	ND	ND	ND	ND	ND
August 2010	5 000	30 000	79	A	A	P
September 2010	70 000	30 000	>3 000	ND	A	P
October 2010	20 000	50 000	>3 000	P	A	P
November 2010	800	700	36	P	A	P
December 2010	40 000	5 000	149	P	A	A
January 2011	20 000	300	126	P	A	P
February 2011	50 000	600	>3 000	P	A	A
March 2011	500 000	2 000	>3 000	P	A	P
April 2011	8 000	400	154	P	A	P
May 2011	200 000	ND	241	P	A	P
June 2011	100	ND	141	P	A	P

ND = none detected; P = typical growth; A = no growth

Table 21. Counts for post-harvest Tomatoes produce from the Thohoyandou rural market in Limpopo.

Date	ASF (cfu.mL^{-1})	AnSF (cfu.mL^{-1})	IE (cfu.100 mL^{-1})	SA (P/A)	LM (P/A)	Sal (P/A)
May 2010	1 000 000	8 000	>3 000	P	A	A
June 2010	100	14	192	P	A	A
July 2010	ND	ND	ND	ND	ND	ND
August 2010	500 000	700	129	A	A	A
September 2010	200 000	2 000	123	ND	A	A
October 2010	4 000	40 000	>3 000	P	A	P
November 2010	20	100	54	P	A	A
December 2010	4 000	1 000	133	P	A	A
January 2011	900 000	70 000	>3 000	P	A	P
February 2011	70 000	8 000	>3 000	P	A	P
March 2011	600 000	1 000	>3 000	P	A	P
April 2011	70 000	300	>3 000	P	A	P
May 2011	80 000	ND	>3 000	P	A	P
June 2011	30 000 000	ND	>3 000	P	A	P

ND = none detected; P = typical growth; A = no growth

Table 22. API identification of presumptive positive isolates obtained from irrigation water and post-harvest produce from the Thohoyandou rural market in the Limpopo Province.

Date	Site/Sample	API Identification
August 2010	Market: Cabbage	*Aeromonas spp* *Actinobacter spp.*
September 2010	Market: Cabbage	*Serratia marcescens* *Pseudomonas spp* *Proteus mirabilis*
October 2010	Market: Tomato	*Aeromonas spp.* *Pseudomonas spp.*
	Market: Cabbage	*Enterobacter spp* *Klebsiella spp*
November 2010	Market: Cabbage	*Enterobacter spp* *Actinobacter spp.*
January 2011	Market: Tomato	*Enterobacter spp*
	Market: Cabbage	*Aeromonas spp.*
February 2011	Market: Tomato	*Enterobacter spp* *Citrobacter spp.*
March 2011	Market: Tomato	*Aeromonas spp.*
	Market: Cabbage	*Serratia marcescens* *Citrobacter spp.*
April 2011	Market: Tomato	*Aeromonas spp.*
	Market: Cabbage	*Aeromonas spp.* *Vibrio fluvialis*
May 2011	Market: Tomato	*Enterobacter spp*
	Market: Cabbage	*Aeromonas spp.*
June 2011	Market: Tomato	*Enterobacter spp.* *Pseudomonas spp.* *Citrobacter spp.*
	Market: Cabbage	*Non-fermenter spp* *Enterobacter spp*

Table 23. Latex agglutination identification of presumptive positive *Staphylococcus* isolates from irrigation water and post-harvest produce from the Thohoyandou rural market in the Limpopo Province.

Date	Site/Sample	Staphylococcus Latex Agglutination test
May 2010	Market: Tomato	Neg
	Market: Cabbage	Neg
June 2010	Market: Tomato	Neg
	Market: Cabbage	Neg
October 2010	Market: Tomato	Neg
	Market: Cabbage	Neg
November 2010	Market: Tomato	Neg
	Market: Cabbage	Neg
December 2010	Market: Tomato	Neg
	Market: Cabbage	Neg
January 2011	Market: Tomato	Neg
	Market: Cabbage	Neg
February 2011	Market: Tomato	Neg
	Market: Cabbage	Neg
March 2011	Market: Tomato	Neg
	Market: Cabbage	Neg
April 2011	Market: Tomato	Neg
	Market: Cabbage	Neg

Date	Site/Sample	Staphylococcus Latex Agglutination test
May 2011	Market: Tomato	Neg
	Market: Cabbage	Neg
June 2011	Market: Tomato	Neg
	Market: Cabbage	Neg

Neg = negative; Pos = positive

Table 24. Prevalence of commensal and diarrhoeagenic *Escherichia coli* bacteria isolated from post-harvest produce from the Thohoyandou rural market in the Limpopo.

Date	Site/Sample	*Escherichia coli*
May 2010	Market: Tomato	TP / T / A / H
	Market: Cabbage	A / C / TP / H
June 2010	Market: Tomato	TP / I / T / A
	Market: Cabbage	T / TP / A / H / C
August 2010	Market: Tomato	T / A / TP
	Market: Cabbage	-
October 2010	Market: Tomato	TP / H / A / T / AP
	Market: Cabbage	C / AP / T / TP / I / A
November 2010	Market: Tomato	C
	Market: Cabbage	C / AP
December 2010	Market: Tomato	TP / T / A
	Market: Cabbage	C / AP
January 2011	Market: Tomato	C / T / TP
	Market: Cabbage	C
February 2011	Market: Tomato	C
	Market: Cabbage	C
March 2011	Market: Tomato	C / A
	Market: Cabbage	C
April 2011	Market: Tomato	C
	Market: Cabbage	C / TP
May 2011	Market: Tomato	C / TP
	Market: Cabbage	C
June 2011	Market: Tomato	C
	Market: Cabbage	C / TP

Table 25. Specific viruses isolated from post-harvest produce from the Thohoyandou rural market in the Limpopo.

Date	Site/Sample	Real-time RT-PCR analysis		
		NoV GI	NoV GII	HAV
May 2010	Market: Tomato	Neg	Neg	Neg
	Market: Cabbage	Neg	Neg	Neg
June 2010	Market: Tomato	Neg	Neg	Neg
	Market: Cabbage	Neg	Neg	Neg
January 2011	Market: Tomato	Neg	Neg	Neg
	Market: Cabbage	Neg	Neg	Neg
May 2011	Market: Tomato	Neg	Neg	Neg
	Market: Cabbage	Neg	Neg	Neg
June 2011	Market: Tomato	Neg	Neg	Neg
	Market: Cabbage	Neg	Neg	Neg

Neg = negative; Pos = positive

3.7.3 Conclusions

Microbial contamination of vegetables with foodborne pathogens is a growing public health concern. Consumption of fresh fruits and vegetables has been recommended by the WHO (World Health Organization) because they reduce chance of getting sick with several diseases like cancer. Minimally processed foods are easily exposed to contaminants and this increases the risk to the health of consumers. In recent years, numerous outbreaks of gastrointestinal illness have been linked to the consumption of MPF and contamination with bacterial pathogens from human or animal faeces such as *Listeria monocytogenes*, *Salmonella*, *Shigella* and *E. coli O157*, *Staphylococcus*, *Aeromonas* and *Yersinia enterocolitica* (Beuchat, 1996).

Fresh produce or Minimally Processed fruits and vegetables are of nutritional and health benefit to the consumer's life. This has led to the high demand of fresh produce by the consumers, however the consumption of the fresh produce have been associated with the outbreaks of human infection. In addition, recently outbreaks associated with fresh produce or Minimally Processed fruits and vegetables resulted in a greater number of reported illness cases than outbreaks associated with other food vehicles. The outbreaks have been associated with the consumption of contaminated produce such as cilantro, tomatoes, cantaloupes, parsley, carrots, lettuce, cucumber and green onions (CDC, 2010a). In several studies pathogenic heterotrophic bacteria, *E. coli*, and *Salmonella* have been frequently linked to fresh produce outbreaks with *Salmonella* and *E. coli O157: H7* being the leading causes of fresh produce outbreaks in the USA. This has made these microorganisms to be of public health concern. *E. coli* and *Salmonella* inhabit in the intestinal tracts of animals including humans and are more likely to contaminate raw fruits and vegetables through contact with faeces, sewage, untreated irrigation water or surface water mostly in rural areas where there is poor sanitation. Poor sanitation in rural areas increases the potential for foodborne illness associated with MPF products (Harris *et al.*, 2003).

The results of this study indicated the need to follow the hygienic practices in handling practices of the vegetables in rural markets. Microbial contamination of these produce can be considered as a food safety concern. Therefore fresh vegetable can be a vehicle for the transmission of bacterial pathogens capable of causing human illness. Consequently, these vegetables may be a threat for the Sibasa community consumer and may be considered a serious risk to Limpopo public health. It is concluded that the hygienic quality of fresh vegetables should be improved and controlled to minimize the foodborne disease risk. Because of the results found in this study post-harvest contamination of ready to eat foods by environmental and human sources becomes an important factor that needs attention from researchers, authorities and consumers.

In the food products obtained from the Phadzima farming field and the Thohoyandou rural market the result indicated that a variety of pathogens such as *Staphylococcus aureus*, different opportunistic Gram negative Enterobacteriaceae, diarrhoeagenic strains of *E. coli* pathotypes and intestinal *Enterococci* were identified from the food products which indicate the potential risk of infections and diseases such as bacillary dysentery, typhoid, respiratory infections, urinary tract infections, pneumonia, gastroenteritis, endocarditis and food poisoning that could be a serious consequence if hygiene, water and sanitation infrastructures and practices are below standard in these communities (Potgieter *et al.*, 2009). The presence of these organisms in these samples may be attributed to the fact that commercial or small scale farmers generally irrigate their produce with water from nearby Rivers, streams, ponds, wells and dams which do not meet the required standards for irrigation water quality. The high microbial contamination of

vegetable obtained from the rural market observed in this study may be a reflection of the storage conditions and how long these produce were kept before they were obtained for sampling. Bacteria on storage material may transfer to produce and cross contamination between produce is possible particularly were produce are prewashed with the same wash water by the vendor.

3.8 GAUTENG – MICROBIAL QUALITY OF UNPROCESSED AND MINIMALLY PROCESSED LETTUCE, TOMATOES AND SPINACH SOLD BY RETAIL OUTLETS

3.8.1 Specific aim

The objective of this study was to examine the general microbial quality of unprocessed and minimally processed lettuce, tomatoes and spinach sold by retail outlets in Gauteng Province. Lettuce and tomatoes are usually eaten raw, so it is very important to meet the consumer's expectations of fresh and safer vegetable products.

3.8.2 Results

Gas mixture composition in the packages – Figure 7 shows that carbon dioxide levels were highest in the modified atmosphere package used for tomatoes. The modified atmosphere package used for lettuce had the lowest carbon dioxide and oxygen levels. Swiss chard was packaged in higher carbon dioxide and oxygen levels compared to the lettuce.

Pathogens isolated – E. coli, *Staphylococcus aureus*, *Listeria monocytogenes* and *Salmonella* were not detected in any of the samples, both unpackaged and modified atmosphere packaged (MAP).

Aerobic colony counts (ACC) – For ACC a significant ($p<0.05$) interaction was observed (Fig. 7) between vegetable type and packaging. MAP tomatoes had significantly ($p<0.05$) low ACC compared to the unpackaged tomatoes. ACC for the MAP tomatoes were not significantly ($p>0.05$) different from the other MAP vegetables. The ACC for the MAP tomatoes were significantly ($p<0.05$) different from the unpackaged Swiss chard and unpackaged lettuce (Table 26). The unpackaged tomatoes' ACC was not significantly ($p>0.05$) different from the unpackaged Swiss chard, unpackaged lettuce and MAP lettuce. MAP lettuce, MAP Swiss chard, unpackaged Swiss chard and unpackaged tomato were not significantly ($p>0.05$) different.

Table 26. Aerobic plate counts (ACC) on the modified atmosphere packaged and unpackaged vegetables.

Vegetable	Packaging	Mean ACC (log cfu.g^{-1})
Lettuce	MAP	5.39 ^{abc*} (0.386)
Lettuce	Unpackaged	7.35^{b} (0.046)
Swiss chard	MAP	4.62 ^{ac} (0.604)
Swiss chard	Unpackaged	6.89 ^{ab} (0.259)
Tomato	MAP	3.50 ^{c} (2.322)
Tomato	Unpackaged	6.07 ^{ab} (0.294)

*Mean values with different letters differ significantly ($p<0.05$); Standard error is given in parentheses

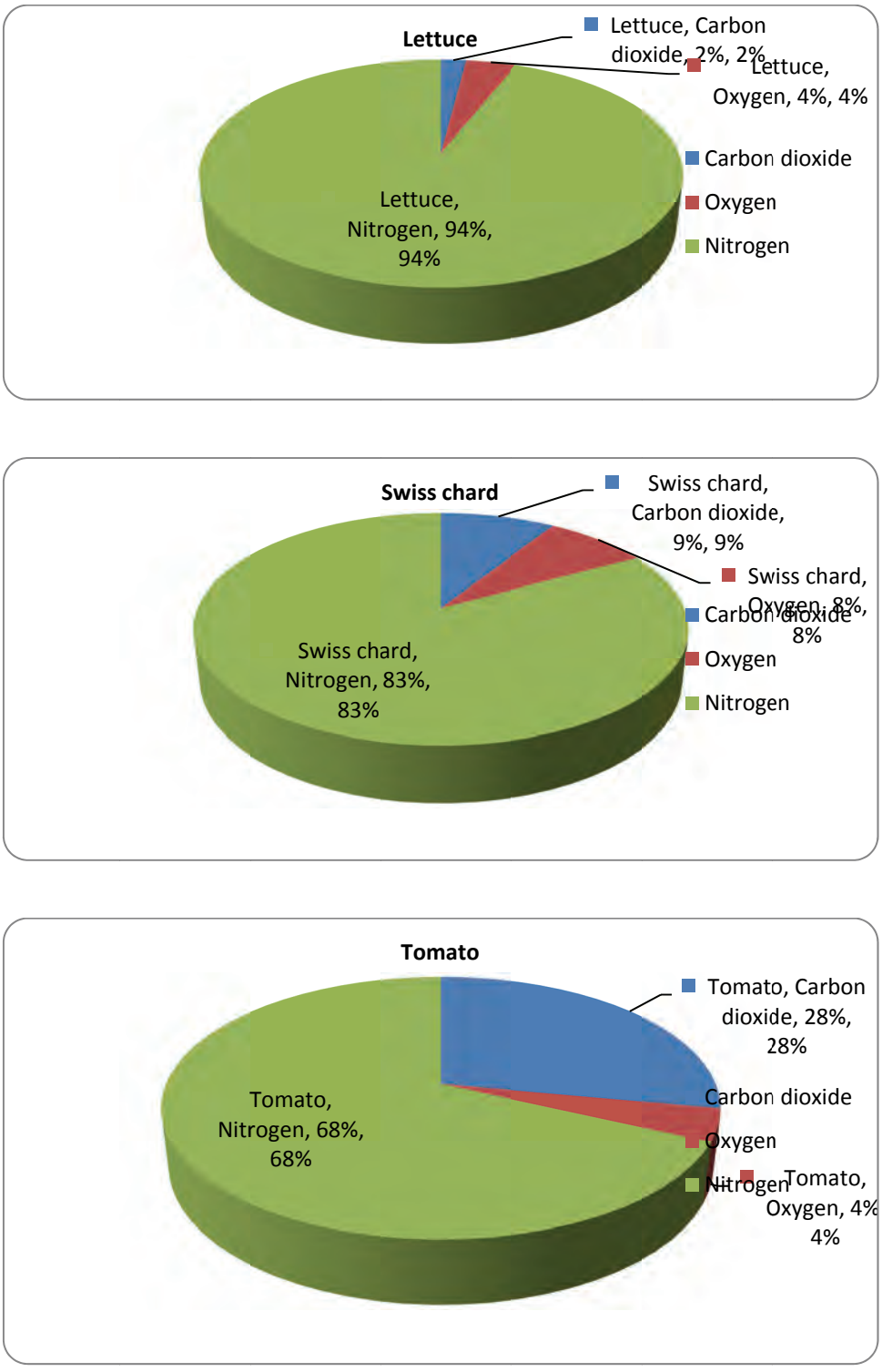


Figure 7. Gas mixture composition in the modified atmosphere packages.

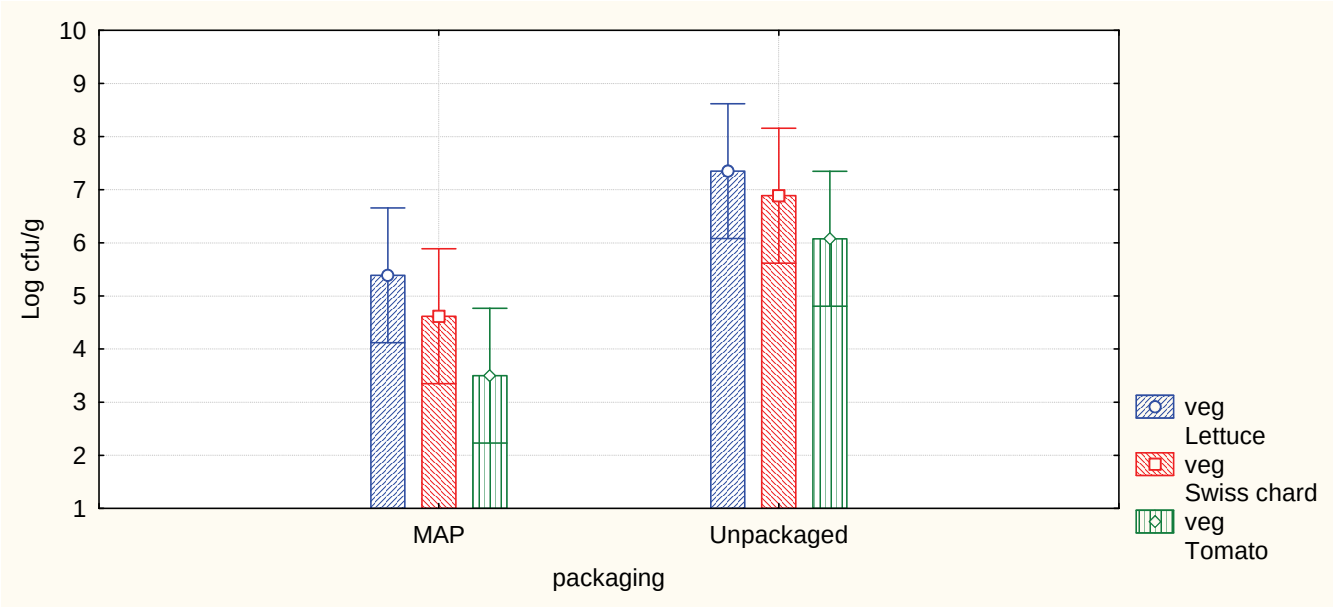


Figure 8. Aerobic plate counts in modified atmosphere packaged and unpackaged lettuce, Swiss chard and tomatoes.

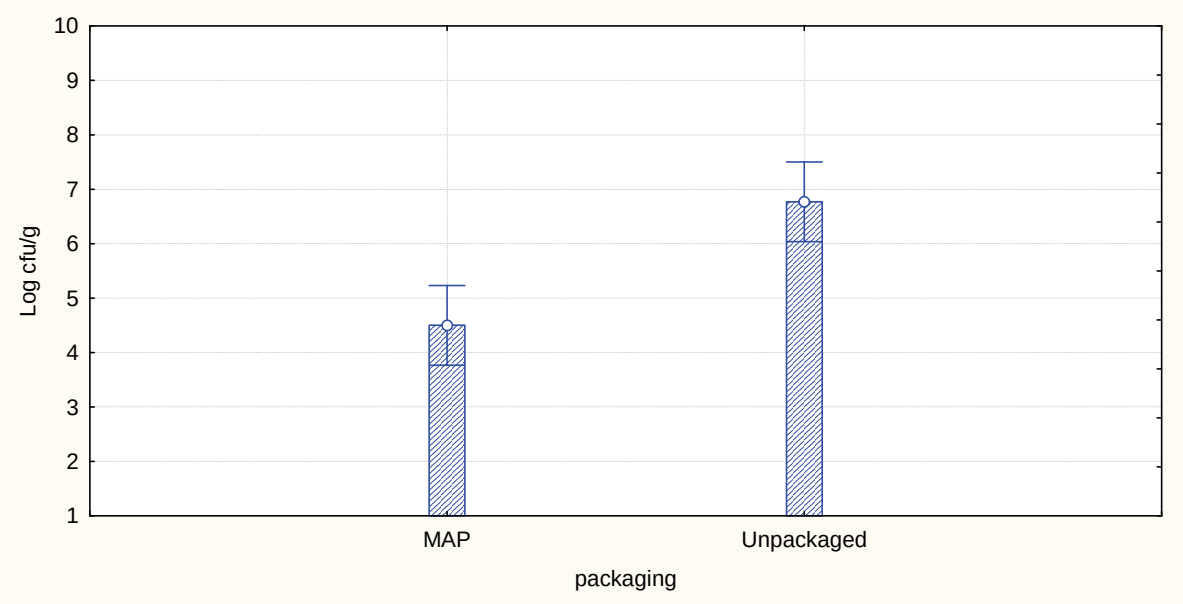


Figure 9. Aerobic plate counts for modified atmosphere packaged and unpackaged vegetables.

Looking at packaging alone, MAP vegetables had a significantly ($p<0.05$) lower ACC than the unpackaged vegetables. There was no significant ($p>0.05$) difference in ACC among the three vegetables.

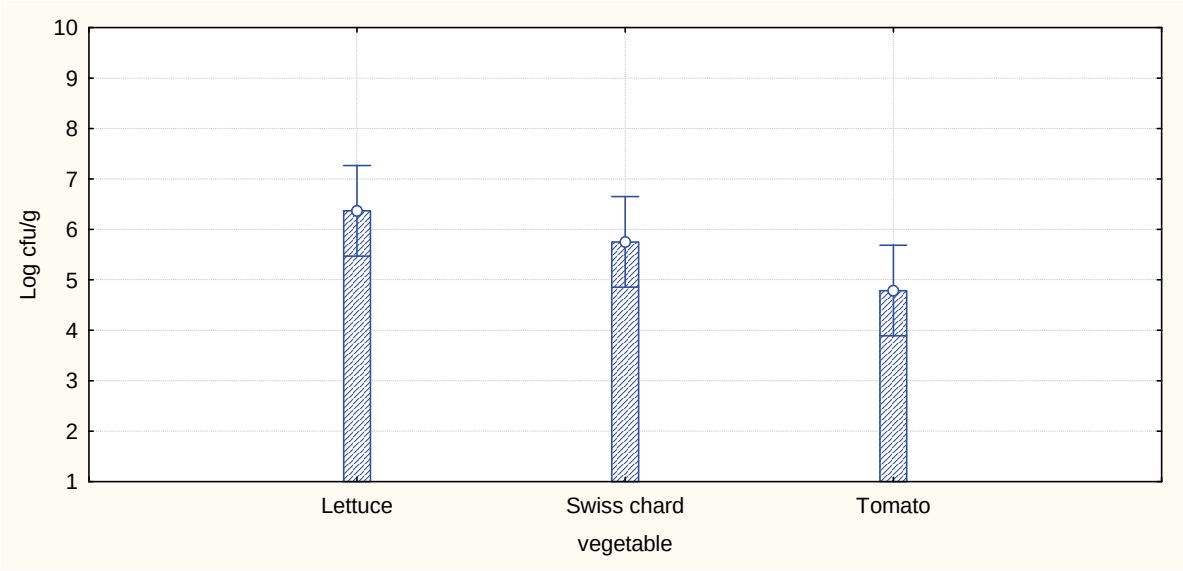


Figure 10. Aerobic plate counts for lettuce, Swiss chard and tomatoes.

Coliform counts – All the vegetables sampled in this study were contaminated with coliforms even though *Salmonella*, *Listeria monocytogenes*, *Staphylococcus aureus* and *E. coli* were not detected in any of the samples.

There was a significant ($p<0.05$) interaction between the vegetable type and packaging observed (Fig. 12, Table 27) in coliform counts. The packaging had no effect on coliform counts on the lettuce and Swiss chard (Fig. 12, Table 27) but it had a significant effect on the coliform levels on the tomatoes. Unpackaged tomatoes had a significantly ($p<0.05$) higher coliform count compared to MAP tomatoes. Coliform counts were not significantly ($p>0.05$) different between MAP tomato and MAP Swiss chard but there was a significant ($p<0.05$) difference between the MAP tomato and MAP lettuce and all the unpackaged vegetables (Fig. 12, Table 27). Coliform counts for the MAP Swiss chard were not significantly ($p>0.05$) different from the counts for all the other analysed vegetables.

Table 27. Coliform counts on the modified atmosphere packaged and unpackaged vegetables.

Vegetable	**Packaging**	**Mean coliform counts (log cfu.g^{-1})**
Lettuce	MAP	3.33 ^{a*} (0.386)
Lettuce	Unpackaged	3.56 ^{a} (0.046)
Swiss chard	MAP	2.17 ^{ab} (0.604)
Swiss chard	Unpackaged	3.75 ^{a} (0.259)
Tomato	MAP	1.00 ^{b} (2.322)
Tomato	Unpackaged	3.65 ^{a} (0.294)

*Mean values with different letters differ significantly ($p<0.05$). Standard error is given in parentheses

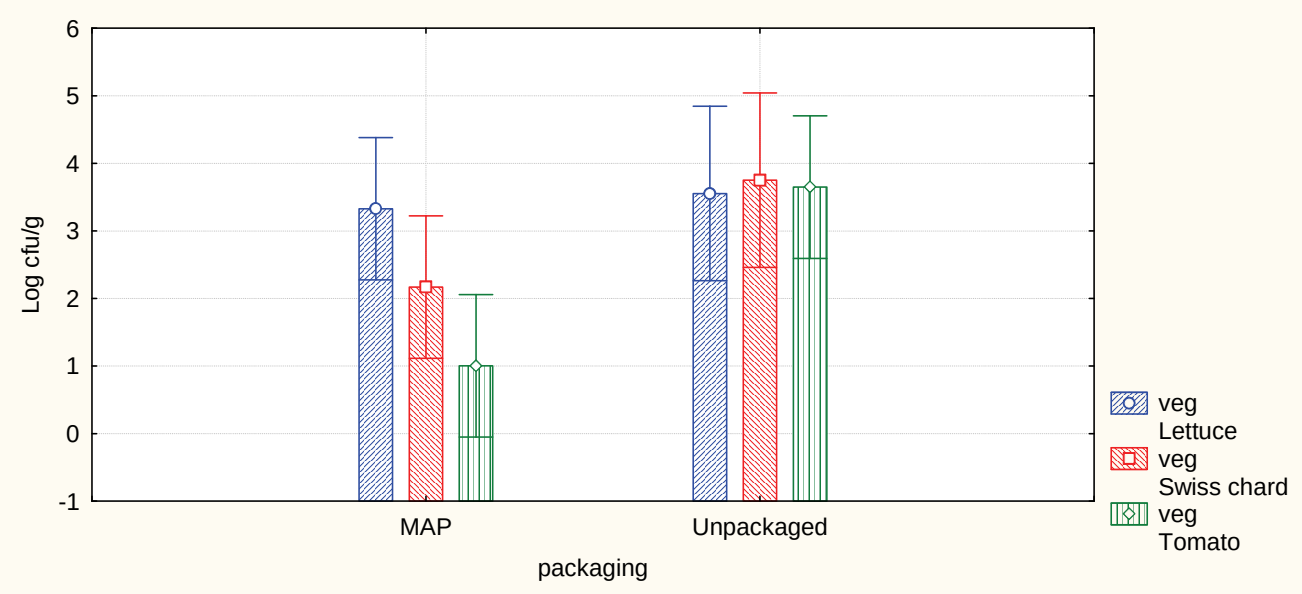


Figure 11. Coliform counts for the modified atmosphere packaged and unpackaged lettuce, Swiss chard and tomatoes.

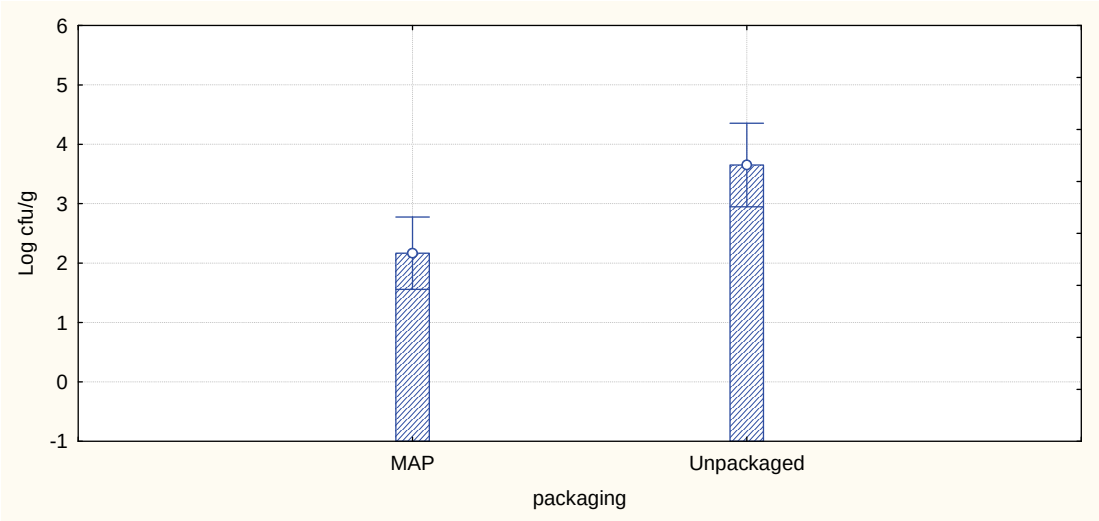


Figure 12. Coliform counts for modified atmosphere packaged (MAP) and unpackaged vegetables.

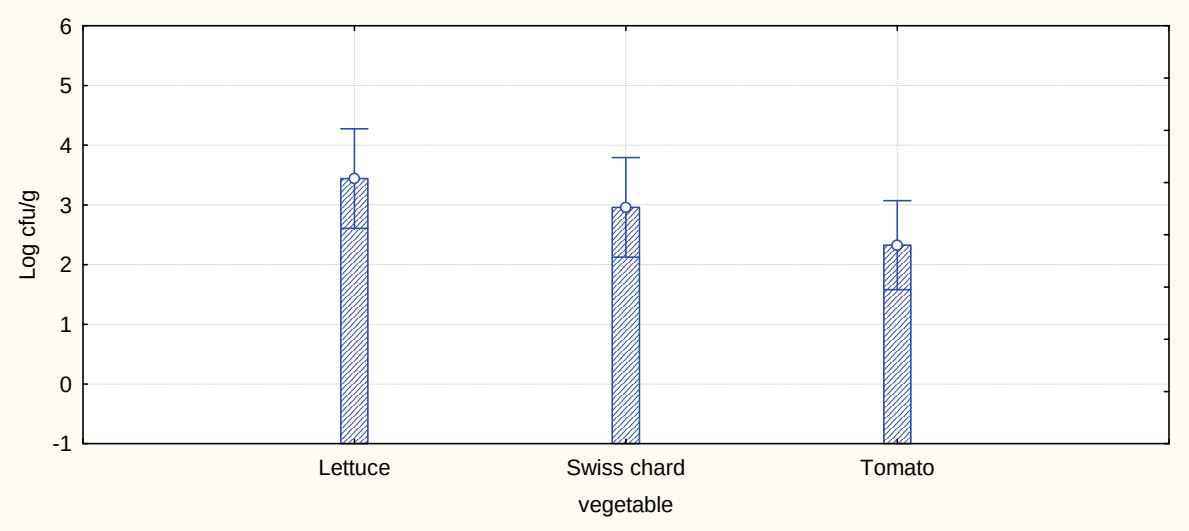


Figure 13. Coliform counts for lettuce, Swiss chard and tomatoes.

Looking at packaging alone, coliform counts were significantly higher ($p<0.05$) on the unpackaged vegetables compared to the modified atmosphere packaged counterpart (Fig. 10, Table 27).

There were no significant ($p>0.05$) differences in coliform counts among the three vegetables; lettuce, Swiss chard and tomatoes (Fig. 11, Table 27).

MPN method – Aerobic plate counts were commensurate to the coliform counts for lettuce and spinach with colony forming units reaching 7.6 log cfu.g^{-1} (Table 28). Although coliform and faecal coliform counts on broccoli were low, high aerobic plate counts of up to 7.2 log cfu.g^{-1} were recorded. The high counts could be due to the presence non-lactose fermenting microorganisms on the broccoli samples. Relatively low aerobic plate counts were recorded for tomatoes and strawberries sampled.

The MPN result for lettuce, spinach, broccoli, tomatoes and strawberries is presented in Table 1. Of all the purchased vegetables tested, the spinach samples had the highest coliform and faecal coliform counts with up to 2.8 x10^{6} MPN.100 g^{-1} for unpackaged spinach leaves. These counts are unacceptably high for vegetables that have presumably been washed and will undergo minimal processing before consumption. These numbers also indicate faecal contamination likely from irrigation water. The cut lettuce also had unacceptably high coliform and faecal coliform numbers. These were washed and MA packaged and ready to eat, which raises serious concern. The high numbers of faecal coliforms suggest the presence of pathogens. The coliform and faecal coliform counts did not vary significantly with time of sampling. The tomato and strawberry samples had the lowest coliform counts with <1.8 MPN.100 g^{-1} recorded. The coliform counts found on broccoli were low ranging from <1.8 to 450 MPN.100 g^{-1}.

Table 28. Bacterial quality of post-harvest fruits and vegetables purchased from retail outlets in Pretoria.

Fruit & Vegetable	**Packaging**	**Gas formers (MPN.100 g^{-1})**	**TC (MPN.100 g^{-1})**	**FC (MPN.100 g^{-1})**	**ACC (Log cfu.g^{-1})**
Cut lettuce (mixed lettuce leaves)	MAP	3 500	3 500	3 500	6.49
Cut lettuce (mixed lettuce leaves)	MAP	>16 000 000	>16 000 000	>16 000 000	7.20
Lettuce	PVC	54 000	54 000	54 000	7.60

Fruit & Vegetable	Packaging	Gas formers (MPN.100 g^{-1})	TC (MPN.100 g^{-1})	FC (MPN.100 g^{-1})	ACC (Log cfu.g^{-1})
Cut lettuce	MAP	490	490	490	6.95
Cut lettuce	MAP	1 300	1300	1 300	5.06
Cut spinach	MAP	16 000 000	16 000 000	16 000 000	7.54
Cut spinach	MAP	>16 000 000	>16 000 000	>16 000 000	7.65
Spinach	Unpackaged	11 000	11 000	11 000	6.32
Spinach	Unpackaged	2 800 000 000	2 800 000 000	2 800 000 000	6.48
Spinach	Unpackaged	35 000	35 000	35 000	6.09
Cut spinach	MAP	54 000	54 000	54 000	-
Baby leaf spinach	MAP	560 000	560 000	560 000	-
Rocket	MAP	3 500	3 500	3 500	6.71
Broccoli	PVC	40	40	40	7.21
Broccoli	PVC	40	40	40	5.90
Broccoli	PVC	360	360	360	5.39
Broccoli	PVC	450	450	450	6.14
Cauliflower & Broccoli florets	MAP	<1.8	<1.8	<1.8	5.06
Baby tomatoes	PVC	<1.8	<1.8	<1.8	3.73
Cherry tomatoes	MAP	<1.8	<1.8	<1.8	4.76
Baby tomatoes	MAP	<1.8	<1.8	<1.8	2.48
Strawberries	MAP	7 000	7 000	7 000	5.27
Strawberries	MAP	<1.8	<1.8	<1.8	2.95

3.8.3 Discussion

The pathogens *E. coli*, *Staphylococcus aureus*, *Listeria monocytogenes* and *Salmonella* were not found on the MAP and unpackaged vegetables. The presence of coliforms does not indicate that pathogens are present (Nguz *et al.*, 2005).

Absence of *Staphylococcus aureus* on any of the samples may be due to employees practising good personal hygiene. According to Nguz (Nguz *et al.*, 2005), the absence of *E. coli* may indicate that vegetables were processed under sanitary conditions while the absence of Salmonella in all the samples may have been due to Good Agricultural Practices (GAP) and Good Manufacturing Practices (GMP). The absence of *Listeria monocytogenes* in all the MAP samples may have been due to effective sanitation treatments. Conventional methods for the detection of species in foods involve the use of selective culture enrichment with subsequent culturing on selective media, followed by biochemical identification. Although used as standard methods these Listeria isolation procedures are not entirely effective, with about 30% of all *L. monocytogenes* positive food samples escaping detection (Pinner *et al.*, 1992). This may be the reason why *Listeria monocytogenes* was not detected in both the MAP and Unpackaged samples. Other possible reasons for its absence could be the overgrowth by other *Listeria* species and/or natural background flora during enrichment (Ryser *et al.*, 1996). Phenotypic properties are sometimes not always expressed resulting in the misidentification of *L. monocytogenes*. Low reactive or atypical listeria occurs frequently in some foods resulting in samples testing negative when in actual fact *L. monocytogenes* present.

The number of microorganisms found on fresh produce can vary according to a number of factors including product quality, product characteristics, normal micro flora of the product, handling, processing, storage, distribution and normal and retail display (Gonzalez-Aguilar *et al.*, 2004). Lettuce and Swiss chard

had higher coliform and aerobic plate counts as they are top soil vegetables while tomatoes had lower counts because they are tree-borne. According to Garcia (Garcia-Armisen & Servais, 2007), it is expected that different microorganisms will be present in produce that grows on top of the soil versus a tree-borne fruit.

Tremendous variation in weight to surface area ratio ($g.cm^{-2}$) could account for the lower coliform and aerobic counts in the tomatoes compared to Swiss chard and lettuce. Lettuce and Swiss chard have a two sided plane providing a greater surface area for the contamination and growth of microbes while tomatoes only have a sided plane (sphere) (Gil *et al.*, 2009). Beuchat and co-workers (Beuchat *et al.*, 2001) described that a decontamination process designed to achieve, for example, a 3-log reduction in $cfu.g^{-1}$ of lettuce or tomato would result, respectively in approximately 0.114 and 18 log reductions in $cfu.cm^{-2}$.

The lower coliform and aerobic plate counts in tomatoes may have been due to its different surface morphology compared to Swiss chard and lettuce. Leaf veins and stomata can be seen on the upper and lower surface of Swiss chard and lettuce while lenticels are evident on the outer surface of the tomato. The dense array of stomata, ribs, trichomes and other cuticular microstructures in lettuce and Swiss chard not only make washing difficult but provide environmentally friendly niches in the form of depressions that protect and favour the survival of microbes (Whipps *et al.*, 2008).

MAP tomatoes had lower coliform counts and ACC compared to the MAP Swiss chard and the MAP lettuce because they have a smooth exposed surface with little micro scale structures and are easily washed unlike lettuce and Swiss chard that have considerable amount of macro scale structure of entwined clusters of curled leaves that make washing difficult (Keeratipibul *et al.*, 2010). Tomatoes have lower counts than Swiss chard and lettuce because of its waxier surface cuticle that makes it difficult for the hydrophilic surfaces of bacterial cells to attach. The hydrophilic injured surfaces of lettuce are more adhesive to the hydrophilic surfaces of bacterial cells. Lettuce and Swiss chard are fragile and easily damaged during harvesting, transportation and handling when compared to a tomato. The damaged sites provide a greater opportunity for bacterial attachment and higher bacterial counts.

Coliforms and ACC were still present in the MAP samples though in lower numbers. This is because effective washing and decontamination of fresh cut vegetables is difficult to achieve and the reduction of microbial counts on fruits and vegetables is typically 100 fold at best. The MAP vegetable samples had lower coliform counts and aerobic plate counts compared to unpackaged vegetables. The washing process during processing may have helped to reduce the total microbial load, with the addition of chlorine exerting an even greater microbial reduction. An atmosphere with low oxygen levels and elevated carbon dioxide levels at 5^0C has been found to inhibit microbial growth although not all microorganism strains or species are sensitive to the antimicrobial effect of carbon dioxide (Oliviera *et al.*, 2010).

In the MAP samples, lettuce was shredded, in Swiss chard only the stalk was cut off and the tomatoes were whole. The cut surfaces in lettuce exude nutrients and provide moisture which facilitates bacterial growth resulting in greater bacterial attachment and higher bacterial counts. The coliform and ACC may be higher in the unpackaged lettuce because of the presence of wrapper or external leaves compared to the MAP lettuce where these leaves are removed during processing. External leaves in lettuce have a high microbial load compared to inner leaves as they are exposed more to the soil and irrigation water.

Lettuce may have had higher coliform counts and ACC because of the lower carbon dioxide content in the packages. Since carbon dioxide is the gas that exerts the antimicrobial effect, low levels of carbon dioxide will not inhibit microbial growth strongly, resulting in the higher aerobic plate counts and

coliform counts (Parry, 1993). Swiss chard had lower coliform and ACC than lettuce because it had higher levels of carbon dioxide. The lower coliform counts in tomatoes may be attributed to the high carbon dioxide levels in the package.

Swiss chard had higher oxygen levels than lettuce. According to Allende (Allende *et al.*, 2004), fresh cut Swiss chard has a higher respiration rate and so requires higher levels of oxygen in the package to maintain their quality while lettuce has a low respiration rate. If oxygen is depleted beyond the extinction point of the commodity, anaerobic conditions within the package can result in the production of off odours and rapid deterioration during shelf life (Allende *et al.*, 2004). According to the Hazard Analysis Critical Control Point- Total Quality Management (HACCP-TQM) Technical guidelines for the microbial quality of raw food: food containing $<10^4$ cfu.g^{-1}, 10^4 to 5 x 10^6 cfu.g^{-1}, 5 x 10^6 to 5 x 10^7 cfu.g^{-1} and >5 x 10^7 cfu.g^{-1} ACC are rated as good, average, poor and spoiled food respectively (Aycicek *et al.*, 2006). In this study, MAP lettuce, MAP Swiss chard and MAP tomato were of good quality. The unpackaged lettuce, Swiss chard and tomatoes were of average quality.

Faecal coliform limits for vegetables eaten uncooked has been defined as 10^5 cfu.100 g^{-1} (Aycicek *et al.*, 2006). Lettuce and tomatoes are eaten raw and coliform counts were within limit with counts in the following ranges: MAP lettuce (5.94 x 10^3 to 1.07 x 10^4), unpackaged lettuce (5.94 x 10^3 to 1.07 x 10^4), MAP tomatoes (0 to 4.12 x 10^3) and unpackaged tomatoes (1.47 x 10^3 to 2.21 x 10^4). Levels of *S. aureus* that are higher than 4 log are potentially hazardous (Beuchat *et al.*, 1998). All the vegetables that were analysed in this survey were safe to eat.

According to Kokkinaki (Kokinakis *et al.*, 2007), most food researchers agree that at least for mixed salads containing fresh vegetables, *Listeria* should be absent in 25 g and *E. Coli* levels are satisfactory if <20 cfu.g^{-1}, acceptable if <100 cfu.g^{-1} and unsatisfactory if >100 cfu.g^{-1}. The Greek salads where tomatoes were taken for this analysis were of satisfactory quality as *Listeria monocytogenes* and *E. coli* were absent. All the vegetable samples were of satisfactory microbiological quality more so the unpackaged vegetables considering the fact that Swiss chard will be cooked and the raw tomatoes and lettuce will be washed and even better sanitized before use.

The MPN result for lettuce, spinach, broccoli, tomatoes and strawberries is presented in table 3. Of all the purchased vegetables tested, the spinach samples had the highest coliform and faecal coliform counts with up to 2.8 x10^6 MPN.100 g^{-1} for unpackaged spinach leaves. These counts are unacceptably high for vegetables that have presumably been washed and will undergo minimal processing before consumption. These numbers also indicate faecal contamination likely from irrigation water. The cut lettuce also had unacceptably high coliform and faecal coliform numbers. These were washed and MA packaged and ready to eat, which raises serious concern. The high numbers of faecal coliforms suggest the presence of pathogens. The coliform and faecal coliform counts did not vary significantly with time of sampling. The tomato and strawberry samples had the lowest coliform counts with <1.8 MPN.100 g^{-1} recorded. The coliform counts found on broccoli were low ranging from <1.8 to 450 MPN.100 g^{-1}.

Aerobic plate counts were commensurate to the coliform counts for lettuce and spinach with colony forming units reaching 7.6 log cfu.g^{-1} (Table 3). Although coliform and faecal coliform counts on broccoli were low, high aerobic plate counts of up to 7.2 log cfu.g^{-1} were recorded. The high counts could be due to the presence non-lactose fermenting microorganisms on the broccoli samples. Relatively low aerobic plate counts were recorded for tomatoes and strawberries sampled.

Bacterial contamination of post-harvest vegetables cannot be attributed to irrigation water alone, there could be other possible sources of contamination along the food chain or they could have been presented with conditions that allowed rapid multiplication of existing bacterial on the produce.

3.8.4 Conclusions

The leafy vegetables lettuce and Swiss chard have a higher microbial load than tomatoes for both the MAP and unpackaged vegetables. In light of this, when sanitizing lettuce and Swiss chard, it is important to use a treatment that is different from the one used for tomatoes. For instance, a combination of treatments may be necessary for lettuce and Swiss chard but a single treatment sufficient for tomatoes. A more effective decontamination process is required for lettuce which is eaten raw unlike Swiss chard which is cooked. It is of paramount importance that the sanitation procedure used be effective because providing a modified atmosphere in the packages may have little or no effect in preventing the growth and proliferation of microbes in some vegetables.

Pathogenic microorganisms will be absent from vegetables if care is taken to prevent contamination during cultivation, harvesting, transportation and processing. Based on the absence of pathogens and low coliform and plate counts, the quality and safety of vegetables analysed in this study were acceptable.

Retailers and manufacturers must know the history of the vegetables they purchase from their suppliers even if they will be processed. This will encourage the farmers to have Good Agricultural Practices in place. Good Manufacturing Practices help to prevent cross contamination from equipment surfaces and handler's hands. Consumers are encouraged to be alert when purchasing MAP vegetables as those purchased after their best before date may have higher bacterial counts. The microbial quality of unpackaged and MAP vegetables must continually be improved to guarantee wholesome and safe products.

3.9 MPUMALANGA, NORTH WEST AND GAUTENG – LINKS BETWEEN CRYPTOSPORIDIUM, GIARDIA IN IRRIGATION WATER AND ON PRODUCE

3.9.1 Specific aim

This study will determine the occurrence of *Cryptosporidium* and *Giardia* in three South African rivers used for the irrigation of fresh produce. Due to the cost and low recovery rate of the isolation methods for *Cryptosporidium* and *Giardia*, it will be necessary to assess the correlation between the presence of faecal indicators such as *E. coli* and faecal coliforms and incidence of *Cryptosporidium* and *Giardia* in order to find a reliable indicator for those two protozoa.

3.9.2 Incidence of indicators and protozoa in the Klip, Moses and Skeerpoort Rivers

The aerobic colony counts (ACC) in the Klip River ranged from 217 and 899 000 cfu.mL^{-1} with an average of 122 667 cfu.mL^{-1} (Fig. 14). Levels of faecal coliforms ranged from 1700 to 350 000 counts.100 mL^{-1} (Fig. 14). *E. Coli* levels ranged from 1700 to 350 000 100 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 (Fig. 14). *Salmonella* spp., *Salmonella* Enteritidis and *Salmonella* 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 (Tables 29 to 32). *Cryptosporidium* and *Giardia* were isolated from the Klip River during both winter and summer months (Fig. 14).

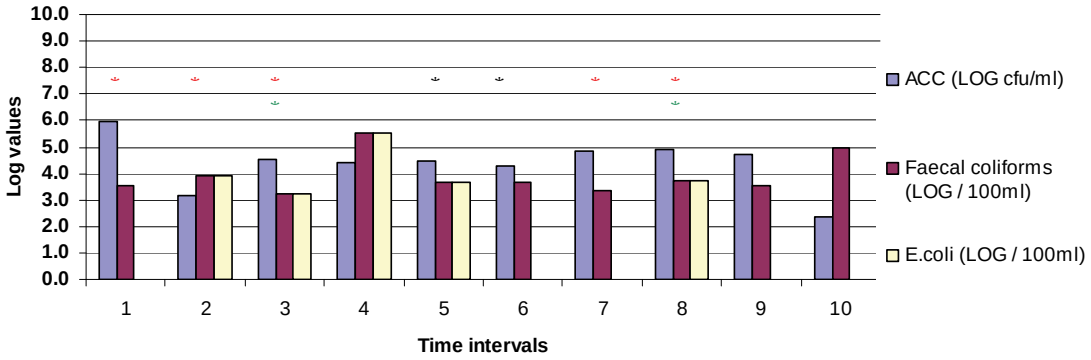
The recovery efficiency for *Giardia* was significantly lower ($p<0.05$) than the recovery efficiency for *Cryptosporidium* with 15.3% recovery for *Cryptosporidium* and 9.2% recovery for *Giardia*. The average recovery efficiency for the 10 positive controls for the isolation of *Cryptosporidium* and *Giardia* was lower than reported in some studies (Robertson & Gjerde, 2001b).

Aerobic colony counts in the Skeerpoort River ranged from 255 to 46 000 cfu.mL^{-1} (Fig. 14). Faecal coliforms in the Skeerpoort River varied greatly between samples and ranged from 7.8 to 100 mL in winter to more than 1 600 000 000 counts.100 mL^{-1} in summer. *E. coli* were isolated from 4 out of the 10 samples and were 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 31). The eae Intimin must have originated from other EHEC not included in the GeneDisc 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 (Fig. 14). *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 (7.8 counts.100 mL^{-1}) as well as high (>1600 000 000 counts.100 mL^{-1}) levels of faecal coliforms (Fig. 14).

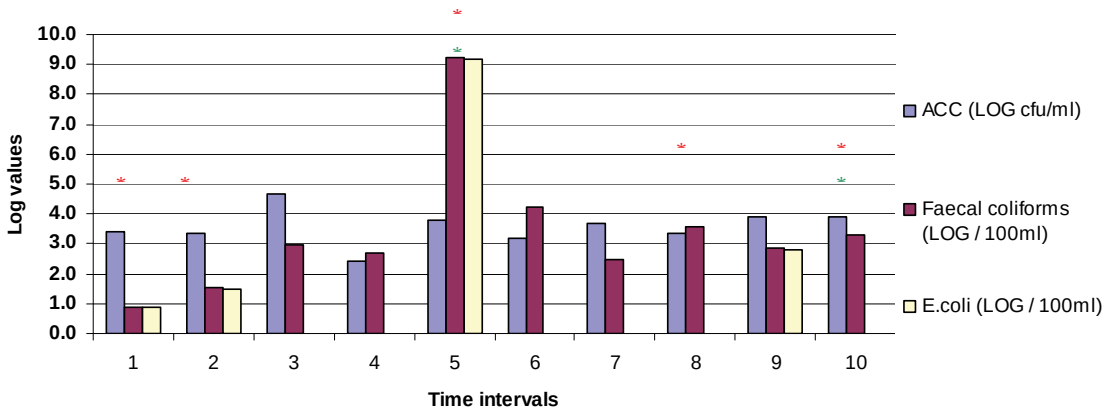
The aerobic colony counts in the Moses River ranged from 250 to 32 350 cfu.mL^{-1} (Fig. 14). Faecal coliforms ranged from 350 to 70 000 counts.100 mL^{-1} and 7 out of the 10 samples had faecal coliform levels above 1000 counts.100 mL^{-1}. An increase in faecal coliforms was observed during the beginning of summer (sampling interval 4-6) which coincides with the first rainfall after a dry winter (Figure 3). *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 (11 000 and 70 000 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 (Tables 25, 26 and 27).

Out of the 10 water samples analysed, 8 tested positive for at least one pathogen and 4 samples were positive for 2 pathogens (Fig. 14). *Salmonella* spp. was found in 6 samples. *Salmonella* Gaminara*, Salmonella* Fulica*, Salmonella* Infantis*, Salmonella* I and *Salmonella* II were isolated from the Moses River (Table 8). *Cryptosporidium* oocysts and *Giardia* cysts were isolated from 3 of the samples (Fig. 14).

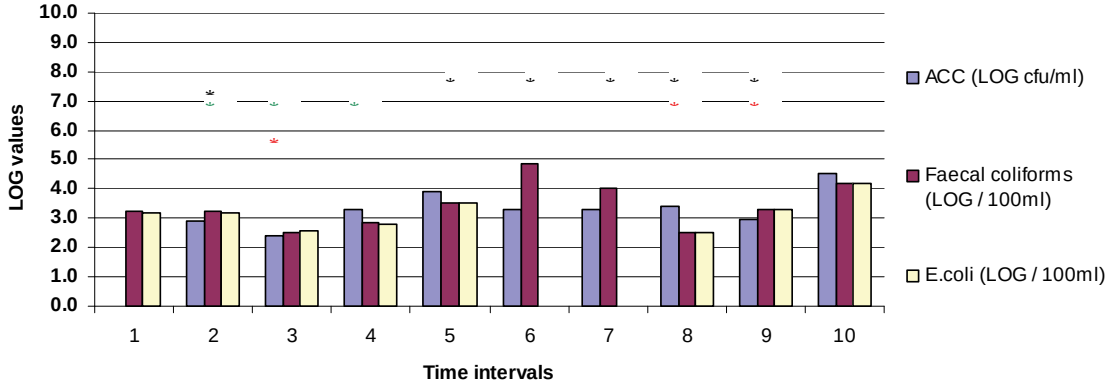
A



B



C



***=** Positive for *Salmonella* spp; ***=** Positive for *Cryptosporidium;* ***=** Positive for *Giardia*

Figure 14. Aerobic colony (ACC), faecal coliforms and *E. Coli* counts and incidence of *Salmonella* spp., *Cryptosporidium* and *Giardia* in the Klip (A), Skeerpoort (B) and Moses Rivers (C) for 10 sampling intervals between June 2009 and April 2010.

The two parasites 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.

3.9.3 Incidence of indicators and protozoa in broccoli and lettuce

The aerobic plate counts for the lettuce head were higher than those of the broccoli but counts on both vegetables were acceptable according to guideline set up by the University of Ghent for vegetable intended for raw consumption (Uyttendaele *et al.*, 2011) (Table 31)**.** Faecal coliform counts were low on both vegetables, *E. Coli* were isolated from the lettuce but not from the broccoli head. None of the samples were positive for *Salmonella*, *Cryptosporidium* or *Giardia*.

Table 29. Indicator parameters and percentage of samples positive for pathogens for the vegetable samples analysed according to vegetable ([#] Standard deviations in brackets).

Produce	No. of samples	ACC (Log cfu.g)	FC (per 100 g)	E. Coli (per 100 g)
Broccoli	3	3.18 (1.02)	44 (74)	0 (0)
Lettuce	3	4.38 (0.98)	37 (14)	22 (22)

3.9.4 Links between Cryptosporidium, Giardia in Skeerpoort River and found on produce

The average temperature of the three Rivers varied 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. No significant differences in temperature between Rivers was observed ($p>0.05$) (Tables 29-33). 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 (Tables 29-33). Aerobic colony counts were on average higher in Klip River than in the Skeerpoort and Moses River. Faecal coliform counts in Skeerpoort River were lower on average than in the Moses and Klip River.

These differences were however not statistically significant ($p>0.05$) (Tables 29-33). Fewer pathogen 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 months 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 ($p>0.05$).

Table 30. Indicator parameters and percentage of water samples (n=30) positive for pathogens collected between June 2009 and May 2010 from the Klip, Moses and Skeerpoort Rivers.

River	Samples	Indicator parameters					% positive		
		Temp (°C)	pH[4]	ACC (cfu.mL^{-1})	FC (MPN. 100 mL^{-1})	*E. coli* (MPN. 100 mL^{-1})	Sal	Crypto	Giar
Klip[1]	10	18.8	7.32	122 667	47 590	36 990	20	50	20
Moses[2]	10	20.6	7.36	5 622	10 740	2 640	60	30	30
Skeerpoort[3]	10	20.0	8.06	8 135	2 670	315	0	50	20
Average	30	19.76 (5.44)[5]	7.58 (0.59)	46 849	20 333	13 315	27	43	23

[1] *Salmonella enteritidis* and *S. adeoye* from the Klip River; [2] *S. gaminara, S. fulica, S. infantis, Salmonella I* and *II* and *E .coli* Shigatoxin 1 from the Moses River;
[3] eae intimin from the Skeerpoort River; [4] Anova performed between Rivers (n=10). Skeerpoort pH was significantly different from Klip and Moses pH (p=0.003);
[5] Standard deviation indicated in brackets

Table 31. Percentage of water sample from the Klip, Moses and Skeerpoort Rivers (n=30) positive for *Salmonella* spp., *Cryptosporidium* and *Giardia* at various levels of faecal coliforms in samples.

FC (MPN.100 mL^{-1})	Samples (%)	% of positive samples			
		Sal	Crypto	Giar	Any pathogen
<100	2 (6.7)[#]	0	100	0	100
100-1 000	7 (23.3)	28	14	28	42
1 000- 10 000	14 (46.7)	21	57	28	78
>10 000	7 (23.3)	42	28	14	57
Total	30 (100)	26	43	23	66

[#] Standard deviations in brackets

Table 32. Percentage of water samples from the Klip, Moses and Skeerpoort Rivers (n=30) positive for *Salmonella* spp., *Cryptosporidium* and *Giardia* at various levels of *E. Coli* in samples.

E. coli (MPN.100 mL^{-1})	Samples (%)	% of positive samples			
		Sal	Crypto	Giar	Any pathogen
<1	12 (40)[#]	25	25	0	50
1-1000	6 (20)	33	50	33	83
1 000- 10 000	9 (30)	22	55	44	77
>10 000	3 (10)	33	66	33	66
Total	30 (100)	26	43	23	66

[#] Standard deviations in brackets

Table 33. Logistic regression analysis of relationship between indicator parameters and presence of *Cryptosporidium* and *Giardia* p-value.

Indicator parameter	p-value	
	Crypto	Giar
Temperature	0.8193	0.7845
pH	0.4865	0.6598
ACC	0.4112	0.8302
FC	0.6174	0.6767
E. coli	0.2141	0.3038

Table 33 shows the percentage of positive water samples for pathogens at various levels of faecal coliforms when looking at the three Rivers together. Two water samples contained less than 100 faecal coliforms.100 mL^{-1}. *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

1000 faecal coliforms.100 mL 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*, *Cryptosporidium* or *Giardia*.

Table 33 shows the percentage of water samples positive for pathogens at various levels of *E. Coli* in water samples when looking at the three Rivers together. 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}.

Predictive relationships between indicators and pathogens were evaluated using binary logistic regression. Results from each River were analysed separately by Rivers or as pooled data set (all Rivers together) to determine if the concentration of any of the indicators were correlated with the presence of *Salmonella* spp. *Cryptosporidium* or *Giardia*. The incidence of *Salmonella* spp., *Cryptosporidium* and *Giardia* did not correlate significantly with the any of the indicator parameters. Aerobic colony counts, faecal coliform counts, *E. Coli* counts, temperature and pH had no significant effect on the presence or absence of the pathogens (Table 33).

3.9.5 Discussions

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). The temperatures recorded did not differ significantly between Rivers and where within the growth range of enteric bacteria (Adams *et al.*, 1989).

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 1000 counts.100 mL^{-1} (WHO, 1989) 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 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 (Moss, 2008). *Salmonella* Gaminara which was isolated from the Moses River is mostly associated with poultry. It has however been the aetiological agent of a foodborne 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. The pH of the Skeerpoort River was more alkaline than the pH of the Moses and Klip River. This could explain the absence of *Salmonella* spp. in the Skeerpoort River as the optimum pH for the survival of S*almonella* is 7.

These results 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 River despite much higher levels of faecal coliforms in the Klip River than in the Skeerpoort River. These suggest that the use of faecal coliforms as indicator of 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, 2001b; 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 and in Norway on the occurrence of *Cryptosporidium* and *Giardia* in raw water (Robertson & Gjerde, 2000; Kay *et al.*, 2008).

The presence of both protozoa in the same sample was observed in 5 out of the 30 samples (17%) analysed. This contrast with the results from Kfir (Kfir *et al.*, 1995) where only 2.9% of surface water sampled in South Africa were positive for both protozoa. This could mean that the incidence of *Cryptosporidium* and *Giardia* in surface water in South Africa is increasing. The risk of contaminating fresh produce with *Cryptosporidium* and *Giardia* through the use of irrigation water is thus increasing as well. Even though, 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.

While lack of seasonality were reported in other studies (Robertson & Gjerde, 2001a)**,** higher incidence of *Cryptosporidium* and *Giardia* in surface water were 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 were also 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 and thus mask the possible effect 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* 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 pathogen independent of the location of the River.

The microbiological quality of the vegetables analysed was good 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 those two protozoa in the water used for their irrigation. The low bacterial count 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 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* were absent of some samples which had high faecal coliform counts and were sometime 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 (Harwood *et al.*, 2005). 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 were 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* suggest 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 good microbiological quality if only faecal indicator 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.

3.9.6 Conclusions

This study demonstrated the widespread presence of *Cryptosporidium* and *Giardia* in surface water used for irrigation fruits and vegetables in three Rivers from different provinces in 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 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. 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 predictor of the presence of *Cryptosporidium* and *Giardia* in water.

3.10 MPUMALANGA – LINKS BETWEEN MICROBES IN WATER AND THOSE ON PRODUCE IN THE LOSKOPDAM IRRIGATION AREA

3.10.1 Specific aim

We seek to determine the effect of source water on the bacterial quality of water in the canal it feeds and also the subsequent contribution to the contamination of fresh vegetables during a 12 month sampling period in Mpumalanga Province. The second aim is to use logistic regression analysis to predict the presence of *Salmonella*, *Listeria* and intestinal *Enterococcus* in irrigation water and on fresh produce.

3.10.2 Physico-chemical properties of water from Loskop-canal, Olifants and Wilge Rivers

The mean turbidity and COD of 36 samples taken Loskop-canal, Olifants and Wilge Rivers were 20 NTU and 55 $mg.L^{-1}$ (Table 34). The mean turbidity level was higher than the international turbidity (1 NTU) standard for water; also mean COD was also higher than the international standard which is 10 $mg.L^{-1}$ (DWAF, 1996b). However, sampling interval and sites had significant effect on the turbidity and COD (Table 34).

3.10.3 Incidence of ACC, ASF and AnSF in the Loskop-canal, Olifants and Wilge Rivers

There was no significant difference on the mean values of aerobic colony count in Loskop canal and the two Rivers; Olifants and Wilge Rivers during the 12 sampling intervals (Table 35). However, sampling interval and sites had significant effect (Table 34). There were some intervals in which there were significant differences in the ACC results. The mean ACC of the 36 samples altogether was 3.02 log $cfu.mL^{-1}$ (Table 34). Unlike the mean values of the aerobic colony counts of water samples, there were significant differences on the mean values of aerobic spore formers and anaerobic spore formers recovered from Loskop canal and the two Rivers; Olifants and Wilge Rivers during the 12 sampling intervals (Table 35). The mean ASF and AnSF of the 36 samples altogether was 1.62 and 1.42 log 10 $cfu.mL^{-1}$ respectively (Table 34).

3.10.4 Prevalence of SA, *E. coli,* IE, Sal and LM in water from surface waters during 12 sampling intervals

The mean % of samples positive for *L. monocytogenes* from the total 36 samples was 53%. While it was 81% for Intestinal *Enterococcus* and 42% for *Salmonella* sp (Table 34). 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 Loskop canal were positive for *S. aureus* (Fig. 15). However the mean *S. aureus* counts of water from the three surface water sampling sites was very low <1 log $cfu.mL^{-1}$ (Table 35). *E. coli* (confirmed with API 20E) was recovered from the two Rivers and Loskop canal during every sampling interval (Fig. 15). 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 and Olifants Rivers, the water samples met the standard during 25% and 30% of the 12 sampling intervals respectively. Intestinal *Enterococcus* was present in all the water samples collected from the Wilge River while incidence was lower in the Olifants River (67%) and Loskop canal (75%) (Fig. 15). Incidence of *Salmonella* (50%) was highest in Loskop canal than in Wilge River and Olifants (33% and 42% respectively), however the incidence of *L. monocytogenes* (58%) in Wilge River was higher than the 50% incidence observed in both Loskop canal and Olifants during the 12 sampling intervals (Fig 15).

Table 34. Indicators and proportion of samples positive for various pathogens for 36 surface water samples collected between November 2007 and November 2008 in Mpumalanga.

Sampling time	Samples /sites	pH	Temp (^oC)	Turbidity[a]	Rain-fall	COD[a] ($mg.L^{-1}$)	ACC[a]	ASF[a]	AnSF[a]	% positive for pathogens				
										SA[b]	*E. coli*	LM [b]	IE[a]	Sal[b]
Nov 2007	3	8.40	16.50	9.58	0	59.90	3.98	1.45	1.36	0	100	0	100	0
Dec 2007	3	8.30	16.30	39.05	0	63.64	3.18	2.09	2.78	0	100	33	67	100
Jan 2008	3	7.55	18.80	16.08	0	49.71	2.07	2.49	1.88	0.72	100	100	100	100
Feb 2008	3	7.43	24.00	8.75	0	102.66	3.51	2.02	1.59	1.42	100	67	100	67
March 2008	3	7.23	17.60	31.6	0.5	66.19	3.47	2.25	2.18	0.59	100	33	33	67
April 2008	3	7.43	21.60	66.85	0	78.69	3.87	2.14	2.07	0.76	100	0	100	0
May 2008	3	7.22	16.4	26.59	0	93.59	3.68	1.54	2.23	0	100	0	100	0
July 2008	3	7.37	10.50	5.00	0	42.80	2.44	0.51	0.91	0.19	100	100	67	0
Aug. 2008	3	7.03	13.90	6.35	0	15.64	1.98	1.17	0.44	0.13	100	100	67	33
Sept. 2008	3	7.26	19.90	6.56	0	38.50	2.59	1.23	0.52	0.38	100	100	100	0
Oct. 2008	3	7.28	23.20	5.78	0	20.50	2.64	0.85	0.75	0.07	100	100	100	33
Nov 2008	3	7.27	25.30	17.78	2	34.11	2.86	1.72	0.33	0.26	100	0	33	100
Total	36	7.48	18.67	20.00	0.21	55.49	3.02	1.62	1.42	0.40	100	53	81	42

[a]P value for the effect of sampling interval and sampling site is <0.05 (ANOVA); [b]P value for the effect of sampling interval is <0.05 and sampling site is >0.05 as determined by ANOVA

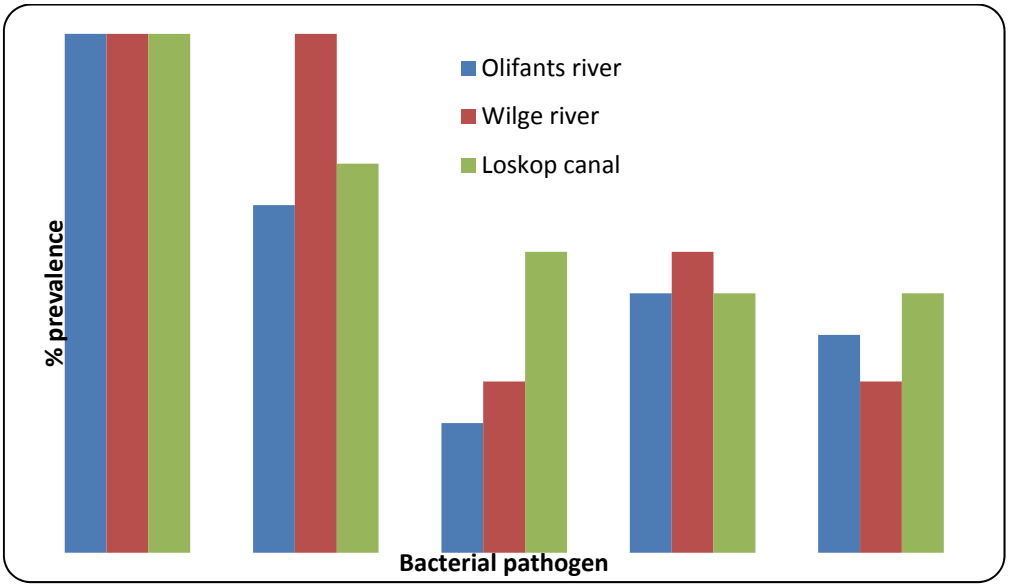


Figure 15. Prevalence of bacterial pathogens in the three water sources during twelve sampling intervals.

Table 35. Mean counts (Log cfu.mL^{-1}) of ACC, ASF, AnSF and *S. aureus* recovered from Loskop canal, Olifants and Wilge Rivers during the 12 intervals.

Sampling site	ACC	ASF	AnSF	SA
Loskop canal	2.96^{a}	1.33^{a}	1.33^{ab}	0.46^{a}
Olifants River	3.04^{a}	1.59^{ab}	1.13^{a}	0.32^{a}
Wilge River	3.06^{a}	1.91^{b}	1.74^{b}	0.31^{a}

Means with the same letter are not significantly different.

3.10.5 Incidence of ACC, ASF and AnSF on broccoli and cauliflower

The average ACC on cauliflower was 3.8 log cfu.g^{-1} while it was 4.1 log cfu.g^{-1} on broccoli. Similarly, the average ASF and AnSF were also higher on broccoli. ASF on broccoli and cauliflower were 2 log cfu.g^{-1} and 1.5 log cfu.g^{-1} respectively while AnSF on broccoli and cauliflower were 1.6 and 1.4 log cfu.g-1^{-1} respectively. There was no significant difference between the mean aerobic bacteria count of broccoli and cauliflower from the three farms whereas the mean anaerobic spore counts and aerobic spore counts differed significantly (P≤0.05) (Table 36). However, there was significant difference in aerobic colony count, aerobic spore counts and anaerobic spore counts in the two vegetables from the individual farm (Table 36).The average ACC in the three water samples from the Loskop canal, Wilge and Olifants Rivers was lower than that on the two vegetables however the average ASF and AnSF were almost the same i.e., ≤0.2. Average ACC, ASF and AnSF in the water samples were 3.0, 1.6, 1.4 log cfu.mL^{-1} (Table 34) while they were 3.9, 1.8 and 1.5 log cfu.g^{-1} respectively on vegetables (Fig. 16).

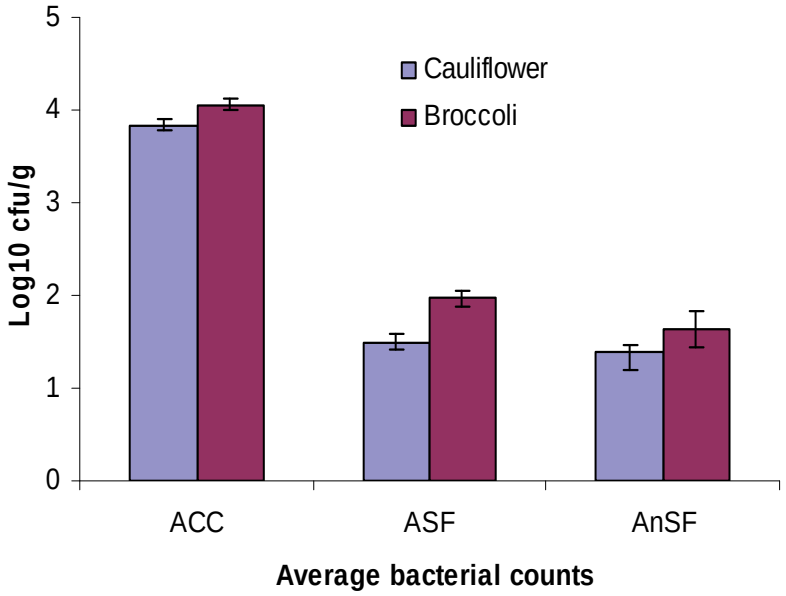


Figure 16. The average ACC, ASF, and AnSF on broccoli and cauliflower during three sampling intervals.

Table 36. Analysis of variance for ACC, ASF, and AnSF of broccoli, cauliflower and irrigation water from the Loskop- canal at 3 intervals for a period of three months.

Effect	Degrees of freedom	ACC	ASF	AnSF
Sampling interval	2	0.266	0.001	0.002
Source	2	0.001	0.003	0.024
Sampling interval and source	4	0.001	0.001	0.101

Statistical significance of main factor and interaction: $p \leq 0.05$

3.10.6 Incidence of Sa, *E. coli,* IE, Sal *and* LM on cauliflower and broccoli

Incidence of *S. aureus* on broccoli (67%) was higher than on the cauliflower (33%). However, the average *S. aureus* counts on the vegetables during the three month sampling period was very low <1 log cfu.mL^{-1} (Fig. 17). *E. coli* was recovered from Loskop canal, cauliflower and broccoli during the three sampling intervals (Fig. 17). Incidence of Intestinal *Enterococcus* on broccoli was higher than that on cauliflower. The incidence was 44 and 33% respectively, it was however 67% in Loskop canal. Also, the incidence of *Salmonella* (33%) in Loskop canal was higher than the 11% incidence observed on broccoli and cauliflower (Fig. 17). Only broccoli was positive for *L. monocytogenes* during the three sampling intervals however *L. monocytogenes* were recovered from Loskop canal at other sampling intervals when vegetables were not examined. Also, with an exception of *L. monocytogenes* which was not recovered from cauliflower, all the bacterial pathogens isolated from the three water sources were also isolated from the two vegetables.

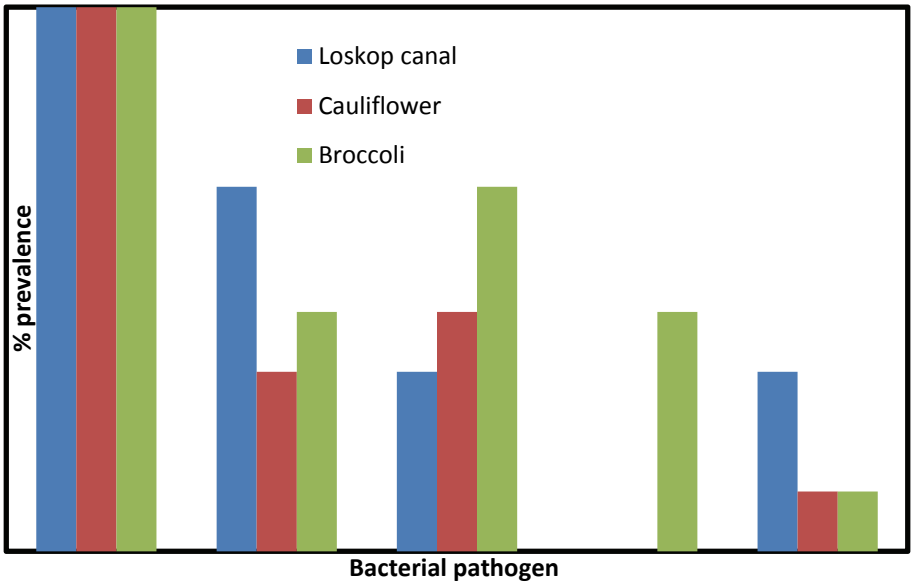


Figure 17. Prevalence of pathogens in the Loskop canal and two vegetables during 3 sampling intervals.

3.10.7 Predictive relationships between predictors

A pooled data set from Loskop canal, Olifants and Wilge Rivers were analysed to determine if the concentrations of any of the indicators, total coliforms, faecal coliforms, *S. aureus*, aerobic spore formers, anaerobic spore formers and aerobic colony counts, were correlated with each other and with physico-chemical parameters (turbidity and chemical oxygen demand). High significant correlations were observed between FC coliforms and TC ($r = 0.999$; $p - value < 0.0001$), ASF and AnSF ($r = 0.535$; $p - value < 0.0001$), Sa and ASF ($r = 0.498$; $p - value < 0.0001$), ACC and AnSF ($r = 0.354$; $p - value = 0.0002$), ACC and Sa ($r = 0.345$; $p - value = 0.0003$); and a significant correlation was observed between AnSF and Sa ($r = 0.203$; $p - value = 0.0354$). Except between turbidity and Sa, COD and TC, COD and FC; significant correlations were observed between the concentrations of any of the indicators with physico-chemical parameters. Binary logistic regression was used to test the hypothesis that FC, location, COD and turbidity were predictive of the presence of LM, Sal and IE in irrigation water. Binary logistic regression was also used to test the hypothesis that ACC, ASF, AnSF, Sa, FC and TC were predictive of the presence of LM, Sal and IE on vegetables.

3.10.8 Prediction of LM, Sal and IE in water samples from Loskop canal, Wilge and Olifants Rivers

Results of logistic regression indicated that only one predictor, COD, was statistically reliable (p≤0.05) to predict the presence *L. monocytogenes.* Turbidity was found to be statistically significant (p≤0.05) to predict the presence of Intestinal *Enterococcus* while faecal coliforms and coliforms however were found to be significant (p≤0.05) to predict the presence of *Salmonella* in the water samples from three sources. The estimates of regression coefficients of the predictors $\hat{\beta}$, Wald statistic and p-values are presented in Table 37.

Table 37. Prediction of LM, IE and Sal in irrigation water with Logistic regression analysis.

Predictors	$\hat{\beta}$	**Wald**	**p-value**
LMFaecall			
Coliform	-0.0014	0.5785	0.4469
Turbidity	0.0001	0.5194	0.4711
COD	-0.0199	0.6958	0.4042
	-0.0399	9.4825	0.0021
IE			
Faecal	0.0013	0.4224	0.5157
Coliform	-0.0001	0.3564	0.5505
Turbidity	-0.0544	5.7643	0.0164
COD	0.0264	2.4581	0.1169
Salmonella			
Faecal	0.0048	3.8008	0.0500
Coliform	-0.0005	3.8038	0.0500
Turbidity	0.0105	0.3399	0.5599
COD	0.0123	1.3747	0.2410

A p-value of ≤0.05 was considered statistically significant

3.10.9 Prediction of LM, Sal, and IE on vegetables

The result of logistic regression analysis shows that two predictors, ACC and *S. aureus* were statistically dependable (p≤0.05) to predict the presence *L. monocytogenes* on vegetables. Also, from the result of the logistic regression analysis, ACC and AnSF were observed to be significant (p≤0.05) to predict the presence of Intestinal *Enterococcus* and *Salmonella* respectively. The estimates of regression coefficients of the predictors $\hat{\beta}$, Wald statistic and p-values are also shown in Table 38.

3.10.10 Discussion

The temperature and pH values of the Loskop canal and the two Rivers which were conducive for bacterial growth may have influenced the survival of indigenous and bacterial pathogens in the water sources. According to (Park *et al.*, 2006), 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, 1996a), 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 result of Fatoki (Fatoki *et al.*, 2003). They also found high turbidity levels in surface water indicated that soil erosion and run-off could be source of high turbidity in the water system. The soil erosion and run off could have been caused by the informal settlement around the two Rivers. The COD results for all the three water samples from Loskopdam, 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 which may have originated from the informal settlements and mines around the region where Rivers are located.

Table 38. Prediction of LM, IE and *Salmonella* in vegetables with Logistic regression analysis.

Predictors	$\hat{\beta}$	**Wald**	**p-value**
LM			
ACC	-1.8486	17.9433	0.0001
ASF	-0.2353	0.3620	0.5474
AnSF	-0.0767	0.0586	0.8088
S. aureus	0.9414	6.9747	0.0083
Faecal	-0.0004	0.0855	0.7700
Coliform	0.0001	0.0830	0.7733
IE			
ACC	-0.7971	6.2123	0.0127
ASF	0.0152	0.0016	0.9682
AnSF	0.7324	5.2992	0.0213
S. aureus	-0.1662	0.2770	0.5986
Faecal	-0.0020	3.1176	0.0775
Coliform	0.0002	3.3093	0.0689
Salmonella			
ACC	-1.2487	9.7924	0.0018
ASF	0.1181	0.0932	0.7602
AnSF	0.6926	4.2584	0.0391
S. aureus	0.5469	2.4546	0.1172
Faecal	0.0007	0.3020	0.5827
Coliform	-0.0001	0.2633	0.6079

A p-value of ≤0.05 was considered statistically significant

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

The recovery of aerobic spore formers from the three water samples is similar to the work of Fournnelle (Fournnelle, 1967) who recovered them from Alaska water at the same low level. The level of anaerobic spore formers observed in our water samples was however lower than has been reported by Molongoski (Molongoski & Klug, 1976). They recovered up to 6 log of anaerobic spore formers from fresh water lakes. Although low aerobic spore formers level was observed in the water samples, it may not be suitable for irrigation of fresh produce because of the possibility of microbial growth and cell division after attachment and infiltration on the vegetables.

Recovery of *S. aureus* from water samples is low. *S. aureus* was not expected to be recovered from Loskop-canal, Wilge River and Olifants River because its natural habitat is nasal cavity (Jay, 1997). The presence of *S. aureus* in the two Rivers and Loskop-canal also show 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 coliform 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 coliform are indicators of faecal pollution (Garcia-Armisen & Servais, 2007).

Contamination of water sources with other bacterial pathogens i.e. *L. monocytogenes* and *Salmonella* show that the two Rivers and canal are of poor microbiological quality and may be 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 (Garcia-Armisen & Servais, 2007; Lyautey *et al.*, 2007). 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). Other safety concern with these pathogens is that they can form biofilms on the produce thereby making sanitizers' ineffective (Fonseca, 2006).

Although the Loskop canal may be a potential source of broccoli and cauliflower of food pathogens, the incidence of the pathogens on the two vegetables did not correspond. Lower incidence of *S. aureus*, *Salmonella*, Intestinal Enterococci and absence of *Listeria monocytogenes* on cauliflower compared to broccoli show the possibility of differences in surface characteristics of the two produce affecting pathogen attachment and survival. Broccoli among some other vegetables has been reported to pose a higher risk of being associated with listeriosis because of enhanced *L. monocytogenes* attachment (FDA/CFSAN, 2008).

The result of the prediction of LM in irrigation water signifies that there is direct relationship between *Listeria monocytogenes* and COD in irrigation water. Higher COD results in water may result in high concentration of LM in irrigation water. The result also signifies that there is direct relationship between Intestinal Enterococcus and turbidity. The reason why COD could not predict the presence of IE or turbidity predicting LM is not well understood yet.

Faecal coliform and coliforms have long been known as indicator of enteric bacteria in water generally. The logistic regression result proved that faecal coliform and coliform that can be used to predict the presence of *Salmonella* in water and that there is relationship between faecal coliform and *Salmonella.* This is similar to the observation of Polo (Polo *et al.*, 1999) who showed that there is a direct relationship between the presence of *Salmonella* and indicators of faecal pollution i.e., coliforms and faecal coliforms in Rivers, fresh water reservoirs and sea water. Ferguson (Ferguson *et al.*, 1996) also observed that the higher the concentration of faecal coliform, the higher the recovery of *Salmonella* spp in aquatic habitat.

The reason why faecal coliforms and coliforms were not significantly associated with LM and IE may be because they are not usually found in human faeces unlike *Salmonella.* According to Gildreich (Gildreich & Kenner, 1969), human faeces contain higher faecal coliforms counts, while animal faeces contain higher levels of faecal enterococci. Wild birds and animals have also been shown to be the main source of contamination with *L. monocytogenes* (Weiss & Seeliger, 1975).

The study clearly indicates the potential effect of contaminated surface water (i.e. River water) on irrigation water sources and pre-harvest vegetables. Also, logistic regression analysis may therefore be used as a tool for predictive microbiology model which has an immediate practical application to predict microbial produce safety and quality, and provide quantitative understanding of the microbial ecology of irrigation water and produce (Ross, 2006).

3.10.11 Conclusion

The water used for irrigation in our study is a likely source of contamination of broccoli and cauliflower with bacterial pathogens and it 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 (GAP) during production of fresh vegetables. Also, faecal coliforms and coliforms can be used to indicate high probability of *Salmonella* presence in water and they may be used as risk parameters. There is relationship between physiochemical properties of water i.e., COD and turbidity and certain bacterial pathogen i.e., *L. monocytogenes* and Intestinal *Enterococcus*. Low aerobic colony counts however can be used to indicate high probability of *L. monocytogenes*, Intestinal *Enterococcus* and *Salmonella* on vegetables.

3.11 VIRUSES – VIRAL DETECTION IN IRRIGATION WATER, FRESH PRODUCE AND PRODUCE COLLECTED AT-RETAIL

3.11.1 Specific aim

To determine the extent of virus contamination in irrigation water, samples will be collected pre-harvest or at-harvest, with or without paired irrigation water samples, in Limpopo and the Western Cape and analysed for NoVs and HAV. In addition, the extent of contamination found on fruit and vegetables post-harvest will be assessed using fresh produce purchased from vendors and commercial outlets from the Tshwane Metropolitan Area, Gauteng, Limpopo and the Western Cape and analysed for NoVs and HAV.

3.11.2 Pre-harvest fresh produce samples

From April 2008 to June 2011, 52 fresh produce samples (cabbage, tomato, spinach, lettuce and beans) were collected from commercial and subsistence farms (Table 39). Among fresh produce samples collected pre-harvest or at harvest, 0/18 samples with smooth surfaces and 2/34 (6%) samples with rough or convoluted surfaces were positive for the presence of one enteric virus.

Table 39. Summary of the number and type of fresh produce samples collected according to their source and province of origin.

	Cabbage ***(n = 41)***	**Spinach** ***(n = 4)***	**Lettuce** ***(n = 5)***	**Rocket** ***(n = 1)***	**Onion** ***(n = 2)***	**Tomato** ***(n = 32)***	**Beans** ***(n = 2)***
Gauteng							
Commercial Farm	0	0	0	0	0	0	0
Subsistence Farm	0	0	0	0	0	0	0
Street Vendor	2	2	1	0	0	5	0
Commercial outlet	5	1	1	1	1	5	0
Limpopo							
Commercial Farm	1	1	0	0	0	0	0
Subsistence Farm	26	0	0	0	0	18	0
Street Vendor	3	0	0	0	1	4	0
Commercial outlet	0	0	1	0	0	0	0
Western Cape							
Commercial Farm	3	0	1	0	0	0	2
Subsistence Farm	0	0	0	0	0	0	0
Street Vendor	0	0	0	0	0	0	0
Commercial outlet	0	0	0	0	0	0	0
Farm stall	1	0	1	0	0	0	0

Limpopo: No viruses were detected on fresh produce with smooth or rough convoluted surfaces collected in the field from either Farmer 2 or Site 1 (Phadzima community farm). Norovirus GII (untypable) was detected on spinach (fresh produce with rough surface) from Farmer 1's field (Tables 40 and 41).

Table 40. Summary of results of viral analysis of fresh produce samples from Limpopo.

Sample site, name, location	**Fresh Produce**	**Sample date**	**Real-time RT-PCR analysis**			
			NoV GI	**NoV GII**	**HAV**	**Mengo virus**
Farmer 1	Spinach (a)	2008.09.23	Neg	Neg	Neg	NT
	Spinach (b)		Neg	Neg	Neg	NT
	Spinach (c)		Neg	Pos	Neg	NT
Farmer 2	Cabbage (a)	2008.09.23	Neg	Neg	Neg	NT
	Cabbage (b)		Neg	Neg	Neg	NT
Site 1	Tomato	2008.10.27	Neg	Neg	Neg	NT
	Tomato	2008.11.17	Neg	Neg	Neg	NT
	Tomato	2008.12.10	Neg	Neg	Neg	NT
	Tomato	2009.01.26	Neg	Neg	Neg	NT
	Tomato	2009.02.16	Neg	Neg	Neg	NT
	Tomato	2009.03.16	Neg	Neg	Neg	NT
	Tomato	2009.04.27	Neg	Neg	Neg	NT
	Tomato	2009.05.18	Neg	Neg	Neg	NT
	Tomato	2009.06.24	Neg	Neg	Neg	**Pos**
	Tomato	2009.07.20	Neg	Neg	Neg	Neg
	Tomato	2009.08.17	Neg	Neg	Neg	Neg
	Tomato	2009.10.13	Neg	Neg	Neg	**Pos**
	Tomato	2009.11.02	Neg	Neg	Neg	NT
	Tomato	2010-12-06	Neg	Neg	Neg	**Pos**
	Tomato	2011-01-03	Neg	Neg	Neg	**Pos**
	Tomato	2011-02-14	Neg	Neg	Neg	**Pos**
	Tomato	2011-05-03	Neg	Neg	Neg	**Pos**
	Tomato	2011-06-06	Neg	Neg	Neg	**Pos**

NT – not tested

Table 41 *continued.* Summary of results of viral analysis of fresh produce samples from Limpopo.

Sample site, name, location	Fresh Produce	Sample date	Real-time RT-PCR analysis			
			NoV GI	NoV GII	HAV	Mengo virus
Site 1:	Cabbage C*	2008.10.27	Neg	Neg	Neg	NT
	Cabbage C*	2008.11.17	Neg	Neg	Neg	NT
	Cabbage C*	2008.12.10	Neg	Neg	Neg	NT
	Cabbage C*	2009.01.26	Neg	Neg	Neg	NT
	Cabbage C*	2009.02.16	Neg	Neg	Neg	NT
	Cabbage C*	2009.03.16	Neg	Neg	Neg	NT
	Cabbage C*	2009.04.27	Neg	Neg	Neg	NT
	Cabbage C*	2009.05.18	Neg	Neg	Neg	NT
	Cabbage C*	2009.06.24	Neg	Neg	Neg	**Pos**
	Cabbage C*	2009.07.20	Neg	Neg	Neg	**Pos**
	Cabbage C*	2009.08.17	Neg	Neg	Neg	**Pos**
	Cabbage C*	2009.09.07	Neg	Neg	Neg	**Pos**
	Cabbage C*	2009.10.13	Neg	Neg	Neg	**Pos**
	Cabbage C*	2009.11.02	Neg	Neg	Neg	NT
	Cabbage C*	2011-01-03	Neg	Neg	Neg	**Pos**
	Cabbage T†	2008.10.27	Neg	Neg	Neg	NT
	Cabbage T†	2008.11.17	Neg	Neg	Neg	NT
	Cabbage T†	2008.12.10	Neg	Neg	Neg	NT
	Cabbage T†	2009.01.26	Neg	Neg	Neg	NT
	Cabbage T†	2009.02.16	Neg	Neg	Neg	NT
	Cabbage T†	2009.03.16	Neg	Neg	Neg	NT
	Cabbage T†	2009.04.27	Neg	Neg	Neg	NT
	Cabbage T†	2009.11.02	Neg	Neg	Neg	NT
	Cabbage	2010-12-06	Neg	Neg	Neg	**Pos**
	Cabbage	2011-01-03	Neg	Neg	Neg	**Pos**
	Cabbage	2011-08-08	Neg	Neg	Neg	**Pos**

C*: Variety Conquest; T†: Variety Tennessee; NT: not tested

Western Cape: No viruses were detected on cabbage (rough convoluted surface) collected in the field and which had been irrigated with water from the Mosselbank River (Site 2), or from beans irrigated with tap water drawn from a dam filled from the Plankenburg River (Table 42). Norovirus GII.1 was detected on a cabbage sample, at harvest, which had been irrigated with water from the Berg River (Site 17: R-farm).

Table 42. Results of viral analysis of fresh produce samples from Western Cape (NT = not tested.

Sample site, name, location	Fresh Produce	Sample date	Real-time RT-PCR analysis			
			NoV GI	NoV GII	HAV	Mengo virus
Site 3b/B-K) Mosselbank	Cabbage	2008.09.08	Neg	Neg	Neg	NT
Site 3b/B-K) Mosselbank	Cabbage	2008.09.08	Neg	Neg	Neg	NT
Twkloof /Berg 2	Cabbage	2008-08	Neg	**Pos:GII.1**	Neg	**Pos**
Twkloof/Berg 2	Lettuce	2008-08	Neg	Neg	Neg	NT
Site 11: R-tap	Beans		Neg	Neg	Neg	NT
Site 11: R-tap	Beans		Neg	Neg	Neg	NT

3.11.3 Viral detection in irrigation water samples

Eleven irrigation water samples were drawn at the same time as the fresh produce samples were collected.

Limpopo: No viruses were detected in the River water sources (refer Volume II). 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).

Western Cape: Norovirus GII genotype 2 (NoV GII.2) and HAV were detected in the Mosselbank River (Site 1) in September 2008, while no viruses were detected in the irrigation water (Site 2). No viruses were detected in the tap water from Site 10a/11 which was used to irrigate beans (Table 43).

Table 43. 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	Mengo Virus
Site 1: River Water	2008.05.05	Neg	Neg	Neg	NT
(B-R)	2008.06.17	Neg	**Pos: GII.4**	Neg	Neg
(Mosselbank River)	2008.09.08	Neg	**Pos: GII.2**	**Pos**	**Pos**
Site 11: Irrigation tap water	2008.05.19	Neg	Neg	Neg	Neg
(R-tap)	2008.12.?	Neg	Neg	Neg	**Pos**

3.11.4 Viral detection from linked irrigation water and fresh produce

Limpopo: Irrespective of the farms from which they were collected, there was no correlation between the occurrence of viruses in the irrigation water samples and in the linked fresh produce samples collected in the field or at harvest (Table 44).

Table 44. Summary of results of viral analysis of linked irrigation water and fresh produce samples from Limpopo.

Sample site, name, location	Samples	Sample date	Real-time RT-PCR analysis			
			NoV GI	**NoV GII**	**HAV**	**Mengo virus**
Set 1: Farmer 1	River Water	2008.09.23	Neg	Neg	Neg	NT
	Spinach (a)		Neg	Neg	Neg	**Pos**
	Spinach (b)		Neg	Neg	Neg	**Pos**
	Spinach (c)		Neg	**Pos**	Neg	**Pos**
Set 2: Farmer 2	River Water	2008.09.23	Neg	Neg	Neg	NT
	Cabbage (a)		Neg	Neg	Neg	NT
	Cabbage (b)		Neg	Neg	Neg	**Pos**
	Cabbage (c)		Neg	Neg	Neg	**Pos**
Set 3: Site 1	Canal water	2008.10.27	Neg	**Pos**	Neg	NT
	Tomato		Neg	Neg	Neg	NT
	Cabbage C*		Neg	Neg	Neg	NT
	Cabbage T†		Neg	Neg	Neg	NT
Set 4: Site 1	Canal water	2009.06.24	Neg	Neg	**Pos**	NT
	Tomato		Neg	Neg	Neg	NT
	Cabbage C*		Neg	Neg	Neg	NT
Set 5: Site 1	Canal water	2009.07.20	Neg	Neg	Neg	NT
	Tomato		Neg	Neg	Neg	NT
	Cabbage C*		Neg	Neg	Neg	NT
Set 6: Site 1	Canal water	2009.08.17	Neg	Neg	Neg	NT
	Tomato		Neg	Neg	Neg	NT
	Cabbage C*		Neg	Neg	Neg	NT
Set 7: Site 1	Canal water	2009.09.07	Neg	Neg	Neg	NT
	Cabbage C*		Neg	Neg	Neg	NT

C*: Variety Conquest; T†: Variety Tennessee; Canal water: Irrigation canal water; NT: not tested

Western Cape: No correlation between the occurrence of viruses in the irrigation water samples and in the linked fresh produce samples collected in the field or at harvest noted (Table 45).

Table 45. Results of viral analysis of linked irrigation water and fresh produce samples from Western Cape.

Sample site, name, location	Fresh produce	Sample date	Real-time RT-PCR analysis			
			NoV GI	NoV GII	HAV	Mengo virus
Site 1: (B-K) Mosselbank	River water	2008.09.08	Neg	**Pos:GII.2**	**Pos**	NT
	Cabbage		Neg	Neg	Neg	NT
	Cabbage		Neg	Neg	Neg	NT
Site 10: R-Tap	Tap water		Neg	Neg	Neg	NT
	Beans		Neg	Neg	Neg	**Pos**
	Beans		Neg	Neg	Neg	**Pos**

NT: not tested

3.11.5 Viral detection from fresh produce collected at-retail

Among fresh produce samples collected at retail, 2/16 (6%) samples with smooth surfaces and 5/20 (25%) samples with rough or convoluted surfaces tested positive for one or more enteric viruses, respectively.

Gauteng: Enteric viruses were detected on fresh produce from both commercial outlets and the informal sector. However, from Tables 46, 47 and 48 it was evident that fresh produce available from street vendors was more likely to be contaminated with enteric viruses than fresh produce from the formal commercial sector. Multiple enteric viruses were detected on the fresh produce from street vendors (street vendors 7 and 8) from Marabastad, Pretoria, Tshwane Metropolitan Area. A lettuce sample from street vendor 7 yielded untypable NoVs and HAV on two of the three items which comprised a sample. No viruses were detected on the third item of the sample. The tomato sample from street vendor 8 yielded NoV GII.9 and HAV IB on one of the three items which comprised the single sample.

Table 46. Summary of results of viral analysis of fresh produce samples from Gauteng at-retail.

Sample site, name, location	Fresh produce	Sample date	Real-time RT-PCR analysis			
			NoV GI	NoV GII	HAV	Mengo virus
Greengrocer (Housewives' market)	Tomato (a)	2009.07.22	Neg	Neg	Neg	NT
	Tomato (b)		Neg	Neg	Neg	NT
	Tomato (c)		Neg	Neg	Neg	NT
	Cabbage (a)	2009.07.22	Neg	Neg	Neg	NT
	Cabbage (b)		Neg	Neg	Neg	NT
	Cabbage (c)		Neg	Neg	Neg	NT
	Spinach (a)	2009.07.22	Neg	Neg	Neg	NT
	Spinach (b)		Neg	Neg	Neg	NT
	Spinach (c)		Neg	Neg	Neg	NT
	Tomato (a)	2009.08.15	Neg	Neg	Neg	NT
	Tomato (b)		Neg	Neg	Neg	NT

Sample site, name, location	Fresh produce	Sample date	Real-time RT-PCR analysis			
			NoV GI	NoV GII	HAV	Mengo virus
	Tomato (c)		Neg	Neg	Neg	NT
	Cabbage (a)	2009.08.15	Neg	Neg	**Pos**	NT
	Cabbage (b)		Neg	Neg	Neg	NT
	Cabbage (c)		Neg	Neg	Neg	NT
	Spinach (a)		Neg	Neg	Neg	NT
	Spinach (b)	2009.08.15	Neg	Neg	Neg	NT
	Spinach (c)		Neg	Neg	Neg	NT
Supermarket 1 (Upmarket food store)	Rocket (a)		Neg	**Pos**	Neg	NT
	Rocket (b)	2009.08.15	Neg	Neg	Neg	NT
	Rocket (c)		Neg	Neg	Neg	NT
Supermarket 2	Tomato (a)		Neg	Neg	Neg	**Pos**
	Tomato (b)	2009.09.19	Neg	Neg	Neg	**Pos**
	Tomato (c)		Neg	Neg	Neg	**Pos**
Supermarket 3	Tomato (a)		Neg	Neg	Neg	NT
	Tomato (b)	2009.09.19	Neg	Neg	Neg	NT
	Tomato (c)		Neg	Neg	Neg	NT
Farmer's market (Boeremark)	Tomato (a)	2009.07.	Neg	Neg	Neg	NT
	Tomato (b)		Neg	Neg	Neg	NT
	Tomato (c)		Neg	Neg	Neg	NT
	Onion (a)	2009.07.	Neg	Neg	Neg	NT
	Onion (b)		Neg	Neg	Neg	NT
	Onion (c)		Neg	Neg	Neg	NT
	Lettuce (a)	2009.07.	Neg	Neg	Neg	NT
	Lettuce (b)		Neg	Neg	Neg	NT
	Lettuce (c)		Neg	Neg	Neg	NT

NT: not tested

Table 47 (*continued).* Summary of results of viral analysis of fresh produce samples from Gauteng at retail.

Sample site, name, location	Fresh produce	Sample date	Real-time RT-PCR analysis			
			NoV GI	**NoV GII**	**HAV**	**Mengo virus**
Farmer's market (Boeremark)	Cabbage (a)	2009.10.10	Neg	Neg	Neg	**Pos**
	Cabbage (b)		Neg	Neg	Neg	**Pos**
	Cabbage (c)		Neg	Neg	Neg	**Pos**
	Cabbage (a)	2009.10.10	Neg	Neg	Neg	**Pos**
	Cabbage (b)		Neg	Neg	Neg	**Pos**
	Cabbage (c)		Neg	Neg	Neg	**Pos**
	Cabbage (a)		Neg	Neg	Neg	**Pos**
	Cabbage (b)	2009.10.10	Neg	Neg	Neg	**Pos**
	Cabbage (c)		Neg	Neg	Neg	**Pos**
Street Vendor 1	Tomato (a)		Neg	Neg	Neg	**Pos**
	Tomato (b)	2009.09.19	Neg	Neg	Neg	**Pos**
	Tomato (c)		Neg	Neg	Neg	**Pos**
Street Vendor 2 (Arcadia)	Tomato (a)		Neg	Neg	Neg	**Pos**
	Tomato (b)	2009.09.26	Neg	Neg	Neg	**Pos**
	Tomato (c)		Neg	Neg	Neg	**Pos**
Street Vendor 3 (Sunnyside)	Tomato (a)		Neg	Neg	Neg	**Pos**
	Tomato (b)	2009.09.26	Neg	Neg	Neg	**Pos**
	Tomato (c)		Neg	Neg	Neg	**Pos**
Street Vendor 4 (Central)	Tomato (a)		Neg	Neg	Neg	**Pos**
	Tomato (b)	2009.09.26	Neg	Neg	Neg	**Pos**
	Tomato (c)		Neg	Neg	Neg	**Pos**
Street Vendor 5 (Marabastad)	Spinach (a)	2009.10.17	Neg	Neg	Neg	**Pos**
	Spinach (b)		Neg	Neg	Neg	**Pos**
	Spinach (c)		Neg	Neg	Neg	**Pos**
Street Vendor 6 (Marabastad)	Cabbage (a)	2009.10.17	Neg	Neg	Neg	**Pos**
	Cabbage (b)		Neg	Neg	Neg	**Pos**
	Cabbage (c)		Neg	Neg	Neg	**Pos**
Street Vendor 7 (Marabastad)	Lettuce (a)		Neg	**Pos**	**Pos**	**Pos**
	Lettuce (b)	2009.10.17	Neg	**Pos**	**Pos: 1B**	**Pos**
	Lettuce (c)		Neg	Neg	Neg	**Pos**
Street Vendor 8 (Marabastad)	Tomato (a)	2009.11.07	Neg	Neg	Neg	**Pos**
	Tomato (b)		Neg	Neg	Neg	**Pos**
	Tomato (c)		Neg	**Pos:GII.9**	**Pos: 1B**	**Pos**

Sample site, name, location	Fresh produce	Sample date	Real-time RT-PCR analysis			
			NoV GI	NoV GII	HAV	Mengo virus
Street Vendor 9 (Marabastad)	Spinach (a) Spinach (b) Spinach (c)	2009.11.07	Neg Neg Neg	Neg Neg Neg	Neg Neg Neg	**Pos** **Pos** **Pos**

NT: not tested

Table 48 (*continued).* Summary of results of viral analysis of fresh produce samples from Gauteng at retail.

Sample site, name, location	Fresh produce	Sample date	Real-time RT-PCR analysis			
			NoV GI	NoV GII	HAV	Mengo virus
Street Vendor 10 (Marabastad)	Cabbage (a) Cabbage (b) Cabbage (c)	2009.11.07	Neg Neg Neg	Neg Neg Neg	Neg **Pos** Neg	**Pos** **Pos** **Pos**

Western Cape: Although only a limited number of post-harvest fresh produce samples were tested, no viruses were detected in the fresh produce from a farm stall which had been irrigated with water from Plankenburg River (Table 49).

Table 49. Summary of results of viral analysis of fresh produce samples from Western Cape at-retail

Sample site, name, location	Fresh produce	Sample date	Real-time RT-PCR analysis			
			NoV GI	NoV GII	HAV	Mengo virus
Friends farm	Lettuce Cabbage	2008.08.08	Neg Neg	Neg Neg	Neg Neg	NT **Pos**

NT: not tested

Limpopo: Viruses were detected on two vegetable samples, namely one lettuce sample from a supermarket and an onion sample from a street vendor, both from Thohoyandou (Table 50).

Table 50. Summary of results of viral analysis of fresh produce samples from Limpopo at-retail.

Sample site, name, location	Fresh produce	Sample date	Real-time RT-PCR analysis			
			NoV GI	NoV GII	HAV	Mengo virus
Supermarket (Thohoyandou)	Lettuce (a) Lettuce (b)	2008.09.26	Neg Neg	Neg **Pos**	Neg Neg	NT NT

Sample site, name, location	Fresh produce	Sample date	Real-time RT-PCR analysis			
			NoV GI	NoV GII	HAV	Mengo virus
Street Vendors (Thohoyandou)	Tomato (a)	2008.09.26	Neg	Neg	Neg	NT
	Tomato (b)		Neg	Neg	Neg	NT
	Tomato (c)		Neg	Neg	Neg	NT
	Onion (a)	2008.09.26	Neg	Neg	Neg	**Pos**
	Onion (b)		Neg	**Pos**	Neg	**Pos**
	Onion (c)		Neg	Neg	Neg	NT
	Cabbage	2011.01.24	Neg	Neg	Neg	**Pos**
	Tomato	2011.01.24	Neg	Neg	Neg	**Pos**
	Cabbage	2011.05.03	Neg	Neg	Neg	**Pos**
	Tomato	2011.05.03	Neg	Neg	Neg	**Pos**
	Cabbage	2011.06.06	Neg	Neg	Neg	**Pos**
	Tomato	2011.06.06	Neg	Neg	Neg	**Pos**

NT: not tested

3.11.6 Viral characterisation

Norovirus: BLAST analysis of a 250 bp region of the 5' end of the capsid region of the NoV GII strains from the fresh produce revealed the presence of four genotypes, namely GII.1, GII.4, GII.6 and GII.9. Pairwise analysis of the nucleotide sequences indicated that the most of the strains from irrigation water and fresh produce showed the highest percentage genetic relatedness to other South African clinical and environmental strains (Fig. 18).

Strain GII.1 from a cabbage sample from the Western Cape showed a 99% nucleotide and 100% amino acid sequence identity to a strain, 8738, from a case of sporadic gastroenteritis in a hospitalised paediatric patient from Gauteng. The NoV GII.2 strain detected in the Mosselbank River in 2008 had the highest genetic relatedness (94% nucleotide and 92% amino acid sequence identity) to a strain, 1605, detected in Russia in 2009. Norovirus GII.4 strains from the Berg River (Lady Loch) and the Mosselbank Rivers in the Western Cape showed 100% nucleotide and 100% amino acid sequence identity to each other and a 99% nucleotide and 100% amino acid sequence identity to a strain, 8483, from a case of sporadic gastroenteritis in a hospitalised paediatric patient from Gauteng and strains 0399 and 1296 detected in Korea and Russia in 2009 and 2010, respectively.

The GII.6 strain from the Berg River (Franschhoek) showed a 99% nucleotide and 100% amino acid sequence identity to two strains, 08-06-02b and 08-05-26, detected in River water in the Vaal catchment area of SA and a 99% nucleotide and 100% amino acid sequence identity to a strain, 9088, detected in Japan in 2008. The GII.9 strain (SA-2009-Tom) detected on a tomato purchased from a street vendor in the Tshwane Metropolitan area, Gauteng showed a 98% nucleotide and 96% amino acid sequence identity to a strain, 08-08-18b-RV2, detected in the Rietspruit River in the Vaal catchment area in 2008 (Mans *et al.*, 2010).

Hepatitis A virus: Pairwise analysis of the nucleotide sequences of a 168 bp region of the putative VP1/P2A junction of the HAV strains detected on lettuce (SA-2009-Let) and tomatoes (SA-2009-Tom)(Table 8.6) with one another and to representative strains of all genotypes and sub-genotypes

revealed that the strains from South African fresh produce showed >92.5%, i.e. 95.96% sequence identity to the type strain, HM 175 and could therefore be grouped within genotype IB. These strains showed a 98.3% nucleotide identity to each other and 98-99% nucleotide sequence identity to the most closely related HAV strains in GenBank, i.e. 034, 126 and 166, which were from German tourists who had acquired hepatitis A in SA. Phylogenetic analysis showed that these South African strains from the fresh produce and the German tourists, together with previously characterised South African strains SA-2333 and SA-JVR, formed a distinct cluster within sub-genotype IB (Fig. 19). Pairwise analysis of the amino acid sequences for the same putative VP1/2A junction region indicated that the HAV strains from the fresh produce showed a 92% amino acid sequence identity to the type strain, HM175 and a 99-100% amino acid sequence identity to strains 034, 126 and 166 (results not shown).

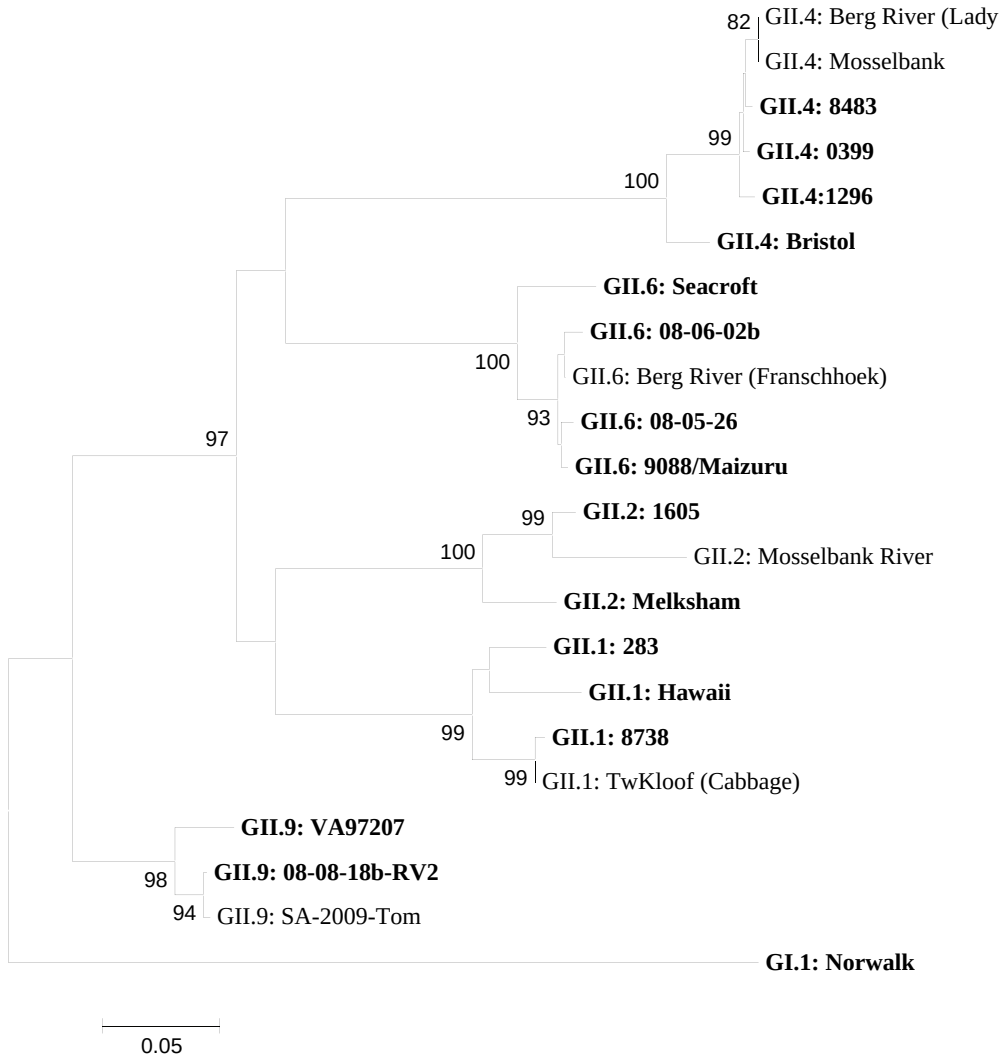


Figure 18. Neighbour-joining phylogenetic tree based on 250 base-pair fragment of the capsid region of the NoV strains from irrigation water and fresh produce and reference NoV GII strains using NoV G1.1 as an out-group. The bar indicates nucleotide changes per site and bootstrap percentages are indicated.

3.11.7 Discussion and Conclusions

Fresh produce may be contaminated with pathogenic microorganisms pre-harvest in the field through the use of polluted irrigation water or improperly treated manure (Steele & Odumeru, 2004; Gerba & Choi,

2009), at harvest or post-harvest during handling, processing and distribution (Beuchat, 2002; Carter, 2005; Berger *et al.*, 2010). In this investigation several factors may account for the viral contamination detected on the fresh produce analysed.

The findings of the present study showed that 3.8% (2/52) of the pre-harvest irrigated produce had detectable enteric virus contamination, with NoV GII.1 being detected on a cabbage sample from the Western Cape. These results are higher than the 1.7% for group A rotaviruses on fresh produce reported by van Zyl and colleagues (Van Zyl *et al.*, 2006) and the 3% reported for fresh vegetables in South Korea (Cheong et al., 2009). Although a direct link between contaminated irrigation water and contamination of fresh produce could not be shown, polluted irrigation water was a possible source of contamination of fresh produce. Norovirus GII.1 was detected on a cabbage sample irrigated with water from the Berg River (Site 17), and although no NoV was detected in the irrigation water at this site, NoV GII.6 was detected upstream (Site 16) and NoV GII.4 downstream (Site 18) in the Berg River during the same time period (August-September 2008).

The detection of identical or closely related NoV GII strains would have provided strong evidence of a direct link between viral contamination on fresh raw produce and irrigation water. The identification of distinct NoV GII strains, however, does not preclude the possibility of a link between the irrigation water and contamination of the produce as both the faecally contaminated irrigation water and the fresh produce may have been contaminated with multiple NoV strains (Le Guyader & Atmar, 2008; CDC, 2011). The infectious and outbreak potential of NoV GII.1 detected on the cabbage is highlighted by the first report of possible foodborne NoV-associated gastroenteritis in SA which was ascribed to NoV GII.1 (Hawaii) (Taylor *et al.*, 1993). In addition during the period 1990-2005 lettuce and salad greens contaminated with NoV were associated with ~25% of produce- associated foodborne outbreaks of NoV infection (Patel *et al.*, 2009). In spite of the fact that very few samples were collected from Site 1 in the Western Cape, human pathogenic viruses were detected twice in the Mosselbank River water, i.e. NoV GII.4 in June 2008 and NoV GII.2 and HAV in September 2008. Similarly, NoV GII and HAV were detected concurrently in the Plankenburg River in October 2008, with HAV being detected in consecutive samples (October 2008 and December 2008). These results indicate that these water sources are subject to continual human faecal pollution, confirmed by concurrent bacteriological data and would therefore pose a risk if used for irrigation.

The identification of HAV IB on the lettuce and tomato sample confirms that the contamination on the fresh produce was of human origin as HAV 1B has only been described in association with human infection (Robertson *et al.*, 1992; Nainan *et al.*, 2006; Pinto, 2007) and subtype IB was the most prominent type detected in clinical specimens in SA (Taylor, 1997). In SA, HAV is endemic with epidemiological features of both lower socio-economic and higher socio-economic communities being evident (Venter, 2007).

Hepatitis A virus has been detected in a variety of water sources (Taylor *et al.*, 2001; Venter, 2007) and the potential risk of infection to the different South African communities has been quantified (Venter, 2007). Extrapolating from the latter study, fresh produce contaminated with HAV would pose a minimal risk to the predominantly immune lower socio-economic community as the majority of this population group are immune to HAV infection. In this community only the very young, (children <10 years), the immunocompromised or non-immune individuals would be at risk of infection. In the higher socio-economic income urbanised predominantly white communities the risk of clinical infection would be greater.

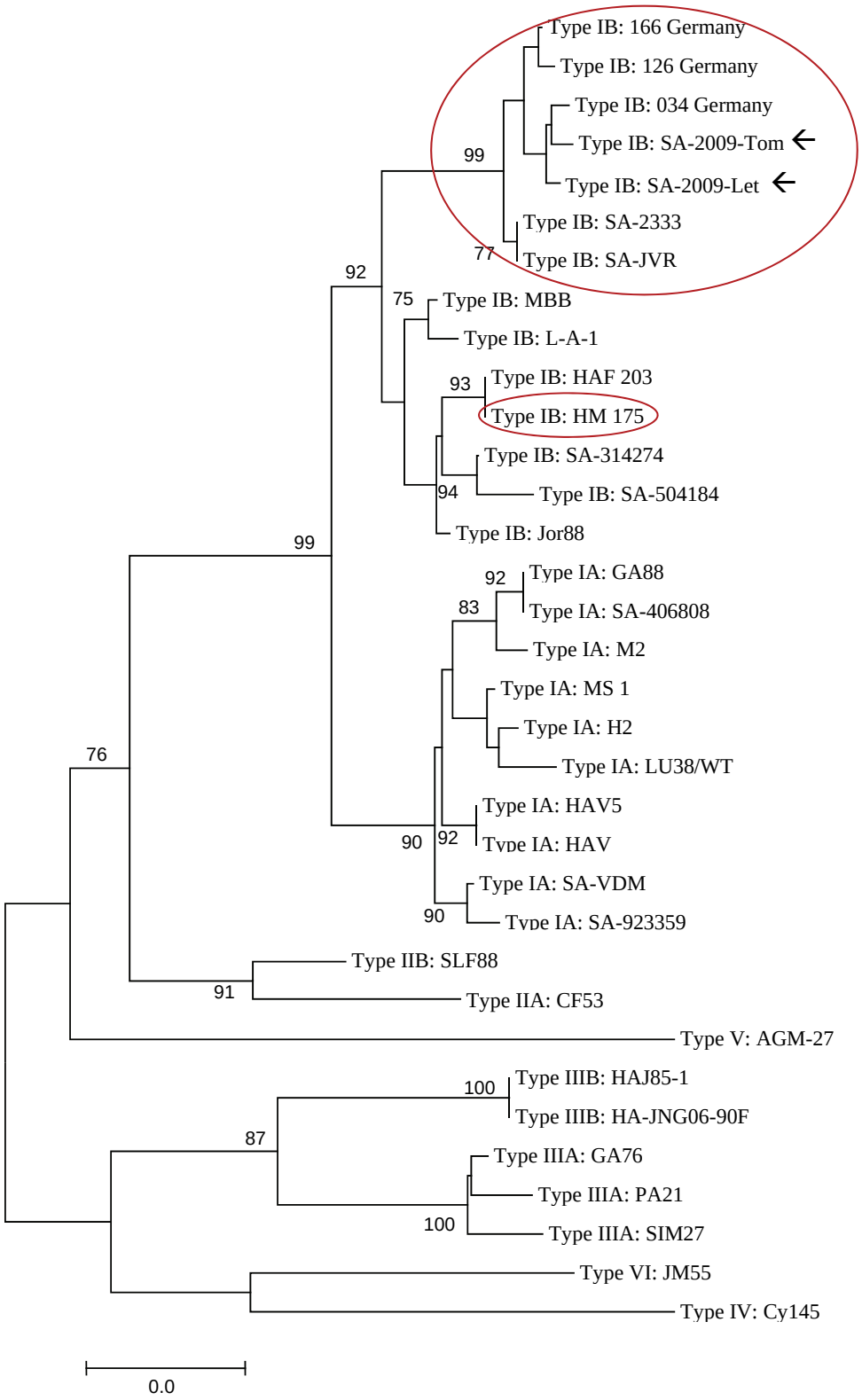


Figure 19. Neighbour-joining phylogenetic tree based on 168 base-pair fragment of the putative VP1/P2A region of the HAV strains from fresh produce and reference HAV strains. The bar indicates nucleotide changes per site and bootstrap percentages are indicated.

The potential health impact of foodborne HAV from fresh produce would not be limited to the South African geographical region as non-immune unvaccinated tourists from non-endemic areas are also at risk

of infection (Faber *et al.*, 2009) which could be fatal (Oltmann *et al.*, 2008). The economic impact of contaminated South African fresh produce therefore has important implications for the travel industry.

From this study it was evident that the fresh produce samples collected post-harvest at retail were more likely to be contaminated with enteric viruses, with either NoV or HAV or both viruses being detected on 19% (7/36) samples tested. These results suggest that most of the viral contamination of the fresh produce occurred during harvesting, processing and packaging. Food handlers have typically been implicated in the contamination of foods implicated in foodborne outbreaks of NoV infection (Tuan Zainazor *et al.*, 2010; CDC, 2011) and HAV infection (Fiore, 2004). It was noted that viral contamination was more prevalent on post-harvest fresh produce with rough convoluted surfaces, being present on ~25% of the samples, than on fresh produce with smooth surfaces where only ~12.5% of samples showed viral contamination. At pre-harvest enteric viruses were only detected on fresh produce with rough convoluted surfaces, i.e. cabbage and spinach (Tables 36 and 37). The higher viral contamination on produce such as lettuce, cabbage and spinach may be due to the large surface area of the leaves which also have many folds. Rotavirus and HAV have been detected on lettuce samples post-harvest in Costa Rica (Hernandez *et al.*, 1997), while NoV was detected on a pre-harvest spinach sample in Korea (Cheong *et al.*, 2009). The most common foods implicated in outbreaks of NoV and HAV infection are those ready-to-eat minimally processed foods with rough surfaces such as leafy vegetables and green onions and berry fruits (Rosenblum *et al.*, 1990; Cuthbert, 2001; Fiore, 2004; Shan *et al.*, 2005; Wheeler *et al.*, 2005; Berger *et al.*, 2010; CDC, 2011).

Lettuce has been known to have a high adsorption capacity for HAV and in a laboratory-based study, HAV was still detectable up to nine days after artificial contamination (Croci *et al.*, 2002). The fresh produce from street vendors 7 and 8 from Marabastad, which included leafy green vegetables and tomatoes, was contaminated with both NoV GII and HAV. Outbreaks of NoV and HAV infection are thought to result predominantly from contamination of food via unwashed hands of infected food workers (Berger *et al.*, 2010; CDC, 2010b; Tuan Zainazor *et al.*, 2010) suggesting that the lack of proper toilet and hand washing facilities and/or unhygienic practices of street vendors may be the cause of the post-harvest contamination of the fresh produce.

Due to the low titres of viruses in water and on food samples the analysis of food and water is a complex process. As most food- and waterborne viruses have a low infectious dose, ranging from 1 to 100 particles, it is essential that sensitive and specific methods are applied to the viral analysis of food and water (Mattison & Bidawid, 2009). The sampling procedures required to obtain a representative sample for viruses on fresh produce are critical to obtain a high level of analytical sensitivity (Bosch *et al.*, 2011). In this study the approach was to use a sample comprised of three randomly selected items of produce from the same batch, crate or field. The detection of viruses in one or two items in a sample comprised of three items (Tables 36-40) indicates the necessity of collecting a representative sample to get a true reflection of the virological quality of a particular crop. Effective quality assurance/quality control procedures are also required to exclude false positive or false negative results (Bosch *et al.*, 2011). False negative results usually result from poor virus recovery, inefficient nucleic acid extraction and inhibitory substances that affect the reverse transcription or amplification reaction, while false positive results could be due to cross-contamination (Bosch *et al.*, 2011). In this study mengovirus was included as a process control to control for viral recovery and nucleic acid extraction efficiency. From Table 84 it is evident that items of fresh produce from street vendors 7 and 8 from Marabastad, which tested negative for the target viruses, namely

NoV GI, NoV GII and HAV, tested positive for mengovirus highlighting the efficiency of the viral recovery and nucleic acid extraction methodology applied, and therefore the results are correctly reported as negative for the target viruses. Mengovirus was, however, not detected in a number of irrigation water or on a number of fresh produce samples submitted from collaborating laboratories, e.g. Table 80. This could have been due to incorrect seeding, viral decay on the seeded vegetable and/or suboptimal and inefficient viral recovery in the referring laboratory or due to the presence of inhibitory substances in the extracted nucleic acid. This may have resulted in false negative results in selected samples where amplification of the target virus was inhibited, and therefore these results may represent an under-estimation of the true extent of viral contamination in the irrigation water and on the fresh produce.

From this study it was evident that in selected areas of SA, irrigation water and fresh produce, which is often eaten raw, was contaminated with potentially pathogenic human viruses, namely HAV and NoV GII. Irrigation water and food handling were identified as possible sources of contamination of the fresh produce. The detection of both NoV GII.9 and HAV on a single tomato sample (Table 84) is of concern as HRVs were previously been detected on tomatoes in SA (Van Zyl *et al.*, 2006), indicating the vulnerability of tomatoes to viral contamination. As this study represented an exploratory study to ascertain the extent of viral contamination in irrigation water and on fresh produce, further in-depth studies are required to determine; i) the viral load on the fresh produce and the associated health risk; ii) where in the supply chain, from farm to fork, the contamination occurs, iii) what intervention strategies can be implemented to ensure the safety of the fresh produce for the consumer, and iv) the economic impact on health, agricultural and tourism industries.

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:

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