CHAPTER 4: GENERAL DISCUSSION

4.1 INTRODUCTION

In South Africa fruit and vegetables are produced on a large scale by commercial farmers who depend on surface water for their cultivation. However, the surface water, i.e., rivers have been reported to be heavily contaminated with *E. coli* and feacal coliforms (Barnes, 2003; Tshivhandekano, 2006). There is also a serious concern that contaminated surface water used for irrigation may also contaminate fresh vegetables which may also have a negative effect on the export of vegetables to the EU and USA. Consumption by South Africans of vegetables contaminated with foodborne pathogens might lead to outbreaks of foodborne illnesses, bearing in mind that a large proportion of the citizens have immune-system compromised diseases such as HIV and tuberculosis. According to the CDC (2006), immune-compromised people, elderly people, pregnant women, and children are reported to be most vulnerable to foodborne diseases. The last group of people that may be negatively affected because of the contaminated surface water are those who are directly and indirectly associated with the production of fresh vegetables such as pickers, handlers, packers and farmers that participate in the production of vegetables during pre-harvest and post-harvest. It was reported that contaminated surface water/irrigation water not only results in health risks to these groups of people but also that it has a more negative effect on their families, especially on young children (Ait & Hassani, 1999; FDA/CFSAN, 2001). The overall objective of this study was first of all, 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 bacterial contamination of fresh vegetables. In addition, the effect of attachment time on the survival of *L. monocytogenes* and the effect of chlorine on *L. monocytogenes* attached to vegetables were also determined.

4.2 REVIEW OF METHODOLOGY

4.2.1 Bacterial analyses

Conventional methods were used to enumerate total coliforms, faecal coliforms, *E. coli, L. monocytogenes, Salmonella* sp., *Enterococcus, S. aureus*, aerobic sporeformers, anaerobic sporeformers and aerobic colony counts in our study. McMahon and Wilson (2001) also used conventional methods, namely, different enrichment and selective media to screen 86 organic vegetable samples for the presence of *Aeromonas* and enteric pathogens. Teltsch, Dalgaard and Tienungoon (1980) used M-endo Broth with 15% Agar (Difco) for determination of the total coliform count. The most probable number (MPN) method was used by them to estimate quantitatively the levels of *Salmonella* in wastewater.

Detection, differentiation and identification of microorganisms can be performed by numerous methods including: phenotypic, biochemical and immunological assays and nowadays, routinely applied as well, molecular techniques (Settanni & Corsetti, 2007). According to them, the reason why molecular techniques like real time PCR are preferred is that they are believed to overcome problems associated with selective cultivation and isolation of microorganisms from natural sources and because they are generally characterized by their simplicity, speed and reliability. The potential automation of real time PCR is another advantage compared to the conventional method (Bleve *et al.,* 2003). Multiplex PCR, for example, is undoubtedly useful to rapidly identify several isolates and with respect to denaturing gradient gel electrophoresis (DGGE), it enables the selection of various species and represents the fastest culture-independent approach for strain-specific detection in complex matrices (Settanni & Corsetti, 2007).

However, we could not use PCR or real time PCR for detection and identification of bacterial pathogens because of the cost implication. PCR is

not cost effective when the study involves the identification and quantification of many bacterial pathogens as in our study. PCR methods also have some disadvantages. One disadvantage of conventional PCR is that it does not distinguish among viable, viable but non-culturable and dead cells. However, this is not the case with real time PCR (Bleve *et al.,* 2003). PCR can also present some limitations when used for the identification and enumeration of microorganisms in a natural sample that are viable (Rompre *et al.,* 2002). Frequent inhibition of the enzymatic reaction, i.e., humic substances is a major challenge and limitation to PCR analysis of environmental samples. Humic substances, which are known as polymerization enzyme inhibitors and colloid matter, have a high affinity for DNA. Their presence in irrigation water, for example, can considerably decrease the amplification yield of PCR applied to the detection of greatly diluted bacteria (Rompre *et al.,* 2002).

MPN methods were used for the enumeration of coliforms and faecal coliforms in our study. One merit of MPN is that its results are accurate especially when coliforms and *E. coli* are present at low levels. The limitation of this method is that it is cumbersome and time consuming. However, the Membrane Filter method, which we used for the enumeration of intestinal *Enterococci*, could have been used for the determination of coliforms and faecal coliforms. According to Rompre *et al.* (2002), the Membrane Filter method is also used for the enumeration of coliforms and feacal coliforms and it is simple to perform, inexpensive, requires at least an overnight incubation period and a confirmation test. Impedance is another method that could have been used for the enumeration of coliforms and faecal coliforms. According to Madden and Gilmour (2008), two main benefits of impedance compared to MPN are that results are obtained faster and there is a marked reduction in the use of consumables and staff time.

4.2.2 Microscopy

The ability of LM to attach to the surface of spinach and tomato before and after chlorine washing has been studied with a scanning electron microscope (SEM). However, we did not get convincing results when a confocal laser microscope (CLM) was used for the same study. One of the main problems faced was a strong autofluorescence of the sections, mainly caused by chlorophyll of the vegetables. It may nevertheless be possible to solve this challenge in future by staining the sections after immunolabeling with the dye Sudan Black B, which may completely block the autofluorescence **(**Romijn *et al.,* 1999)**.**

4.3 OVERALL DISCUSSION

The result of heavy contamination of the three water sources and subsequently irrigated fresh vegetables with *E. coli*, faecal coliforms, intestinal *Enterococcus*, *L. monocytogenes*, *Salmonella* sp and *S. aureus*, show that surface water as irrigation water is an important pre-harvest source of contamination and also a public health risk in the sampled area.

The surface water pollution in our study may have originated from both human and animal sewage disposal by the informal settlement that lacks proper sanitation. According to Vuuren (2010), lack of proper sanitation usually leads to disposal of both human and animal wastes in the wrong places including surface water. While most African countries have an ambition to halve the number of people without access to sanitation by 2015, the continent as a whole is lagging far behind (Vuuren, 2010).

Others reasons that may be responsible for the prevalence of human bacterial pathogens in the surface water were given by Sigge and Fitchet (2009). According to Sigge and Fitchet (2009), 98% of South African water resources are fully utilized while 80% of its municipal sewerage systems are

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overburdened. In addition, according to the *Business Day* newspaper of April 28, 2010, only seven per cent of South Africa's wastewater treatment systems comply with international standards. The poor condition of the wastewater system may be the reason for the heavy microbial contamination of surface water observed in our study.

According to NWRS (2004), deterioration of the quality of the South African surface water resources is one of the major threats the country is faced with. The Minister of Water Affairs and Forestry has stated that bacteriological contamination and pollution of the surface water, which originates from the absence of poorly maintained sanitation facilities, is widespread in the country (NWRS, 2004).

Increasing rates of urbanization, industrialization and population growth have also led to stress on water resources and to pollution.

According to Vuuren (2009b), one of the major sources of faecal pollution of surface water is the large number of un-serviced informal settlements that have been established near rivers in the last two decades. Another major contributor to the menace is the failing sewage disposal systems of a large number of villages, towns and cities (Vuuren, 2009b). According to a newspaper report in *Rekord* (Stuijt, 2008), a water crisis in SA is on the increase daily: 'Only 23 out of 283 municipalities countrywide have sufficient operating water services while another 23 municipalities are facing a full-scale water crisis.' Also, according to the report, 2 million litres sewerage per day reach the Hartbeespoort Dam and later flow downstream.

Contaminated irrigation water is also a cause of public health concern in other countries and is one of the greatest problems encountered by producers of fresh produce the world over (Bumos, 2003).

Broccoli and cauliflower sampled in our study may be a health risk for the local consumers because bacterial pathogens were isolated from them. This

is possible since they are eaten raw or consumed after minimal processing which may not eradicate the bacterial pathogens.

The result of our study also shows that aerobic bacteria levels alone are not a good determinant of the microbiological quality of irrigation water and produce because a higher incidence of bacterial human pathogens was observed in the vegetables and in the water sampled. The levels of aerobic bacteria in the water and vegetables sampled were 2 log lower than has been reported internationally (Johnston *et al.,* 2006; Ruiz *et al.,* 1987; Ukuku *et al.,* 2005). The incidence of bacterial pathogens in water and vegetables was not significantly related to the aerobic bacterial level because vegetable and water samples with a high incidence of bacterial pathogens carried lower numbers of bacteria.

Recovery of the same type of pathogens found in irrigation water sources and the vegetables supported the hypothesis that such pathogens may be able to attach to and infiltrate the surfaces of the produce. Bacterial pathogens from the irrigation water might have attached to cauliflower and broccoli during irrigation at pre-harvest. According to Brandl (2006), attachment is the first step in the establishment of pathogenic bacteria on the plant surface.

Our work also showed that *L. monocytogenes* attach to vegetables within 30 min of coming into contact with them in irrigation water or other sources. Other workers have reported attachment time could take place just after 5 min of pathogens touching produce (Li *et al.,* 2002; Ukuku & Fett, 2002; Milillo *et al.,* 2008; Ells & Hansen, 2006; Solomon *et al.,* 2006).

It was evident from our work that pathogen *L. monocytogenes* has a preference of adhering to certain vegetables. While *L. monocytogenes* was isolated regularly from broccoli, this was not the case with cauliflower. Broccoli has been reported to be one of the vegetables with a higher risk of

being associated with listeriosis because of enhanced *L. monocytogenes* attachment (Ukuku *et al.,* 2005; FDA/CFSAN, 2008).

The results of this work also showed the difficulty of sanitizing pathogens that have become internalized into the subsurface structures of vegetables and fruits. Internalization is one of the factors that aid survival of pathogens on fresh produce even after sanitizing (Heaton & Jones, 2008). Chlorine is less effective on internalized pathogen because it is not able to access the subsurface structures effectively, where the pathogens are located (Doyle & Erickson, 2008; Fonseca, 2006). Entrapped or internalized pathogens are not readily accessible to chlorine because of the components, namely, liquids leaking from subsurface structures or wounds. The liquid is able to neutralize some of the chlorine before it reaches the microbial cells (Seymour *et al.*, 2002; Bhagwat, 2006).

Out of ground water, surface water and human wastewater that are commonly used for irrigation, ground water is the best source of water of good quality available for the cultivation of produce (Steele & Odumeru, 2004). It would be a very sound development for South Africa to increase the use of ground water for the cultivation of especially fresh produce. At the moment, only 8% of water used for agricultural purposes is from ground water while the highest percentage, namely, 77% of water used in South Africa, is sourced from surface water (Vuuren, 2009a). Although South Africa has the goal of increasing the percentage use of ground water to 10% by 2040 (Vuuren, 2009a), it is our opinion that this increase is too small, bearing in mind the advantages of ground water compared with surface water. Contamination in ground water is easily controlled because irrigation wells are easily maintained (Buck *et al.*, 2003). Other benefits of ground water are that proper design and construction can be carried out, adequate wellcovers can be put in place and periodic microbial well monitoring is easier (FDA/CFSAN, 2001).

This work also showed that step-wise logistic regression analysis can be used to determine the microbiological quality and safety of irrigation water and of vegetables. This is possible after determining some predictor variables like COD and faecal coliforms in irrigation water and also ACC on vegetables.

This work has been able to show that irrigation water in South Africa is a potential source of contamination of fresh produce. Also, while chlorine washing is more helpful on pathogens of the surface than on the subsurface structures of fresh produce, it is not reliable to remove pathogens effectively. The logistic regression model also showed that there is a direct relationship between physico-chemical properties (COD and turbidity) of irrigation water and bacterial pathogen incidence. This may aid a faster determination of the microbiological quality of irrigation water.

There is need for more research on the bacterial adhesion to fruits and vegetables which may lead to the development of more effective washing treatments to control microorganisms on whole produce and fresh-cut pieces. Future research should be focused on improving the identification and detection of foodborne pathogens and toxins in fresh produce. More rapid and precise testing methods are important to minimize the spread of foodborne disease once it occurs. There should also be a continuous study of possible intervention or hurdle strategies, such as the use of thermal treatment and irradiation, which could be applied to fresh produce products to reduce the level of bacteria and viruses that are in or on the product. For example, irradiation has been proven as an effective food safety measure for more than 50 years of research, although there is an unfounded safety controversy inhibiting its broad acceptance and uses (Gjessing & Kaellgust, 1991; Brackett, 2009). Research into cost-effective methods of irrigation and water purification should also be carried out.

CHAPTER 5: CONCLUSION AND RECOMMENDATIONS

Physico-chemical parameters (turbidity and COD) and the presence and high incidence of faecal coliform and other bacterial pathogens showed that the two rivers and the canal were of poor bacteriological quality. This shows that the management of water resources and wastewater disposal are of paramount importance. This study also confirms that though chlorine was not 100% effective to sanitize produce contaminated with pathogens, its efficacy on surface pathogens was more significant than on subsurface pathogens. More research should be done on the possibility of noroviruses and hepatitis A virus in irrigation water attaching to the surface of produce. Although not reported, it was observed that the sampled irrigation water sources were also contaminated with these viruses. Further work should be done on the mechanism of internalization of produce pathogens into the subsurface structures of vegetables. In particular, the way pathogens gain entrance through the naturally occurring surface apertures, namely, stomata, lenticels, stem scar, wounds and roots requires more information. Another challenge facing the produce industry is the problem of microbial stress-adaptation, which makes it difficult for hurdles to be effective against pathogens. Little is known about this phenomenon on produce and both the problem and solution require extensive research. Finally, it will be necessary to develop a suitable sanitizer that will be effective and environmentally friendly for use in the produce industry.