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Effect of irrigation water quality on the microbiological safety of fresh vegetables

by

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DECLARATION

I declare that the thesis which I hereby submit for the degree of PhD at the University of Pretoria is my own work and has not previously been submitted by me for a degree at any other university or institution of higher education.

Oluwatosin Ademola Ijabadeniyi

January 2010

DEDICATION

This thesis is dedicated to Almighty God, Jesus Christ and Holy Spirit for being my strength and helper.

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ABSTRACT

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Department: Food Science
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Irrigation water is perhaps the leading pre-harvest source of contamination of fresh vegetables in the world. In this thesis, the effect of source water from the Olifants River and the Wilge River on the bacterial quality of water in the Loskop Canal that they feed and also the subsequent contribution to the bacterial contamination of fresh vegetables was determined for a period of twelve months. Also effect of attachment time on the survival of *Listeria monocytogenes* and the effect of chlorine on *L. monocytogenes* attached to vegetables were determined. Finally, a step-wise logistic regression analysis was made to determine whether various predictor variables could be used to predict the occurrence of *L. monocytogenes*, *Salmonella* spp and intestinal *Enterococcus* in irrigation water and vegetables (i.e., cauliflower and broccoli).

COD and turbidity were higher in the Olifants River and the Wilge River than in the Loskop Canal that they feed, according to the water guidelines set by the World Health Organisation (WHO) and the Republic of South Africa (RSA). The level of the COD and turbidity were significantly different in terms of the two rivers in comparison with the canal. Levels of faecal coliforms and *Escherichia coli* were also higher than the WHO standard. *Staphyloccocus aureus*, intestinal *Enterococcus, Salmonella, L. monocytogenes* were recovered from the two rivers and the canal. Apart from *L. monocytogenes*

that was not recovered from cauliflower, all bacterial pathogens recovered from the surface water were recovered from the vegetables. This study also indicated that *L. monocytogenes* could attach to both surface and subsurface structures of both tomatoes and spinach within 30 min, and that even after 72 h, it still remained viable. It also indicated that chlorine treatment is more effective against surface *L. monocytogenes* compared with subsurface inoculated *L. monocytogenes.*

Finally, the logistic regression analysis of the sampled data showed that COD was statistically reliable to indicate a high probability of *L. monocytogenes*, turbidity reliable to indicate a high probability of intestinal *Enterococcus* and faecal coliforms and coliforms reliable to indicate a high probability of *Salmonella* in irrigation water. Low aerobic colony count (ACC) was statistically significant for the prediction of the three pathogens on vegetables.

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CHAPTER 1: GENERAL INTRODUCTION

1.1 PROBLEM STATEMENT

Outbreaks of food infections associated with consumption of ready-to-eat vegetables have been increasing (Beans *et al.,* 1997; Parish, 1997; De Roever, 1998; Beuchat, 2002; Sivapalasingam *et al.,* 2004; IFT, 2007; Pezzoli *et al.,* 2008; Fynn, 2009; Schreck, 2009). In September 2006, pre-packaged fresh spinach was recalled by the Food and Drug Administration (FDA) in the United States of America (USA) as a result of an *Escherichia coli (E. coli)* outbreak in California, USA. Also, in the same month, fresh tomatoes consumed at restaurants in the USA were responsible for an outbreak of *Salmonella* Typhimurium. In addition, there was an *E. coli* O157:H7 outbreak linked to lettuce from Taco Bell restaurants in the northern USA (IFT, 2007).

The increase in outbreaks of foodborne illnesses due to fresh produce is as a result of changes in dietary habits, including a higher per capita consumption of fresh or minimally processed fruits and vegetables and the increased use of salad bars and meals eaten outside the home (Altekruse & Swerdlow, 1996; Alzamora, Lopez-Malo & Tapla, 2000). According to Alzamora *et al.* (2000), yearly consumption of fresh fruits and vegetables in the USA increased by 20 pounds per person from 1988 to 1996 mostly because of the belief that fruits and vegetables are healthier. Changes in production and processing methods; agronomic, harvesting; distribution and consumption patterns and practices are other factors that have also contributed to the increase (Hedberg, MacDonald & Osterholm, 1999; Beuchat & Ryu, 1997).

Other reasons given by the Food and Agriculture Organisation (FAO) and World Health Organization (WHO) (2006) for increased foodborne infection/poisoning outbreaks are: microbial adaptation; increase in international trade; increase in susceptible population and increase in travel; change to a lifestyle of convenience and consumer demands regarding

healthy food with no chemical preservatives and with an extended shelf life; changes in human demographics and behaviour.

Surface water (dams and rivers) used for the irrigation of vegetables in South Africa (SA) are susceptible to contamination with pathogens because there are informal settlements around that use them for waste and sewage disposal. In addition, the water is not treated before it is used for irrigation. Irrigation water used in agriculture in SA is mostly untreated water while home gardeners have access to treated water of high quality (SAWQG, 1996.)

The Berg River used for irrigation of vegetables in SA has also been reported to fall below the European Union (EU) microbiological standard allowed for food production according to the *Cape Times* (Britz *et al.,* 2007). The *Landbouweekblad* magazine, of 24 August 2007, reported that the water in Loskop Dam contained poisonous heavy metals and *E. coli* as a result of mines and municipalities dumping wastes in the rivers that feed the dam. The magazine reported that Mr Johan van Stryp, manager of the Loskop Dam Irrigation Board had indicated that the water quality was not according to quality standards set. Farmers in the area, according to the report, feared the effect of the water on the safety and quality of the fruit and vegetables produced.

This problem of the contamination of irrigation water and subsequently, of vegetables might lead to a suspension of exports to the EU and USA, leading in turn to lost markets, reduction of foreign exchange earnings and job losses. This should be discouraged from happening because South Africa's local and export trade in fresh and processed fruit and vegetables is steadily growing. Exports from the Western Cape Province in particular have grown to R8 billion (WESGRO, 2006).

Furthermore, consumption by South Africans of vegetables contaminated with foodborne pathogens might lead to outbreaks of foodborne illnesses, bearing

in mind that a large proportion (i.e., more than 7 million) of the citizens have immune system compromised diseases such as HIV and tuberculosis (Suarez, 2009). Immune-compromised people, elderly people, pregnant women and children are reported to be the most vulnerable to foodborne diseases (CDC, 2006).

Apart from a fear of the safety of consumers from contaminated vegetables as a result of contaminated irrigation water, there is concern over the safety of pickers, handlers, packers and farmers that participate in the production of vegetables during pre-harvest and post-harvest. It has been reported that young children from families of farming communities are the most vulnerable to *Salmonella* infection as a result of sewage irrigation (Ait & Hassani, 1999; FDA/CFSAN, 2001).

There are few reports on the irrigation water quality in the Loskop Dam irrigation area, Mpumalanga Province, SA. Little is also known regarding the contribution of irrigation water to the contamination of ready-to-eat vegetables at harvest.

The increasing demand for fresh produce presents a challenge for government, researchers and processors to ensure the microbiological quality and safety of fruits and vegetables (Garcia, Mount & Davidson, 2003). Therefore, this study seeks to determine the effect of source water from the Olifants River and the Wilge River on the bacterial quality of water in the Loskop Canal they feed and also the subsequent contribution to the bacterial contamination of fresh vegetables. The effectiveness of chlorine as a sanitizer of vegetables and regression analysis as a tool for predictive microbiology model were also considered.

CHAPTER 2: LITERATURE REVIEW

2.1 IMPORTANCE OF FRESH AND MINIMALLY PROCESSED VEGETABLES

Fresh and minimally-processed vegetables and fruits provide most of our daily requirements for vitamins, minerals and fibre and their role in reducing the risk of lifestyle associated illnesses such as heart disease, diabetes and cancer has resulted in a further increase in their desirability and consumption. FDA and WHO have recommended 5–9 servings of fruits and vegetables to be taken daily because correct fresh produce intake alone could save 2.7 million lives a year because 31% of heart disease cases are due to an insufficient intake of fresh produce (Johnston *et al.*, 2006). As a result of this recommendation, fruit and vegetable consumption increased by 29% per capita in the USA between 1980 and 2000 (Matthews, 2006). Also, in SA, the Department of Health is promoting the consumption of fruits and vegetables through its '5-a-Day' eating programme, namely, consumption of least five portions of vegetables and fruit every day (Badham, 2010).

However, unlike in the USA, where they are generally consumed by the majority of the population, fruits and vegetables are seldom consumed by economically and socially deprived communities in developing countries. Instead dietary intakes consist of plant-based staple foods (Chada & Oluoch, 2003). In contrast to what obtains in poor communities in most developing countries, in SA the majority of the population generally consume vegetables and fruits; in fact, vegetables are referred to as 'poor people's food' in some countries of southern Africa (FAO, 2006)

2.2. ECONOMY OF VEGETABLES IN SOUTH AFRICA

SA has a market economy that is largely based on services, manufacturing and mining. In 2002 the agricultural and horticultural sector contributed 3.4%

to the GDP, while the agro-industrial sector contributed 15%. In 2003 agriculture contributed 3.8% to the GDP, USD 159.9 billion, with a projected annual growth of 3% (FAO, 2005).

SA is the major and leading exporter of fresh fruits and vegetables in Africa. Ndiame & Jaffee (2005) reported that 73% of fruits and vegetables exported to the USA in terms of the African Growth and Opportunity Act (AGOA) were from SA. SA is the largest third world supplier of fruits and vegetables to the European Union (EU) with a 31% of imported fruit market share (Ndiame & Jaffee, 2005). Several countries in sub-Saharan Africa export vegetables but three, Cote d'Ivoire, Kenya and SA, account for nearly 90% of the trade in the region for the international market with SA the leading exporter (Ndiame & Jaffee, 2005).

For some produce, especially fruits, SA ranks between number one and number 20 among the world's fresh produce exporting countries in terms of monetary value (FAO, 2004). According to a 2006 agriculture sector brief report on fruit processing, the fruit industry is very important to the South African economy contributing 20% or four million tons to total agricultural production (WESGRO, 2006). SA was ranked the 2nd largest southern hemisphere exporter of deciduous fruit, apples and pears, and stone fruit, nectarines, peaches and plums, after Chile. For citrus fruit, SA was ranked 3rd in the world after Spain and the USA. Apart from the exported fresh fruit, 20% is consumed locally, while the remaining 20% is processed into juices (WESGRO, 2006).

Of the nine provinces, the Western Cape has the highest rate of growth and development in agriculture, especially in fruits and vegetables. About 25% of the South African agricultural sector's total gross income was generated by the Western Cape Province and it also accounts for more than 50% of exported produce (WESGRO, 2006). This is made possible because of the suitable climatic and physical geographic conditions in the Western Cape.

Seventy percent of fruit produced in SA is from various areas in the Western Cape. For example, apples and pears are mostly produced in Ceres. Elgin is known for apple production. The Little Karoo is renowned for apricots, plums, peaches and nectarines and the Hex River Valley for grapes. The Western Cape produces 15–20% of the total citrus fruit produced in South Africa that constitutes 8.5% of total world export (WESGRO, 2006).

Apart from the cultivation of fruit, the Western Cape is also the leading province in the production of vegetables, representing 12% of the total vegetable production in SA. Examples of vegetables produced by commercial farmers in the region are onions, potatoes, carrots, cabbages and brassica (WESGRO, 2006). It is not the international market alone that has a high demand for fruit and vegetables from SA's commercial farmers. Fruit and vegetable sales in local supermarket chains in SA have increased due to the high preference of SA consumers for the fruit and vegetables produced in SA (WESGRO, 2006).

After consideration of the economic importance of fresh vegetables in SA, it is essential to elaborate on the pathogens that may contaminate them during pre-harvest which may later predispose them to become causative agents of infectious diseases to both local and international consumers.

2.3 FOOD PATHOGENS ASSOCIATED WITH VEGETABLES

Vegetables are among the food groups implicated with greater frequency in recent years as causative agents of enteric diseases (Beuchat, 2006). All types of produce have the potential to harbour pathogens (Brackett, 1999). *Shigella* spp, *Salmonella* spp, enterotoxigenic and enterohemorrhagic *Escherichia coli, Campylobacter* spp, *Listeria monocytogenes*, *Yersinia enterocolitica*, *Bacillus cereus*, *Clostridium botulinum,* viruses and parasites such as *Giardia lamblia, Cyclospora cayetanensis*, and *Cryptosporidium parvum* are of public health concern (Beuchat, 1996; Ortega *et al.,* 1997; De

Roever, 1998; Beuchat, 2002). Most of these bacterial pathogens have been associated with foodborne illnesses (Beuchat, 2002).

According to Beuchat (1998), the occurrences of pathogens in vegetables vary. The prevalence of *Campylobacter* is <3%, whereas the prevalence of *Salmonella* is higher, between 4 and 8%. *E. coli* O157:H7 and *L. monocytogenes* were more frequently isolated from vegetables compared to *Salmonella* (ECSCF, 2002). In some studies, tested pathogens were not detected at all on raw vegetables. For instance, in a survey done by McMahon and Wilson (2001) on 86 organic vegetable samples in Northern Ireland, no *Salmonella, Campylobacter, E. coli, E. coli* O157:H7 or *Listeria* spp were found on the organic vegetables examined.

Factors responsible for the emergence and prevalence of produce-linked outbreaks must be clearly understood for effective control and prevention. According to Tauxe *et al.* (1997), such factors include the following:

- Changes in the produce industry such as intensification and centralization of production;
- Wider distribution of produce over greater distances;
- Introduction of minimally processed produce; and
- Increased importation of fresh produce.

Other factors include changes in consumer habits, for example, the increased consumption of meals outside the home, increased popularity of salad bars and increased consumption of fresh fruits and vegetables and fresh fruit juices. In addition, other updated factors given by Tauxe *et al.* (1997) are the increased size of at-risk population (elderly people, children, immuno-compromised people), enhanced epidemiology surveillance, improved methods to identify and track pathogens and lastly, emerging pathogens with low infection dose.

Reported outbreaks of foodborne illnesses as a result of the consumption of fresh produce will therefore vary from the developed countries to the developing countries. From the responsible factors stated above, developed countries such as USA and those in Europe may have higher reported cases of foodborne outbreaks. For example, these countries have enhanced epidemiology surveillance in place unlike countries from the developing world.

In the USA alone, 164 foodborne outbreaks due to fresh produce (excluding salads) were reported to the CDC from 1973 to 1997 (Beans *et al.,* 1997; Tauxe *et al*., 1997). The mean number of produce-associated outbreaks nearly tripled from 4.0 per year from 1973 through to 1982 to 11.8 per year from 1993 through to 1997 (Beans *et al.,* 1997; Tauxe *et al*., 1997). However, no foodborne outbreak due to fresh produce has been reported in most developing countries. According to the FDA (2009), the increase of reported produce-borne outbreaks in developed countries such as the USA is mainly due to improved surveillance that is lacking in most developing countries. The United Kingdom (UK) is another country where the surveillance of foodborne illness is extensive and because of this, a significant proportion of outbreaks have also been associated with fresh produce (Brandl, 2006). Salad, vegetables and fruit caused 6.4% and 10.1% of foodborne outbreaks in the periods of 1993–1998 and 1999–2000 respectively in England and Wales (Brandl, 2006).

According to Chang & Fang (2007), risk associated with the consumption of fresh produce because of the possibility of foodborne infections is a problem in both industrialized nations and developing countries. In a survey carried out on spring onions, lettuce and cabbage cultivated with poor quality irrigation water in Ghana, Amoah *et al.* (2006) found them to be heavily contaminated with faecal coliform (between 4.0×10^3 to 9.3×10^8 MPN/g). The lettuce, cabbage, and spring onions were also contaminated with an average of 1.1, 0.4, and 2.7, helminth eggs g^{-1}, respectively. The eggs were identified as those of *Ascaris lumbricoides*, *Ancylostoma duodenale*,

Schistosoma heamatobium, and *Trichuris trichiura* (Amoah *et al.,* 2006). These studies have given rise to a growing awareness that fresh or minimally processed fruit and vegetables can be sources of disease-causing bacteria, viruses, protozoa, and helminths (Steele & Odumeru, 2004). The continuous rise in the number of outbreaks of foodborne illness linked to fresh fruit and vegetables challenges the notion that enteric pathogens are defined mostly by their ability to colonize the intestinal habitat **(**Brandl, 2006).

Outbreaks of foodborne illnesses as a result of consumption of fruits and vegetables are given in Table 1.

Table 1: Outbreaks of bacterial infections associated with fruits, unpasteurized fruit and vegetables (Wood *et al*., 1991; Hedberg *et al*., 1999; Burnett & Beuchat, 2001;Beuchat, 2002; Watchel et al., 2002; Mahbub *et al*., 2004; Nuorti *et al*., 2004; Bowen *et al*., 2006; CDC, 2006; IFT, 2007; Greene *et al*., 2008; Pezzoli *et al*., 2008; Schreck, 2009; Flynn, 2009)

Bacteria	Year	Country	Vegetables source
Bacillus cereus	1973	USA	Seed sprouts
C. botulinum	1987	USA	Cabbage
E. coli 0157: H7	1991	USA	Apple cider
	1995, 2002	USA	Lettuce
	1996	USA	Apple juice
	1997	Japan	Radish sprouts
	1997	USA	Alfalfa sprouts
	2002, 2006	USA	Spinach
E. coli (enterotoxigenic)	1993	USA	Carrots
L. monocytogenes	1979	USA	Celery, lettuce, tomato,
	1979	Canada	cabbage
Salmonella			
S. miami	1954	USA	Watermelon
S. typhimurium	1974, 2009	USA	Apple cider
S. oranienburg	1979	USA	Watermelon
S. saintpaul	1988	UK	Mungbean sprouts
S. chester	1989–90	USA	Cantaloupes
S. javiana	1990	USA	Tomatoes
S. poona	1991	USA/Canada	Cantaloupes
S. montevideo	1993	USA	Tomatoes
S. bovismorbificans	1994	Sweden/Finland	Alfalfa sprouts
S. hartford	1995	USA	Orange juice
S. stanley	1995	USA	Alfalfa sprouts
S. montevideo	1996	USA	Alfalfa sprouts
S. typhi	1998–1999	USA	Mamey
S. mbandaka	1999	USA	Alfalfa sprouts
S. senftenberg	2007	UK	Prepacked basil
S. newport	2007	USA	Tomatoes
Shigella flexneri	1998	UK	Fruit salad
S. sonnei	1986	USA	Lettuce
	1994	Norway	Lettuce
	1998	USA	Parsley
	1995	USA	Scallions
Vibrio cholera	1970	Israel	Vegetables
	1991	USA	Coconut
Yersinia pseudotuberculosis	2003	Norway	Iceberg lettuce

2.3.1 Bacterial pathogens associated with food and waterborne diseases

Escherichia coli

E. coli, a widely studied genus of bacteria, has a wide distribution in food environments in low numbers as a potential food pathogen (Jay, 2000). It is a common inhabitant of the intestinal tract of mammals (Jones *et al.,* 2006). This has resulted in the almost universal use of *E. coli* as the standard indicator for faecal contamination (Francis, Thomas & O'Beirne, 1999). *E. coli* is known to be able to withstand highly acidic environments and can survive at pH ranges as low as 3.3–4.2 (Johnston *et al.,* 2006).

E. coli O157:H7 along with *Salmonella* spp have been reported to be the most common bacterial enteropathogens associated with fruits and vegetables (CDC, 2006; Elizaquivel & Aznar, 2008; Greene *et al.,* 2008). *E. coli* O157:H7 has been identified as the causative agent in several foodborne outbreaks. If ingested, this strain commonly results in haemorrhagic colitis, gastroenteritis and kidney failure (Francis *et al.,* 1999). Thrombocytopenic purpura and haemolytic uremic syndrome may in few cases result and may be fatal (Gil & Selma, 2006). Outbreaks of enterohemorrhagic *E. coli* O157:H7 infections associated with lettuce and other leaf crops have been reported (Watchel *et al* 2002; Mahbub *et al.,* 2004). Spinach and leafty greens have also been associated with *E. coli* O157:H7 (Calvin, 2003).

Symptoms of enteropathogenic *E. coli* which include malaise, vomiting, diarrhoea with stool containing mucus but not blood may occur 12–36 h after consumption food contaminated with the pathogen (Khetarpaul, 2006).

The food safety concern associated with *E. coli* O157:H7 is its low infective dose and its ability to form biofilms on vegetables that it makes difficult to be sanitized (Somers, Schoeni & Wong, 1994; Bhagwat, 2006; Fonseca, 2006).

Listeria monocytogenes

L. monocytogenes is widely distributed in the environment, where it is associated with decaying vegetation, soil, sewage and faeces of animals (Beuchat, 1996; Beuchat, 2002). *L. monocytogenes* was not considered to be a major problem in the food industry before 1980 (Jones *et al.,* 2006). It has the ability to survive in a wide range of environmental conditions including high moisture concentrations, low oxygen concentrations and at refrigeration temperatures as low as 5 ℃ (Francis *et al.,* 1999; Johnston *et al.,* 2006), making it an ideal waterborne pathogen (Maciorowski *et al.,* 2007). It has been isolated from celery, lettuce, tomato and cabbage in USA and Canada (Beuchat, 1996; Beuchat, 2002). *L. monocytogenes* is a produce-safety concern because it grows very well under refrigeration storage conditions and it can form biofilms on produce which it makes difficult to be sanitized (Bhagwat, 2006; Somers *et al.,* 1994, Fonseca, 2006). It has also been reported to cause death (CDC, 2006).

Incubation periods for listeriosis vary from one day to as long as 90 days with some having an incubation period of a few weeks; a situation that makes the identification of food vehicles difficult if not often impossible (Khetarpaul, 2006). Symptoms of the disease that may likely develop in pregnant women, children, the elderly and the immuno-compromised include flu-like illness, meningitis and meningoencephalitis (Khetarpaul, 2006).

Prazak *et al.* (2002) looked at the prevalence of *L. monocytogenes* during the production and post-harvest processing of cabbage and they found that from 425 cabbage, 205 water and 225 environment sponge samples examined, *L. monocytogenes* was isolated from 3% of all samples. Twenty of these isolates were obtained from cabbage, three from water samples and another three were environmental sponge samples of packing shed surfaces.

Salmonella spp

Salmonellas are motile, Gram-negative, non-sporing rods (Hayes, 1992). The genus comprises five pathogenic strains namely *S. typhimurium, S. enteriditis, S. heidelberg, S. saintpaul* and *S. montevideo* (Francis *et al.,* 1999). *Salmonella* is a highly resistant pathogen and it is well able to survive outside the intestine, particularly at water activities between 0.43 and 0.52 (Maciorowski *et al.,* 2007). It is usually carried by animals such as pigs or poultry or insects and is passed on to humans when undercooked meats, eggs or milk are consumed (Johnston *et al.,* 2006).

Alternatively, non-animal products that have made contact with faeces of these infected animals as a result of animals grazing over the crops or of fertilization with manure can also carry *Salmonella* (Maciorowski *et al.,* 2007). *Salmonella* are facultative anaerobes biochemically characterized by their ability to ferment glucose with the production of acid and gas (Hayes, 1992). Moreover, they can exist over a diverse range of pH i.e., 4.1 to 9.0 and temperatures of 7 ℃ to 59 ℃ (Jones *et al.,* 2006). According to Beuchat (1996) and Hedberg *et al.* (1999), *Salmonella* spp. have been isolated from raw vegetables in the USA, Canada, Sweden and Finland.

The incubation period for *S. enteriditis* is typically between 6 and 48 h. The principal symptoms are mild fever, nausea, vomiting, abdominal pain and diarrhoea that may last for 3–7 days. However, typhoid fever, a food infection cause by *S. typhi* has an incubation period between 10 and 20 days (Khetarpaul, 2006).

Shigella

Shigella is another widespread foodborne pathogen of the family Enterobacteriaceae. The four species, namely *S. sonnei, S. boydii, S. dysenteriae* and *S. flexneri* have been reported to cause gastroenteritis

(Francis *et al.,* 1999). *Shigella* are regarded as fragile organisms which do not survive well outside their natural habitat (Gil & Selma, 2006). However, some strains are capable of survival below pH 6 and, for example, *S. sonnei* can survive at low temperatures such as 10 ℃ (Gil & Selma, 2006). The organisms can tolerate salt concentrations of up to 6% and are relatively heat sensitive (Frazier & Westhoff, 1988). *Shigella* has a very low infectious dose (i.e., 10 cfu) (Gil & Selma, 2006). Its pathogenicity involves the release of a lipopolysaccharide endotoxin that affects the intestinal mucosa (Frazier & Westhoff, 1988).

Infection occurs only at 37 ℃ in which secretion of an exotoxin takes place and it normally occurs through faecally contaminated water or food (Smith & Buchanan, 1992). Where water is contaminated with faeces of animal origin, *Shigella* is likely to present (Savichtcheva & Okabe, 2006). Brackett (1999) considers *Shigella* species to be a very serious threat to human health in cases where fresh produce is irrigated with contaminated water and then consumed raw. Transmissions of this organism usually occur by person-to-person, but several outbreaks have been reported due to consumption of contaminated water and foods particularly raw vegetables (Stine, 2004).

There are also reports that sliced fresh vegetable and fruits, including watermelon and papaya can support the growth of all species of *Shigella* (Johnston *et al.,* 2006; Gil & Selma, 2006). Foodborne outbreaks of the disease are usually linked to the use of raw, contaminated products in salads or foods that have not been properly cooked before consumption (Johnston *et al.,* 2006; Gil & Selma, 2006).

Streptococcus

The genus *Streptococcus* is a Gram-positive spherical, non-spore forming, facultatively anaerobic, catalase negative and homofermentative microbe. Species such *S. pyogenes* and *S. pneumoniae* are human pathogens (Hardie

& Whiley, 1997). Although it has not been reported to cause outbreaks of foodborne illnesses from vegetables, Turantas (2002) isolated faecal *Streptococcus* from 41 (75%) frozen vegetables out of 55 frozen vegetables. His result is in agreement with Insulata, Witzeman and Sunya (1969) who recovered *Streptococci* from frozen vegetables. Vegetables irrigated with wastewater were also reported to contain equal numbers of *S. faecium* and *S. faecalis* (Sadovski & Ayala, 1980). After 2–36 h after consumption of produce contaminated with *S. faecium* and *S. faecalis,* symptoms such as diarrhoea, abdominal cramps, nausea, vomiting, fever, chills and dizziness may occur (Khetarpaul, 2006).

Staphylococcus aureus

There are currently 27 species and several subspecies of the genus *Staphylococcus* but enterotoxin production is principally associated with *S. aureus, S. intermedius* and *S. hivicus. S. aureus* poisoning is a major cause of foodborne disease all over the world (Harris *et al*., 2003). *S. aureus* exists in air, dust, sewage, food, food equipment, environmental surfaces, humans and animals. However, its primary reservoirs are humans and animals (Khetarpaul, 2006). *S. aureus* is present in the nasal passages, throat, hair and skin of 50% or more of healthy individuals (Jones *et al*., 2006).

Staphylococcus food poisoning is caused by the ingestion of enterotoxins produced in the food by some strains of *S. aureus*. About 10^5 cfu/g of the organism is sufficient to cause food intoxication and the most common symptoms are nausea, vomiting, retching, abdominal cramping and prostration (Khetarpaul, 2006).

Although *S. aureus* is associated with food handlers and has been isolated from vegetables and prepared salads, there has been no reported outbreak due to the consumption of vegetables contaminated with *S. aureus* (Harris *et al.,* 2003). However, vegetable-associated outbreaks due to *Staphylococcus*

could occur under conditions that favour the growth of the organisms and subsequent toxin production.

Vibrio

Historically cholera has been one of the diseases most feared by mankind. It was endemic to the Indian subcontinent where it was estimated to have killed more than 20 million people during the 20th century (Kaysner *et al.,* 1992). Recently, there was a severe cholera epidemic in Zimbabwe, in which more than 90,000 people were infected and 4100 people died as a result (Vuuren, 2009b). A total breakdown of water and sanitation infrastructure was reported to be main cause of the epidemic (Vuuren, 2009b). The genus *Vibrio* includes at least three species that are known as human pathogens: *Vibrio cholerae* that is the etiological agent in cholera; *V. parahaemolyticus* that is often found in seafood and seawater and *V. vulnificus* that causes septicaemia (Kaysner *et al.,* 1992). These organisms are gram-negative, curved, motile rods that do not form spores. They can also ferment glucose without the formation of gas and are oxidase and catalase positive (Kaysner *et al.,* 1992).

Most cholera patients contract the disease via the faecal-oral route through the ingestion of contaminated water, or by eating minimally processed or raw vegetables that were either irrigated with contaminated water, or fertilized using contaminated manure or faeces. Furthermore, outbreaks of the disease are also associated with raw or undercooked sea food (Van Elfen, 2001). Vast amounts of the organism are isolated from the excreta of infected individuals (Kaysner *et al.,* 1992) and animals (Hurst *et al.,* 2002). If these excreta were to contaminate irrigation water, consumers could be at great risk of contracting the disease (Brackett, 1999).

Yersinia enterocolitica

Y. enterocolitica is a small Gram-negative rod which has the unusual property of being non-motile at 37 ℃ but motile, with peritrichous flagella below this temperature (Hayes, 1992). Another unusual attribute of this pathogen is its ability to grow at 4 ℃ with most strains growing down to 1 ℃ or even below (Hayes, 1992). There have not been reported outbreaks of foodborne illness due to the contamination of vegetables with *Yersinia* but it has been isolated from several raw vegetables (Harris *et al.,* 2003). In a survey done on 58 samples of grated carrots in France, 27% of the samples were contaminated with *Y. enterocolitica* serotypes and of these 7% were *Y. enterocolitica* serotypes pathogenic to humans (Harris *et al.,* 2003).

Spore-forming pathogenic bacteria

Endospores of members of the genera *Bacillus* and *Clostridium* (*B. cereus*, *C. botulinum* and *C. perfringens*) can contaminate vegetables especially when they are processed and packaged under conditions for spore germination, i.e. vegetables minimally processed and packaged under modified atmospheres (Harris *et al.,* 2003). Cabbage and sliced onions are able to support the growth of *C. botulinum*. Mixed seed sprouts have caused an outbreak due to *B. cereus,* while salad contaminated with *C. perfringens* was also associated with an outbreak (Harris *et al.,* 2003)

B. cereus is found widely as it occurs naturally in the soil as well as on plants. It is a spore-former meaning that extra care must be taken to store products testing positive for it under the correct storage conditions in order to prevent the spores from resuming their vegetative stage (Johnston *et al.,* 2006).

The two members of the genus *Clostridium* that are of major pathogenic concern are *C. botulinum* and *C. perfringens* and they are commonly found in the faeces of both humans and animals (Johnston *et al.,* 2006). *C. botulinum*

was only seen as a threat in the canned food industry previously but with the increase in popularity of packaging fresh produce with MAP, ideal growth and survival conditions for the pathogen have been created (Francis *et al.,* 1999). Fresh produce that has been associated with the toxin is cabbage, asparagus, broccoli, tomatoes, lettuce and melons (Francis *et al.,* 1999; Britz, 2005). The neurotoxigenic *C. botulinum* is the etiological agent for botulism. Although the outbreaks occur only on rare occasions, when they do so they are fatal (Kautter *et al.,* 1992)

The symptoms of *B. cereus* diarrheal-type food poisoning include watery diarrhea, abdominal cramps occuring 6–15 h after the consumption of contaminated foods. *C. perfringens* food poisoning symtoms are similar to those of *B. cereus.* However, the onset of the symptoms is between 8–24 h after the consumption of food containing large numbers of the vegetative organism, i.e., 10^6–10^8 cfu/g (Khetarpaul, 2006).

2.3.2 Viral food pathogens

A large number of food and waterborne viruses found in the human intestinal tract are potential pollutants of surface water. The three disease categories that are associated with them are: gastroenteritis, caused by human rotavirus (HRV), human caliciviruses (HuCV) which include the noroviruses (NoV) and the sapoviruses (SaV), human astroviruses (HAstVs) and enteric adenoviruses; hepatitis, caused by the faecally transmitted hepatitis viruses, namely hepatitis A virus (HAV) and hepatitis E virus (HEV); and other severe illnesses such as myocarditis, caused by enteroviruses which include polioviruses, coxsackie A and B viruses, echoviruses and enteroviruses 68–71 (Koopmans & Duizer, 2004; Butot, Putallaz & Sánchez, 2007). Although viruses have been recovered from surface water, there is a lack of information on the attachment and survival of specific viruses on fresh produce (Fonseca, 2006). However, group A rotaviruses (Rvs), the cause of acute viral gastroenteritis in infants and young children were detected in irrigation water

and raw vegetables in South Africa (Van Zyl *et al.,* 2006). Rvs were detected in 14% of irrigation water samples and 2% of raw vegetables treated with the irrigation water (Van Zyl *et al.,* 2006). Examples of important food and waterborne viruses and the associated clinical syndrome are shown in Table 2.

Food and waterborne viruses are an important cause of illnesses all over the world (Koopmans & Duizer, 2004; Richards, 2005). The true health risk and economic impact of these viruses are underestimated because of under-reporting, the prevalence of many asymptomatic or mild infections and the fact that the health effects of the disease are not specific (Marx, 1997; Parashar & Monroe, 2001). According to WHO, 70% of diarrhoea is caused by biologically contaminated food (Satcher, 2000).

Table 2: Most important food and waterborne viruses and the associated clinical syndrome

Likelihood of food and waterborne transmission	**Gastroenteritis**	**Hepatitis**	**Other**
Common	Norovirus	Hepatitis A virus	
Occasionally	Enteric adenovirus Rotavirus Sapovirus Astrovirus Coronovirus Aichivirus	Hepatitis E virus	Enterovirus

In addition, the Center for Disease Control and Prevention (CDC) estimates that there are 76 million cases, 325,000 hospitalisations and 5000 deaths associated with foodborne disease annually in the USA (Bresee *et al.,* 2002; Jones *et al.,* 2006). Although some of the problems above are caused by food and waterborne viruses, there is no reason to believe that risks of food and waterborne disease in SA are any different from those in the rest of the

world. For example, a common source of viral foodborne outbreaks of gastroenteritis has been reported by Taylor *et al.* (1993) in SA.

Hepatitis A virus

Hepatitis A virus belongs to the family Picornavidae and is the sole member of the genus Hepatovirus (Carter, 2005; Richards, 2005). It is further divided into six genotypes. While genotypes 1, 11 and 111 are found in humans, genotypes 1V, V and V1 are recovered from simians. Genotype 1 is the most common worldwide with genotype 1A being more common than 1B (Jothikumar *et al.,* 2005). Hepatitis A virus has an incubation period of 15–45 days and is present in the blood and faeces a few days after exposure and before the onset of symptoms (Richards, 2005). Hepatitis A virus is one of the leading causes of foodborne illness (Butot *et al.,* 2007). It is non-enveloped, resistant to heat, disinfection and pH changes and because it cannot replicate outside a living host like bacteria, it cannot replicate in food and water (Koopmans & Duizer, 2004). HAV like many other enteric viruses are extremely infectious. For example, 10–100 infectious virus particles are sufficient to infect a human host (Guévremont *et al.,* 2006). Hepatitis A virus, has been detected in raw and treated water sources in South Africa (Taylor *et al.,* 2001).

Noroviruses

Noroviruses belong to the family Caliciviridae which is divided into four genera: Vesivirus and Lagosvirus which are associated with veterinary infections, and Norovirus (formerly called Norwalk-like viruses) and Sapovirus (formerly called Sapporo-like viruses) which cause human infections (Chiba *et al.,* 2000; Martinez *et al.*, 2006). Noroviruses have been found to be the most important cause of non-bacterial acute gastroenteritis in both developing and developed countries (Moreno-Espinosa, Farkas & Jiang, 2004). Richards (2005) reported that the symptoms of gastroenteritis caused by NoVs and

SaVs are similar. However, they differ epidemiologically because NoVs cause illness in people of all age groups whereas the effect of SaVs is limited to children (Koopmans & Duizer, 2004). Like HAV, Noroviruses are resistant to low pH (4–5), free chlorine (0.5–1mg/litre) and heat treatment (30 min at 60 ℃) (Koopmans & Duizer, 2004).

2.3.3 Protozoan: *Cryptosporodium*

An example of protozoan that can cause foodborne illnesses if consumed with vegetables is *Cryptosporodium parvum (*Beuchat, 1996; De Roever, 1998; Beuchat, 2002). It has been detected in both irrigation water and vegetables alike (Roy *et al.,* 2004). It is known to cause diarrhoea in both immuno-competent and immuno-compromised hosts and it is transferred through the faecal-oral route (Ortega *et al.,* 1997). Out of the total number of vegetables examined in Peru for the *Cryptosporodium*, 14.5% contained *C. parvum* oocysts. Robertson and Gjerde (2001) also examined 475 vegetables from some markets in Norway. Nineteen of the samples were positive for *C. parvum* oocysts. Out of these positive samples, 5 (26%) were found in lettuce while 14 (74%) were found in mung bean. Fayer *et al.* (1992), reported that 72% of surface water samples taken in the USA tested positive for *Cryptosporodium* oocysts. *Cryptosporodium* oocysts may be associated with some other protozoa, in particular *Giardia* cysts and *Microsporidia* in irrigation water and vegetables (Thurston-Enriquez *et al.,* 2002). In a survey conducted on irrigation water samples from US and several Central American countries, 28% of the irrigation water samples tested positive for *Microsporidia,* 60% for *Giardia* cysts and 36% for *Cryptosporidium* oocysts (Thurston-Enriquez *et al.,* 2002).

Having looked at different bacterial pathogens that may cause foodborne illnesses if ingested with vegetables, it is appropriate to discuss ways by which they might likely come in contact with vegetable production during pre-harvest and post-harvest.

2.4 SOURCES OF CONTAMINATION

Contamination of vegetables can be divided into pre-harvest and post-harvest contamination (Beuchat & Ryu, 1997; Beuchat, 2002). Pre-harvest and post-harvest sources of pathogenic microorganisms on fresh and vegetables are given in Table 3. Potential pre-harvest sources include soil, faeces, irrigation water, water used to apply fungicides and insecticides, dust, insects, inadequately composted manure, wild and domestic animals, human handling, among others (Beuchat & Ryu, 1997; Beuchat, 2002). Post-harvest sources include faeces, human handling, harvesting equipment, transport containers, wild and domestic animals, insects, dusts, rinse water, ice, transport vehicles, processing equipment, among others (Beuchat & Ryu, 1997; Beuchat, 2002; Beuchat, 2006)

A study of soil and domestic animal faeces indicated that *Listeria* spp is more often present during July to September than other months in the USA (MacGowan *et al.,* 1994; Beuchat & Ryu, 1997). Wild birds and animals can also be sources responsible for the distribution of *L monocytogenes* to fruits and vegetables because 23% of samples collected from wild bird feeding grounds were positive for *L. monocytogenes* (Weiss & Seeliger, 1975).

Table 3: Sources of pathogenic microorganisms on fresh fruit and vegetables (Beuchat, 1997; Steele & Odumeru, 2004; Johnston *et al*., 2006; Beuchat, 2006)

Preharvest	Postharvest
Faeces	Faeces
Soil	Human handling (workers, consumers)
Irrigation water	Harvesting equipment
Water used to apply	Transport containers (field to packing shed)
fungicides, insectices	Wild and domestic animals (including fowl and reptiles)
Green or inadequately	Insects
composted manure	Air (dust)
Air (dust)	Wash and rinse water
Wild and domestic animals	Sorting, packing, cutting, and further processing
(including fowl and reptiles)	equipment
Insects	Ice
Human handling	Transport vehicles
	Improper storage (temperature, physical environment)
	Improper packaging (including new packaging
	technologies)
	Cross-contamination (other foods in storage, preparation,
	and display areas)
	Improper display temperature
	Improper handling after wholesale or retail purchase

Soil samples contaminated with faeces or untreated sewage coming into contact with vegetables might transfer pathogens to them which might survive different treatments during pre-harvest and post-harvest until vegetables are ready for consumption (Beuchat & Ryu, 1997). Examples of waterborne pathogens, major diseases they cause and their primary sources are given in Table 4.

Table 4: Examples of waterborne pathogens, diseases they cause and their primary sources (Adapted from Ashbolt, 2004)

Name of micro-organisms	Major diseases	Major reservoirs and primary sources
Bacteria		
Salmonella typhi Salmonella *paratyphi* *Shigella* spp.	Typhoid fever Paratyphoid fever Bacillary dysentery	Human faeces
Vibrio cholera	Cholera	Human faeces and freshwater zooplankton
Enteropathogenic *E. coli, Yersinia enterocolitica, Campylobacter jejuni*	Gastroenteritis	Human and animal faeces
Legionella pneumophila and related bacteria	Acute respiratory illness (legionellosis)	Thermally enriched water
Enteric viruses		
Polio viruses Hepatitis A virus, Hepatitis E virus	Poliomyelities Infectious hepatitis	Human faeces
Norovirus	Gastroenteritis	Human faeces to fomites and water
Protozoa		
Cryptosporidium homonis, C. Parvum	Cryptosporidiosis (gastroenteritis)	Water, human and other mammal faeces
Entamoeba histolytica	Amoebic dysentery	Human and animal faeces
Giardia lamblia	Giardiasis (gastroenteritis)	Water and animal faeces

Table 4 shows that most water and vegetables are contaminated with bacterial pathogens through human faeces followed by animal faeces. According to Santo-Domingo & Ashbolt (2008), a basic assumption in microbial water-quality risk assessment models is that risk associated with human faecal matter is much greater than that from non-human sources as well as being more manageable because human activities are more easily controlled than animal activities.

Duffy *et al.* (2005) showed that irrigation water is the leading pre-harvest and post-harvest source of contamination of produce. From a total of 22 *Salmonella* isolates found in environmental samples (irrigation water, soil, packing shed equipment), 16 isolates were from irrigation water and 6 from

packing shed equipment. Contaminated irrigation and surface run-off waters, according to Beuchat and Ryu (1997) and Ibenyassine *et al.* (2006), can also be sources of pathogenic microorganisms that contaminate fruits and vegetables in the field. Apart from irrigation water, the use of sewage as a fertilizer could also be a source of pathogens. MacGowan *et al.* (1994) found 84–100% of sewage samples to be contaminated with *L. monocytogenes* or *L. innocua* during a two-year sampling period. *Salmonella, Ascaris* ova and *Entamoeba coli* cysts were isolated from more than 50% of irrigation water samples contaminated with raw sewage or primary treated chlorinated effluents (Wang, Zhao & Doyle, 1996).

According to the Department of Water Affairs and Forestry (DWAF), almost all farmers in Vhembe region, Limpopo Province, South Africa are forced to use wastewater or faecally contaminated surface water sources to irrigate their produce as a result of inadequate water and sanitation infrastructures (DWAF, 1996b). This is a potential health risk for farmers, crop-handlers and consumers who eat the raw produce due to the possible presence of pathogenic microorganisms in the wastewater (Havelaar & Melse, 2001).

Pre-harvest sources may also contribute to post-harvest contamination of vegetables (Beuchat & Ryu, 1997). Johnston *et al*. (2006) carried out a survey on the microbiological quality of fresh produce and concluded that every step from production to consumption may predispose produce to microbial contamination and each of these steps needs to be included in a food safety programme to ensure safety. For instance, workers handling vegetables from the time of harvest through to packaging and processing, even in the home might act as sources of transmission of pathogens (Beuchat & Ryu, 1997).

In summarizing this section, it must be emphasized that fruits and vegetables can become contaminated with foodborne pathogens in various ways during

production, harvest, processing, transportation, in retail and food service and even at home (Harris *et al.,* 2003).

2.5 WATER SITUATION IN SOUTH AFRICA

Water is a scarce commodity and also a multipurpose resource (Meyer, 2007). This problem of scarcity is serious in SA because it lies in a semi-arid region of the world coupled with the fact that there is poor spatial rainfall distribution across the land. These factors make it a country of scarce, disproportionately available and extremely limited water resources (NWRS, 2004). Apart from the average rainfall of 497 mm/year being well below the global average of 860 mm/year, the annual freshwater availability is also stressed, namely, less than 1700 mm^3/person (Vuuren, 2009a).

According to Vuuren (2009b), South Africa's water sector has faced numerous challenges, such as

- water deficit in an increasing number of water management areas
- water pollution and decreasing water quality
- ageing water and wastewater infrastructure
- severe lack of skilled human resources
- impact of climate change on water resources
- illegal use of water, and
- inappropriate use of funds by different spheres of local government.

There is a projection that by 2025, there will be a national shortage of available water. Furthermore, climate change may increase the variability and intensity of rainfall in the eastern escarpment while decreasing it in the western parts of the country (DEAT, 2006). In spite of the many challenges discussed above, there is an increasing demand on the already scarce and stressed water resources (DEAT, 2006; Meyer, 2007). It must be also be emphasized that increasing the limited supply of water for agricultural food

production and food processing operations is affecting most developing countries (Palumbo, Rajkowski & Miller, 1997)

2.5.1 Sources of water available

Surface water is the main source of water for urban, industrial and irrigation requirements in South Africa (NWRS, 2004). About 77% of water used in South Africa in 2008 was sourced from surface water (Table 5). The country has the lowest rainfall conversion ratios in the world, for example, only 8.6% of rainfall is available for use (Walmsley, Walmsley & Silberbauer, 1999). There is also a dam capacity of about 32 400 million cubic metres coupled with ground water which is seriously limited because of the geology of the country (NWRS, 2004). Other sources of water available in South Africa are water recycling and desalination.

Table 5: Combination of main water sources (%) in South Africa (Vuuren, 2009a)

Water source	2008	Mid term (2025)	Long term (2040)
Surface water	77	72	65
Ground water	8	9	10
Water recycling	15	17	22
Desalination	<1	2	3

2.6 QUALITY OF SOUTH AFRICAN SURFACE WATER

The deterioration of the quality of the South African surface water resources is one of the major threats the country is faced with (Sigge & Fitchet, 2009). The Minister of Water Affairs and Forestry has stated that bacteriological contamination and pollution of the surface water, originating from the absence of poorly maintained sanitation facilities, is widespread in the country (Vuuren, 2009b).

Increasing rates of urbanization, industrialization and population growth have led to stress on water resources and pollution. According to Vuuren (2009a), one of the major sources of faecal pollution of surface water is the fact that during the last two decades many un-serviced informal settlements have developed near rivers. Another major contributor to the menace is the failing sewage disposal systems of a large number of villages, towns and cities.

The rivers in the urban areas regularly measure hundreds of thousands or ten millions of *E. coli* organisms per 100 ml water. The Jukskei River in the Gauteng Province was reported in 2003 to measure 13 million cfu/100 ml of *E. coli*, while the Umungeni River was contaminated with 1.1×10^6 cfu/100 ml of *E. coli*. The Berg River below the confluence with the Stiebeuel River in Franschhoek measured 9.2×10^4 cfu/100 ml of *E. coli* while the stormwater ditches joining the Berg River from the informal settlement of Mbekweni at Paarl measured 2.4×10^9 cfu/100 ml of *E. coli* in 2004 (Barnes, 2003). These data show that some South African rivers and streams are unacceptably polluted.

2.7 WATER FOR AGRICULTURAL USE

There is a serious shortage of quality fresh water globally (FDA/CFSAN, 2001). The USA was ranked third with an estimated 13 billion cubic meters of annual water shortage (Postel, 2000). Reinders (2000) reported a water shortage in SA. According to him, out of 19 management areas surveyed in SA, 63% of the areas (12) had a shortage of water for total local consumption including irrigation suggesting that irrigation agriculture will continue to experience increasing pressure to use less quality water (SAWQG, 1996). Zimmerman (2000) also reported that water is a major constraint to agriculture in SA because the country is in a semi-arid region of the world.

In addition to water availability, climate plays an important role in water quality and the potential for direct or indirect contribution to illness and outbreaks.

Sewage spills, run-off from concentrated animal production facilities, storm-related contamination of surface waters, illicit discharge of waste and other sources of pathogens, all threaten the quality of both surface water and ground water used for fruit and vegetable production and therefore the safety of the consumed product (Postel, 2000; FDA/ CFSAN, 2001). In the USA, water availability and multi-user water management planning affects the cost of agricultural water. Including the cost of energy, water availability determines the type of produce, source of water and methods of irrigation farmers will employ. These factors cause the individual grower and packer to alternate water sources during the course of the year (FDA/ CFSAN, 2001).

2.7.1 Importance of irrigation water in agriculture in South Africa

The importance of irrigation water to any type of farming, whether it is commercial or subsistence in South Africa cannot be overemphasized since it is a country that lies in an arid and semi-arid agro-climatologic zone (FAO, 2005). A report by Reinders (2000) showed the importance of irrigation water in SA.

Out of the total 12,871 million m^3 of water used in SA in 2000, 62% (7920 million m^3) was used for irrigation, while the remaining 38% was used for urban, rural, mining, power generation and afforestation needs. According to Reinders (2000), irrigated agriculture is the largest consumer of available water in South Africa. Also according to Zimmerman (2000), a major constraint in South African agriculture is the country's climate and agro-ecological potential that, throughout most of the country, is more suited for livestock grazing than for crop production. Over a 30-year period (1956–1986) as much as 27% of the country was drought-stricken for more than 50% of the time (Cowling, 1991).

The area equipped for irrigation in South Africa is 149 800 ha (FAO, 2005; Thompson, 1999). The distribution of areas equipped for irrigation differs

among the nine provinces in South Africa (Table 6). The main irrigated crops are fodder crops, wheat, sugar cane, vegetables and pulses. The three main irrigation designs available are 55–65% for surface irrigation; 75–85% for mechanized and non-mechanized sprinkler systems and 85–95% for localized irrigation (FAO, 2005).

2.7.2 Modes of irrigation

There is no detailed report on the types of irrigation modes available in SA. In the USA for example the USDA (1998) reports that four main methods of irrigation are common; gravity flow irrigation (flood or furrow), sprinkler irrigation, drip/trickle irrigation and sub-irrigation.

In Germany, three main types of irrigation methods have been used; flush irrigation technologies, sprinkler irrigation and drip irrigation (EWTSIM, 2005). Flush irrigation technologies were used before the 20th century for production of crops like vegetables, potatoes and grain. Starting from the early 20th century, irrigation development moved towards sprinkler irrigation, in the 1950s hand-moved and from 1960 portable sprinklers with quick-coupling pipes. Sprinkler irrigation was only used for vegetable crops. The development continued with the production of hose reel irrigation machines. Drip irrigation was mainly used in vineyard and orchard irrigation (EWTSIM, 2005).

Table 6: Distribution of irrigated area in South Africa per province in 2000 (FAO, 2005)

Province	Commercial irrigation, permanent (ha)	Commercial temporary (ha)	Area equipped for irrigation total (ha)
Eastern Cape	11070	179995	191065
Free State	46	68764	68810
Gauteng	18	16330	16348
Kwazulu-Natal	2747	131974	134722
Mpumalanga	18498	116977	135475
North West	706	114094	114801
Northern Cape	34759	130181	164940
Limpopo	58704	160617	219321
Western Cape	290204	162325	452529
Total	416753	1081257	1498010

The type of irrigation mode used can reduce or increase the amount of pathogens that will get to produce and this may even lead to health risks to farm workers, consumers and nearby residents (WHO, 2006). Spray and sprinkler irrigation carries with it the highest risk of spreading contamination through the produced aerosols compared to drip irrigation. Also, while drip irrigation may be better to reduce health risks, it has certain financial constraints (WHO, 2006). The effect of the irrigation mode on health risks is summarized in Table 7.

Table 7: Effect of irrigation mode on the health risks associated with use of polluted irrigation water (WHO, 2006)

Irrigation mode	Factors affecting choice	Precautions for heavily polluted water
Flood Furrow Spray and sprinkler Subsurface and localized (drip, trickle and bubbler)	Lowest cost Exact leveling not required Low cost Leveling may be needed Medium water use efficiency Leveling not required High cost, high water use efficiency Higher yields Potential for significant reduction of crop contamination. Localized irrigation systems and subsurface irrigation can substantially reduce exposure to pathogens by 2–6 log units.	Thorough protection for field workers, crop handlers and consumers Protection for fieldworkers, possibly for crop handlers Some crops, especially tree fruits, are prone to more contamination Minimum distance of 50–100 m from houses and roads Localized irrigation: selection of non-cloggingemitters; filtration to prevent clogging of emitters

2.7.3 Sources of irrigation water

The common sources of irrigation water used in South Africa are large reservoirs, farm dams, rivers, ground water, municipal supplies and industrial effluent (SAWQG, 1996). According to Bihn and Gravani (2006), irrigation water in agriculture can be diverse, ranging from potable to surface water from sources such as rivers to treated and untreated municipal water.

Among different of sources of irrigation water in the USA, the most common source is deep ground wells, with 51% of the vegetable and 39% of the fruit growers reporting this source of water. Flowing surface water is the next most common source of irrigation water, with 38% of fruit growers and 19% of the vegetable growers drawing water from this source. About 5% of produce growers reported using municipal water (USDA, 1998).

Other sources of irrigation water are run-off water and reclaimed water. There are standard conventions in irrigation management and local or regional incentive programs for collection and recycling run-off water for on-farm or downstream irrigation. A long-standing solution to both wastewater management and water availability needs has been the use of reclaimed water in agriculture, including irrigation of fruits and vegetables. Reclaimed water has been increasingly used for irrigation and to recharge ground water since the 1980s in the USA (Runia, 1995; FDA/CFSAN, 2001).

There is no evidence that reclaimed water is a known source of irrigation water in SA (SAWQG, 1996). WHO recommended that <1000 faecal coliforms/100ml must be in reclaimed water before it can be used for agriculture (WHO, 1989) and the USA Environmental Protection Agency (EPA) has guidelines for water reclamation and agricultural which states that faecal coliforms should not be detected in the water in at least 50% of samples (EPA, 2000; Lambertini *et al.,* 2008).

2.8 IRRIGATION WATER AND PATHOGEN TRANSFER

The microbial quality of irrigation water is critical because poor quality water can introduce pathogens into produce during pre-harvest and post-harvest. Indirect or direct contamination of produce from water or water aerosols of persistent pathogens on harvested vegetables has been long recognized as a potential hazard (FDA/CFSAN, 2001; WHO, 2003). Irrigation water used for agriculture in SA was reported to be mostly untreated water while home gardeners had access to treated water of high quality (SAWQG, 1996).

Though direct evidence of foodborne illness due to contamination of edible horticultural commodities during commercial production is limited, compelling epidemiological evidence involving these crops has implicated specific production practices (Brackett, 1999). The use of animal waste or manure, faecally contaminated agricultural water for irrigation or pesticide/crop

management application and farm labour personal hygiene, leads to direct contamination (Brackett, 1999).

Brackett (1999) suggested that only clean, potable water should be used for irrigation of fruits and vegetables after planting. However, this approach fails to take into account many aspects of water availability, water conservation programmes, irrigation method, geographic diversity, crop diversity, temporal factors, and the significant difficulty inherent in water monitoring for microbial content during production (FDA, 2001).

Steele *et al.* (2005) carried out a survey on 500 irrigation water samples used for the production of fruit and vegetables in Canada and found about 25% of the samples to be contaminated with faecal *E. coli* and faecal *Streptococci.*

Different workers have evaluated the presence or persistence of pathogens conveyed to crops by spray irrigation, irrigation aerosols of sewage effluent (Garcia-Villanova, Cueto & Bolanos, 1987; Teltsch & Katznelson, 1978) or drip irrigation (Sadovski, Fattal & Goldberg, 1978). It was found that detection varied and depended upon the level and nature of environmental stress. Detection was correlated to population densities of target pathogens in the source water and spatial orientation relative to the point source. The level of organic matter in the water affected the survival of pathogens.

Polluted irrigation and contaminated manure have been implicated in the outbreaks of enterohemorrhagic *E. coli* O157:H7 infections. The infections were associated with lettuce and other leaf crops and they are occurring with increasing frequency (Mahbub *et al.,* 2004). *Salmonella* became undetectable on effluent-irrigated lettuce five days after irrigation was terminated, but generic *E. coli* indicator strains persisted (Vazda, Mara & Vargas-Lopez, 1991).

In a survey done by Garcia-Villanova *et al.* (1987), *Salmonella typhimurium; Salmonella kapemba; Salmonella london* and *Salmonella blockey* were the isolated serotypes in the water samples and on the irrigated vegetables.

2.8.1 Infectious doses of bacterial pathogens in irrigation water

Analyses of some river waters in SA have been reported to contain high levels of pathogens that exceed infectious doses by far (Britz *et al.*, 2007). According to Britz (2005), accidental ingestion of such water, even if diluted, could cause serious infections among the population. The number of viruses that are able to cause infection is low compared with bacteria (Barnes, 2003). Also, some microbes infect the host immediately while others infect on a cumulative basis and thus the infection takes a long period to manifest (Legnani & Leoni, 2004). Waterborne pathogens are also able to form microfilms and ingestion of these microfilms or clusters poses a much higher risk of infection because the number of colonies in clusters or microfilms is very likely to exceed the infectious dose of the pathogen (Jamieson *et al.,* 2005).

Infectious doses of pathogens are not the same everywhere. For example, they are lower in developing countries such as SA where a large percentage of the exposed population is immune-compromised because of malnourishment, old age or suffering from HIV/AIDS or tuberculosis (Barnes, 2003). This factor further increases the importance of reducing pathogens in irrigation water in SA since a large percentage of the population has a much higher risk of infection (Barnes, 2003).

2.8.2 Factors affecting prevalence of pathogens in produce after irrigation

According to Stine *et al.* (2005), the factors that affect the transfer of pathogens from contaminated irrigation water to fresh produce are the type of

crop, the irrigation method and the number days between the last irrigation event and harvest.

Results of a survey of *Salmonella, Shigella,* and enteropathogenic *E. coli* on vegetables done in the USA confirmed that the frequency with which target pathogens could be isolated from irrigation water was inversely correlated with crop height (FDA/CFSAN, 2001). Plants, such as spinach and cabbage, had a higher frequency of confirmed positive isolation of pathogens than taller chilli peppers or tomatoes. According to FDA/CFSAN (2001), other factors that may cause the persistence of pathogens are plant surface hydrophobility and contours.

In another study, during a seven-month microbiological survey of vegetables, higher total coliform counts were recorded when the sprinkler irrigation water source was of poor microbiological quality than when water of acceptable microbial quality was used (FDA/CFSAN, 2001).

2.8.3 At risk populations

Young children are most at risk of contacting *Salmonella* infections when they are exposed to contaminated irrigation water during treatment of vegetables (Ait & Hassani, 1999; FDA/CFSAN, 2001). Crop irrigation with untreated wastewater caused a significantly higher rate of infection with *Salmonella* in children from families in farming communities (39%) than in children of non-farming communities (24%). Also, the prevalence of *Salmonella* infection for children exposed to sewage irrigation was 32% compared to 1% for children from an area that does not practise sewage irrigation. Farm workers are also at a high risk of being infected with infectious diseases.

Exposure to risk can be minimized or even eliminated by the use of less-contaminating irrigation modes i.e., drip irrigation and the use of protective clothing such as gloves, shoes and in certain cases, nose or face masks

(WHO, 2006). Adherence to strict personal and domestic hygiene standards and possibly immunizations can also reduce the health risks associated with contaminated irrigation water. Farm workers should have easy access to proper sanitation facilities, adequate and safe water for drinking purposes (Carr, Blumenthal & Mara, 2004).

2.8.4 Control of pathogens in irrigation water

The introduction of pathogenic microorganisms through irrigation water can be controlled by (Buck, Walcott & Beuchat, 2003)

- knowing the origin and distribution of irrigation water
- knowing the history of the land
- maintaining irrigation wells, and
- monitoring all irrigation sources for human pathogens.

Other measures that may be more successful at minimizing contamination of surface and ground water are proper design, construction and protection of wellheads. Periodic microbial monitoring of wells, using *E. coli* as an indicator of recent or persistent faecal contamination is also recommended (Allen *et al.,* 1990; FDA/CFSAN, 2001). The feasibility and performance of various methods of on-farm water treatment are not available (FDA/CFSAN, 2001).

Application of UV irradiation to river water for the irrigation of celery was effective in reducing total coliforms and non-pathogenic *E. coli* but had no effect on foodborne pathogens like *Salmonella* and *Listeria* (Robinson & Adams, 1978). According to Bihn and Gravani (2006), Good Agricultural Practice (GAP) should be implemented during the irrigation of fresh produce. The following are their recommendations:

- If surface water is used, it should be tested for *E. coli* on a regular schedule to monitor microbiological quality and any changes that may occur due to unusual contamination events.

- If water test results indicate a contamination event, attempts should be made to identify the cause and water should not be applied to ripe crops.
- Drip or surface irrigation should be used when possible to prevent direct wetting of the plant or ripe vegetable.
- Potable water should be used for mixing topical protective sprays (i.e. fungicides and insecticides).
- Producers should be active in local watershed management and be aware of factors influencing their watersheds.
- If well water is used, producers should be sure that the well is capped and properly constructed. Wells should be tested at least once a year to monitor microbiological quality.

In addition, apart from the use of a good water source with the reduced possibility of pathogen contamination, factors that determine the risk of infection such as type of crop, irrigation method and days between the last irrigation event and harvest should be understood (Stine *et al.,* 2005). This will aid in the development of irrigation water quality standards and risk assessment for enteric bacteria and viruses associated with fresh produce (Stine *et al.,* 2005). Surface or drip irrigation, for example, reduces the rate of contamination of produce with bacterial pathogens compared to spray irrigation. It is therefore essential for farmers to employ drip irrigation for vegetables that will be consumed raw. In a study carried out by Solomon, Potenski and Matthews (2002), the number of plants that tested positive following a single exposure to *E. coli* O157:H7 through spray irrigation (29 of 32 plants) was larger than the number that tested positive following surface irrigation (6 of 32 plants). But regardless of the irrigation method used, produce can become contaminated; therefore, the irrigation of food crops with water of unknown microbial quality should be avoided (Solomon *et al.,* 2002).

2.8.5 Monitoring microbiological irrigation water quality

To evaluate the microbiological irrigation water quality, enumeration of indicator bacteria (total coliforms, faecal coliforms and recently intestinal *Enterococci*) is routinely determined (Garcia & Servais, 2007). Since these indicator bacteria are abundant in faeces, their abundance in irrigation or surface water signifies a high level of faecal contamination and a risk of the presence of pathogenic microorganisms (Garcia & Servais, 2007). It also indicates that such water may be a health risk if utilized in agriculture.

Faecal pollution of rivers can be of human and animal origin (Garcia & Servais, 2007). Faecal pollution from animals such as wild animals, grazing livestock and cattle manure get into rivers through surface run-off and soil leaching (Tymzcyna, Chmielowiec & Saba, 2000). On the other hand, faecal pollution of human origin is through the direct discharge of untreated sewage into the water system (Pautshwa *et al.,* 2009). There is justification in using intestinal *Enterococci* as indicator bacteria because it has been reported that human faeces contain higher faecal coliform counts, while animal faeces contain higher levels of faecal *Enterococci* (Gildreich & Kenner, 1969; Pautshwa *et al.,* 2009)

2.9 ATTACHMENT AND INTERNALIZATION OF PATHOGENS INTO PRODUCE

Attachment of bacterial pathogens to the surface of the vegetable always precedes contamination of vegetables with bacterial pathogens (Iturriaga *et al.,* 2003; Solomon, Brandl & Mandrell, 2006). They are made possible because of the stomata, lenticels, broken trichomes, bruises and cracks on the skin surface of fruits and vegetables (Burnett & Beuchat, 2001). While mechanisms of attachment of bacterial pathogens to the surface of produce are not fully understood, it is expected that various organs of attachment i.e. flagella, pili or fimbriae may be used to mediate attachment (Ukuku, Liao &

Gembeh, 2005). Also, the mechanism of attachment of plant bacterial cells to the surface of plants has been extensively researched leading to predictability of the way human pathogens will attach to the surface of produce (Ukuku *et al.,* 2005). *Agrobacterium,* an example of plant bacterium, uses cellulose fibrils to enhance attachment (Romantschuk, 1992). According to Solomon *et al.* (2006), non-fibrillar adhesions in foodborne pathogenic bacteria may assist in attachment to produce. According to Sauer *et al.* (2000), most gram negative bacteria are able to attach with their diverse array of pili. *V. cholerae,* for example, uses a toxin-regulated pili and sometimes flagella for attachment and colonization of host (Herrington *et al.,* 1988). On the other hand, aggregative fimbriae may play a role in the attachment of most *Salmonella enterica* and *E. coli* O157:H7 to sprouts (Barak, Whitehand & Charkowski, 2002). Type 111 secretion systems for the delivery of bacterial virulence associated with infective protein into host cells present in pathogenic bacteria such as *Salmonella enterica*, *Y. entercolitica* biotype 1B, *Y. pestis* and enterohemorrhagic *E. coli* may assist in attachment.

Various authors have studied the attachment of *E. coli* O157:H7 on fresh vegetables and they found out that cells attached within 10 minutes after contact with the vegetables (Solomon *et al.,* 2006; Mandrell, Gorski & Brandl, 2006). After attachment, pathogenic bacteria, through a process called internalization are able to gain access into the subsurface structure of the plant or vegetable (Warriner *et al.,* 2003).

Internalization is a major problem in the fresh-produce industry because pathogens present within the subsurface structures of plants or vegetables are protected from the sanitizing effect of antimicrobial agents such as chlorine, hydrogen peroxide and ozone (Solomon *et al.,* 2006). Internalization is possible because of the natural openings such as stem scars, stomata, lenticels and broken trichomes that abound on plants and vegetable (Allen *et al.,* 1990; Quadt-Hallman, Benhamou & Kloepper, 1997; Warriner *et al.,* 2003; Bartz, 2006). Another reason that has been suggested as a possible cause of

microorganisms gaining access into the internal structures of plant and vegetable is the damage of the waxy cuticles on the plant tissues. Solomon *et al.* (2006) have also reported the ability of *Salmonella enterica* and *E. coli* to gain entrance into the growing plants or vegetables through the root system.

2.9.1 Attachment of *L. monocytogenes* onto produce

Different workers have shown that attachment of *Listeria monocytogenes* is possible through the release of an enzyme to the surrounding host tissue or produce to facilitate bacterial attachment and infiltration (Jedrzejas, 2001; Hall-Stoodley & Stoodley, 2005). It has also been reported that extracellular fibrils and flagellin have been used by *Listeria monocytogenes* to enhance attachment (Lemon, Higgins & Kolter, 2007; Kalmokoff *et al.*, 2008). *L. monocytogenes* are also able to form microfilms and release an enzyme to the surrounding host tissue or produce to facilitate bacterial attachment and infiltration (Jedrzejas, 2001; Hall-Stoodley & Stoodley, 2005).

2.10 REMOVAL OF PATHOGENS FROM PRODUCE

Most processors and consumers have assumed that washing and sanitizing fresh fruits and vegetables will reduce the microbial load. However, published efficacy data indicate that these methods cannot reduce microbial populations on produce by more than 90–99% (Beuchat, 1998). While such population reductions are useful and not to be over looked, they are insufficient to assure microbiological safety. Conventional washing technology was developed primarily to remove soil from produce, not microorganisms. Even with newer sanitizing agents such as chlorine dioxide, ozone, and peroxyacetic acid, improvements in efficacy have been made with shortcomings, such as the inability of chlorine dioxide to reduce the population of *E coli* O157:H7 on inoculated apples (Beuchat, 1998).

Alternatives to chlorine were limited in their ability to kill bacteria when realistic inoculation and treatment conditions were used (Sapers, 2001; Fonseca, 2006; Abadias *et al.,* 2008). Nozomi, Matasume and Kenji (2006) showed that a combination of sodium hypochlorite, fumaric acid and mild heat was very effective in killing aerobic bacteria, *E. coli* O157:H7, *Salmonella typhimurium* DT 104 and *S. aureus* on fresh-cut lettuce but it caused browning. Because of these limitations, it is preferable, wherever possible, to avoid contamination of fruits and vegetables by following good agricultural and manufacturing practices rather than by depending on decontamination (Sapers, 2001; Bihn & Gravani, 2006).

Factors that limit the efficacy of washing are: contamination conditions, interval between contamination, attachment in inaccessible sites, biofilms and internalization (Bhagwat, 2006). According to Sapers (2001), *Salmonella* sp survived washing to a greater extent when attached to cut surfaces of apple and green pepper than on unbroken external surface. Fresh produce such as apples, pears, cherries, grapes, potatoes, carrots and lettuce were reported to often have punctures, cuts or splits, providing space for attachment and internalization of foodborne pathogens (Sapers, 2001). *E. coli* was also reported to grow in wounds on apples and was difficult to kill after it was established within the wounds and puncture (Sapers, 2001).

Chlorine is routinely used as a sanitizer in wash, spray and flume waters used in the fresh fruit and vegetable industry (Beuchat & Ryu, 1997; Beuchat, 1998; Hagenmaier & Baker, 1998; Seymour *et al.*, 2002). Antimicrobial activity depends on the amount of free available chlorine in water that comes in contact with microbial cells. Francis *et al.* (1999) studied the effect of chlorine concentration on aerobic microorganisms and faecal coliforms on leafy salad greens. Total counts were markedly reduced with increased concentrations of chlorine up to 50 ppm, but a further increase in concentration up to 200 ppm did not have a substantial additional effect.

The effectiveness of treatment with water containing up to 200 ppm chlorine in reducing numbers of naturally occurring microorganisms and pathogenic bacteria is minimal, often not exceeding 2 log on lettuce (Adams, Hartley & Cox, 1989; Beuchat & Brackett, 1990; Beuchat *et al*., 1998; Beuchat, 1999; Weissinger *et al*., 2000) and tomatoes (Beuchat *et al*., 1998; Weissinger, Chantarapanont & Beuchat, 2000). Several workers have emphasized that chlorine cannot be relied upon to eliminate pathogenic microorganisms such as *L. monocytogens* (Hagenmaier & Baker, 1998; Nguyen-the & Carlin, 1994; Beuchat & Ryu, 1997).

The hydrophobic cutin, diverse surface morphologies and abrasions in the epidermis of fruits and vegetables limit the efficacy of sanitizers (Burnet & Beuchat, 2001). The inaccessibility of chlorine to the microbial cells in the crevices, pockets and natural openings in the skin of the fruits and vegetables contributes to the overall lack of effectiveness of chlorine in killing pathogens (Lund, 1983).

Use of electrolyzed water as a sanitizing agent is a type of chlorination. Electrolysis of water containing a small amount of sodium chloride generates a highly acidic hypochlorous acid solution containing 10–100 ppm available chlorine and was effective in reducing pathogens in apple and lettuce leaves (Sapers, 2001). Other authors have also reported on the application of electrolyzed water in the produce industry (Koseki *et al.,* 2004; Huang *et al*., 2008). However, the reaction of chlorine with organic residues can result in the formation of potentially mutagenic or carcinogenic-reaction products (Hidaka *et al*., 1992; Simpson *et al.,* 2000). A number of alternatives to chlorine such as chlorine dioxide, iodine compounds, ozone and hydrogen peroxide have been examined and some are in commercial use (Sapers, 2001, Zhao, Zhao and Doyle, 2009). Chlorine dioxide has a higher biocidal activity than chlorine but there are still some difficulties in its large-scale application by the fresh-cut produce industry (Bhagwat, 2006). Hydrogen peroxide has been shown to be a promising alternative to chlorine (Ukuku, *et*

al., 2001, Bhagwat, 2006). It was shown that it increased the shelf life of fresh-cut melons by 4 to 5 days compared to that of chlorine-treated melons. However, commercial application of hydrogen peroxide in the produce industry still requires FDA approval (Bhagwat, 2006).

Another potential replacement for chlorine as a sanitizer is ozone (Graham *et al.,* 2004). In 2001 the FDA approved the use of gaseous and aqueous ozone for application as an antimicrobial agent for foods (FDA, 2001). Garcia *et al.* (2003) determined the effectiveness of using ozone in combination with chlorine as a sanitizer in the treatment of minimally processed lettuce. They found that lettuce treated with chlorine, ozone or a combination had a shelf life of 16, 20, or 25 days respectively, indicating that chlorine-ozone combinations may have beneficial effects on shelf life and quality of lettuce salads as well as on the water used for rinsing or cleaning the lettuce. However, ozone treatment was ineffective in reducing decay of pears and foodborne pathogens (Spotts, 1992; Sapers, 2001). Iodine compounds are also more effective sanitizers than chlorine but they predispose surfaces and products to discolouration (Beuchat, 1998).

Other sanitizing agents that have been used for produce are peroxyacetic acid (which was recommended for the treatment of process water) and hydrogen peroxide which is Generally Recognized as Safe (GRAS) for some food applications but has not yet been approved as an anti-microbial wash for produce (Sapers, 2001). It is important to ensure that the quality of process wash water is good to disallow the potential risk of cross contamination during washing of fresh-cut produce (FDA, 2008). Novel sanitizing applications include vacuum infiltration, vapor-phase treatments and surface pasteurization (Sapers, 2001). Advanced Oxidation Processes (AOP) is another novel sanitizing application that is highly effective in reducing pathogenic bacteria from produce (Allende, Tomas-Barberan & Gil, 2006).

Zhao *et al. (*2009) recently formulated a sanitizer that effectively inactivated *Salmonella* and *E. coli* O157:H7. The new sanitizer that has just been developed has great potential for commercialization because it can kill all known pathogens on produce. It is cost effective, works fast, is not injurious to human health and is environmentally friendly. This development would have been a major breakthrough in the produce industry if not for the challenge of internalization. This sanitizer is only effective on surface pathogen. A combination of Sodium hypochlorite, fumaric acid and mild heat was shown to very effective in killing indigenous microflora, *E. coli* 0157:H7, *Salmonella typhimurium* DT 104 and *S. aureus* on fresh-cut lettuce but it caused browning (Nozomi *et al.,* 2006). Because of these limitations, it is preferable, wherever possible, to avoid contamination of fruits and vegetables by following good agricultural and manufacturing practices rather than depending on de-contamination (Bihn & Gravani, 2006; Sapers, 2001).

2.10.1 Mechanism of action of chlorine

Chlorine is normally used for sanitizing produce in three forms: chlorine gas (Cl_2), calcium hypochlorite ($CaClO_2$), and sodium hypochlorite (NaOCl) (Fonseca, 2006). Chlorine is able to reduce microbial population on produce and other surfaces because it is a strong oxidizing agent (Bhagwat, 2006). The efficacy of chlorine, however, is affected by the amount of free available chlorine in solution, the pH, the temperature and the amount of organic matter (Fonseca, 2006). According to Stopforth *et al.* (2004), low pH of internal tissues of fruits and vegetables and high loads of organic matter in the sanitizing solution significantly reduce the antimicrobial activity of chlorine. Also, according to Suslow (2007), “for optimum antimicrobial activity, the pH of the water must be between 6.5 – 7.5 because at this pH range, most of the chlorine is in the form of hypochlorous acid which produces the highest rate of microbial kill and reduces the release of irritating and potentially hazardous chlorine gas.”

2.11 CONTROL AND PREVENTION MEASURES AGAINST FRESH PRODUCE CONTAMINATION

The inability of sanitizers to completely decontaminate pathogens after coming in contact with produce during pre-harvest has been stated above (Nguyen-the & Carlin, 1994; Beuchat & Ryu, 1997; Hagenmaier & Baker, 1998). In spite of the addition of a sanitizer, higher microbial concentrations have been reported after harvest of fresh produce to be influenced by post-harvest processing, importation and seasonal variations (Ailes *et al.,* 2008). The prevention of foodborne diseases related to fresh produce could therefore occur only by preventing initial contamination (Beuchat, 2006). According to Zhu *et al.* (2009), effective and preventive measures are important to avoid contamination of fresh produce. Such measures should include environmental and family health improvement, good personal hygiene and safe food handling practices (Zhu *et al.,* 2009).

Other practical methods should also be employed to reduce, eliminate or prevent multiplication of pathogens on produce. According to De Roever (1998), proper sanitation at all levels in the fresh produce chain, namely, from farm-to-fork should be made mandatory. Also for the preventive measures to be effective, a collaborative approach among the industry, federal and international partners must be used (Unnevehr, 2000; Bowen *et al.,* 2006).

This safety initiative should include the avoidance of the use of untreated manure as fertilizers; proper sanitary systems and hand-washing facilities for farm workers; use of clean equipment and transportation vehicles; good hygiene in the processing facilities and in the kitchen; and measures to prevent cross-contamination (De Roever, 1998). To prevent cross-contamination, persons with an infection should not be allowed to handle produce or equipment since they may transmit the infection to other workers and may contaminate the produce. Also cold storage and transportation

should be employed to discourage the amplification of pathogens (De Roever, 1998).

All stakeholders in the produce industry, namely, growers, harvesters, packers, processors, preparers and even consumers along the food chain from farm-to-fork should be educated on proper way of produce handling (Balsevich *et al.,* 2003; Berdegué *et al.,* 2005; Henson, Masakure & Boselie, 2005). This will include the prevention of cross-contamination, the temperature at which different produce should be stored or kept and their shelf life (De Roever, 1998; Satcher, 2000). Proper consumer handling of fresh produce has also been canvassed by Bruhn (2006) because many consumers believe that produce is already clean and further washing is not important. The following improper food-handling practices, for example, infrequent hand-washing, poor hand-washing techniques, inadequate cleaning of kitchen surfaces, involvement of pets in the kitchen, and frequent touching of the face, mouth, nose and/or hair which Jay (1997) observed, may predispose produce to risk during its preparation by consumers and they should be warned against such practices (Li-Cohen & Bruhn, 2002).

Other measures that have been recommended are the implementation of Good Manufacturing Practices (GMP) programme in the produce industry (Bihn & Gravani, 2006). Good Agricultural Practices (GAPs) for irrigation water have also been recommended to ensure the safety of fresh produce (Figure 1).

A good agricultural practices farm food safety plan should include the following sections

- Irrigation practices
- Manure use
- Worker health, hygiene and training
- Toilet and hand-washing facilities
- Field and packinghouse sanitation
- Pesticide use
- Animal and pest management
- Post-harvest handling
- Crisis management
- Recall and traceback
- Farm biosecurity
- Record keeping

Specialty and niche markets may need to add the following sections

- Direct marketing
- Farm market protocols
- Pick your own/u-pick operations
- Petting zoos including animal health

Figure 1: Key components of GAP farm food safety plan (Bihn & Gravani, 2006)

The summarized recommendations according to Bihn and Gravani (2006) are as follows:

- If surface water is used for irrigation, it should be tested for *E. coli* on a regular schedule to monitor microbiological quality and any changes that may occur due to unusual contamination events.
- Drip or surface irrigation should be used when possible to prevent direct wetting of the plant or ripe fruit or vegetable.
- Potable water should be used for mixing topical sprays.
- If wellwater is used, producers should be sure that the well is capped and properly constructed. Wells should be tested at least once a year to monitor microbiological quality.

Few attempts have also been made to apply Hazard Analysis Critical Control Point (HACCP) principles during production of fresh produce, i.e., sprouted seeds, shredded lettuce and tomatoes but complete validation of the HACCP

plans has not yet been accomplished (NACMCF, 1999). According to Bihn and Gravani (2006), the problem of too many variables, such as weather, wild animals, irrigation water, soil and several other factors that are not easily controlled are responsible for a lack of validation and difficulty in the implementation of HACCP in the production of produce.

In concluding this section, it must be emphasized that for the measures stated above to work and to lead to the reduction of episodes of foodborne illness, there must be a behavioural change on the part of food producers, food processors, food retailers, food service personnel and even consumers (McCabe-Sellers & Beattie, 2004). According to Yiannas (2009), achieving food safety success in this changing environment involves going beyond traditional training, testing and inspectional approaches to managing risks. It requires a better understanding of organizational culture and the human dimensions of food safety. To improve the food safety performance of a retail establishment or a foodservice establishment, an organization with thousands of employees, or a local community, the way people do things or their behaviour must be changed because according to this researcher, food safety equals behaviour (Yiannas, 2009).

2.12 HYPOTHESES AND OBJECTIVES

2.12.1 Hypotheses

1. Spray irrigation of leafy vegetables with water containing food pathogens will lead to attachment of bacterial pathogens onto the surface of vegetables. Pathogenic microorganisms will attach to vegetables with flagella, fimbria and pili (Mandrell *et al.,* 2006).

2. When chlorine water is used to sanitize vegetables, it will significantly reduce the microbial load of pathogens on the surface of the vegetable while it will have little effect on the internalized

pathogens. According to Aruscavage (2007), pathogens that are internalized into vegetables are more difficult to remove by sanitizers compared with pathogens on the surface of the vegetables. Also according to Burnett and Beuchat (2001), the epidermis of leafy vegetables is covered with a multilayered hydrophobic cuticle that limits the efficacy of chlorine.

2.12.2 Objectives

1. To determine the bacteriological and physico-chemical quality of the Loskop Canal and the two rivers that feed it.

2. To determine the bacteriological quality of broccoli and cauliflower irrigated by the Loskop irrigation scheme.

3. To predict the occurrence of *L. monocytogenes Salmonella* spp and Intestinal *Enterococcus* in irrigation water and vegetables with logistic regression analysis.

4. To determine the effect of attachment time followed by chlorine washing on the survival of inoculated *Listeria monocytogenes* on tomatoes and spinach.

CHAPTER 3: RESEARCH

3.1 IRRIGATION WATER AS A POTENTIAL PRE-HARVEST SOURCE OF BACTERIAL CONTAMINATION OF VEGETABLES

ABSTRACT

The bacteriological quality of the irrigation canal from Loskop Dam, the two rivers that feed it and vegetables (broccoli and cauliflower) in Mpumalanga, SA, were investigated with respect to aerobic colony counts, aerobic sporeformers, anaerobic sporeformers and the presence of coliforms, faecal coliforms, *Escherichia coli*, *Salmonella* spp, *Listeria monocytogenes*, intestinal *Enterococci* and *Staphylococcus aureus*. Physico-chemical parameters determined for the surface water were pH, turbidity and chemical oxygen demand (COD). There were significant differences in the levels of COD and turbidity in the two rivers and the canal and the results of the three water samples were higher than WHO and SA water guidelines. Aerobic bacteria, aerobic spore bacteria and anaerobic spore bacteria in the two rivers, the canal and the vegetables followed the same trend. However, the level of aerobic bacteria (3–4 $\log_{10}$ cfu/g/ml), aerobic spore bacteria (1.6 $\log_{10}$ cfu/g/ml) and anaerobic spore bacteria (1.5 $\log_{10}$ cfu/g/ml) in both water and on vegetables during the period of sampling was low. Levels of faecal coliforms and *E. coli* were higher than the WHO standard. *S. aureus,* intestinal *Enterococci*, *Salmonella, L. monocytogenes* were recovered from the two rivers and the canal. Apart from *L. monocytogenes* that was not recovered from cauliflower, all bacterial pathogens recovered from the surface water were recovered from the vegetables. These results show that the rivers may contribute to the contamination in the irrigation canal and that may be a possible pre-harvest source of contamination of broccoli and cauliflower, which may in turn constitute a health risk to consumers.

3.1.1 Introduction

Commercial and small-scale farmers generally irrigate their produce with water from nearby rivers, streams, ponds, wells and dams most of which do not meet the required standard for irrigation (Westcot, 1997). Furthermore, the water is not treated before it is used for irrigation. According to the South African Water Quality Guidelines (SAWQG, 1996), irrigation water used in agriculture is mostly untreated water while home gardeners have access to treated water of high quality. South African's irrigation water sources are perceived to be at risk of contamination with human bacterial pathogens as a result of pollution caused by informal settlements and mines. According to Sigge & Fitchet (2009), 98% of South African water resources are fully utilized while 80% of its municipal sewerage systems are overburdened. South African surface water may be a source of contamination of fresh vegetables with bacterial pathogens due to the reasons given by Sigge and Fitchet (2009). The Berg River used for irrigation of vegetables in the Western Cape Province, SA, has also been reported to fall below the European Union (EU) microbiological standard allowed for vegetable production according to the *Cape Times* newspaper (2005). Similarly the *Landbouweekblad* magazine, of 24th August 2007, reported that water in Loskop Dam contained poisonous heavy metals and *E. coli* as a result of mines and municipalities dumping wastes in the rivers that feed the dam.

Tshivhandekano (2006) reported that irrigation water in the Tshwane metropolitan area of SA was highly contaminated with faecal coliform and *E. coli*. Hepatitis A Virus and rotavirus were also recovered from the Apies River in the same area (Tshivhandekano, 2006). There is also a concern over the safety of pickers, handlers, packers and farmers that participate in the production of vegetables during pre-harvest and post-harvest. It has been reported that young children from families of farming communities are the most vulnerable to *Salmonella* infection as a result of sewage irrigation (Ait & Hassani, 1999; FDA/CFSAN, 2001)

Although the nutritional and other benefits of a regular intake of fruits and vegetables are well documented **(**Fujiki, 1999; Potter, 1999; Lerici, Nicoli & Anese, 2000), internationally, health risk has been associated with the consumption of fresh fruit and vegetables (Beuchat, 1996; Beuchat & Ryu, 1997; De Roever, 1998; Beuchat, 2002). In September 2006, pre-packaged fresh spinach was recalled by the Food and Drug Administration (FDA) in the US as a result of an *E coli* outbreak in California, USA (IFT, 2007). Also, in the same year, fresh tomatoes consumed at restaurants in the USA were responsible for an outbreak of *Salmonella typhimurium*. There was also an *E. coli* 0157:H7 outbreak linked to lettuce from Taco Bell restaurants in the northern USA (IFT, 2007).

The microbial quality of irrigation water is critical because water contaminated with animal or human wastes can introduce pathogens into produce during pre-harvest and post-harvest (FDA/CFSAN, 2001). Indirect or direct contamination of produce from water or water aerosols of persistent pathogens on harvested vegetables has been long recognized as a potential hazard (FDA/CFSAN, 2001; WHO, 2003). The microbiological quality of the fresh vegetables is a significant concern for all stakeholders in the produce industry both local and international (Chang & Fang, 2007). According to Henneberry, Piewthongngam and Qiang (1999), the ten most common fresh vegetables consumed in the USA and other countries are broccoli, cauliflower, carrots, celery, lettuce, onions, tomatoes, cabbage, cucumbers and green peppers (Henneberry *et al*., 1999). The microbiological quality of irrigation water is therefore paramount to the safety of fresh and minimally processed vegetables (Bihn & Gravani, 2006; Solomon *et al.,* 2002).

Ibenyassine *et al.* (2006) reported that contaminated irrigation water and surface run-off water might be major sources of pathogenic microorganisms that contaminate fruits and vegetables in fields. Steele *et al.* (2005) surveyed 500 irrigation water samples used for the production of fruit and vegetables in Canada and found that 25% of the water samples were contaminated with

faecal *E. coli* and faecal *Streptococci*. River water used for both human and animal waste disposal poses a health risk due to contamination with *Salmonella* and *Listeria* when used for the irrigation of produce (Combarro *et al*., 1997; Johnson *et al*., 1997). Combarro *et al*. (1997) isolated different *Listeria* species from river water in Spain. The specie most isolated was *L. monocytogenes,* followed by *L. seeligeri*, *L. velshimeri* and *L. ivanovii*. Similarly, Geuenich *et al.* (1985) and Bernagozzi *et al.* (1994) also recovered mostly *L. monocytogenes*, 73% and 93% respectively, from river water.

The aim of this study was therefore to determine the effect of irrigation water on the bacterial quality of water in the canal it feeds and also the subsequent contribution to the bacterial contamination of fresh vegetables.

3.1.2 Materials and methods

Selection of rivers and vegetables

Due to various reports of contamination (Britz *et al.,* 2007, Tshivhandekano, 2006), the Loskop Dam irrigation scheme in the Mpumaplanga Province of SA was selected as the sampling area for this study.

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 River and the Wilge River. Water from the dam is subsequently released to the Loskop Canal system that is used to irrigate the vegetables. Surface water from the three points was aseptically collected at 12 intervals over a period of 12 months (November 2007 to October 2008) i.e., one interval per month. At each interval, 2 litres each of surface water was collected at the three points.

Three farms cultivating vegetables irrigated with water from the Loskop Dam irrigation scheme were also visited three times over a period of three months

for the collection of vegetables, namely, cauliflower and broccoli. Vegetables were picked randomly from the three farms and 25 g each was used for analyses. Farms were visited only three times because the vegetables are not grown all the year round.

Bacterial and physicochemical analyses of samples

Water and vegetable samples were examined for the presence of total coliforms, faecal coliforms, *E. coli, L. monocytogenes, Salmonella* sp., *Enterococcus, S. aureus*, aerobic sporeformers, anaerobic sporeformers, and aerobic colony counts were done. Apart from bacterial analysis, the following physico-chemical tests: temperature, pH, turbidity and COD, were determined in water samples.

Aerobic colony counts

Dilution series of water samples were prepared using buffered peptone water (BPW) (Oxoid Ltd; Basingstoke, Hampshire, England) and 0.1 ml each of the dilutions were pour-plated with Nutrient Agar (Oxoid) and incubated at 30 ℃ for 72 h (ISO, 1991).

Aerobic and anaerobic sporeformers

Water samples, 20 ml, were heated in a sterile test tube in a water bath (75 ℃) for 20 min (Austin, 1998). Serial dilutions were pour-plated. A set of plates were incubated aerobically at 37 ℃ for 48 h while the other set of plates were incubated an-aerobically in an anaerobic jar with anaerocult (Merck Ltd; Wadeville, Gauteng, South Africa) at 37 ℃ for 48 h.

Coliforms and faecal coliforms

Coliforms and faecal coliforms in the water samples were determined using the Most Probable Number (MPN) method (Christensen, Crawford & Szabo, 2002).

Escherichia coli

Positive *E. coli* Broth (MPN) samples were inoculated onto the surface of L-EMB (Oxoid) Agar plates with inoculating loop and incubated at 37 ℃ for 24 h (Christensen, *et al.,* 2002). Typical colonies from L-EMB were streaked onto *E. coli* Chromogenic Agar (Oxoid). Thereafter colonies were confirmed with API 20E (Oxoid Ltd; Basingstoke, Hampshire, England).

Listeria monocytogenes

Listeria monocytogenes was determined according ISO (2004). A 1 ml water sample was added to 9 ml of ½ Fraser Broth (Oxoid) and incubated at 37 ℃ for 48 h. 0.1 ml of the ½ Fraser Broth culture was then transferred into a test tube containing 10 ml of full Fraser Broth (Oxoid) and also incubated at 37^{o} C for 48 h. Oxford Agar (Oxoid) plates and Palcam (Oxoid) Agar plates were inoculated from culture from Fraser Broth. The plates were placed in an anaerobic jar and incubated microaerobically at 37 ℃ for 24 h. Typical colonies were streaked onto *Listeria* Chromogenic Agar (Oxoid). Thereafter colonies were confirmed with API *Listeria* (Oxoid).

Salmonella

Salmonella sp was determined according to ISO (1993). A 25 ml water sample was added to 225 ml sterile buffered peptone water and incubated at 37 ℃ for 24 h. The pre-enriched sample suspension, 10 ml, was transferred into 100 ml of Selenite cystine medium (Oxoid) and incubated at 37 ℃ for 24

h. About 0.1 ml of the same pre-enriched sample suspension was transferred into 10 ml of RVS (Merck Ltd; Wadeville, Gauteng, South Africa) and incubated at 37 ℃ for 24 h. Phenol Red/Brilliant Green Agar (Oxoid) and XLD (Oxoid) Agar plates were inoculated with cultures from Selenite cystine and RVS medium. The plates were incubated at 37 ℃ for 24 h. Typical colonies were streaked onto *Salmonella* Chromogenic Agar (Oxoid) and thereafter colonies were confirmed with API 20E (Oxoid Ltd; Basingstoke, Hampshire, England).

Staphylococcus aureus

S. aureus was determined according to ISO (1999). About 0.1 ml each of the dilutions were released on Baird Parker (Oxoid) Agar plates containing egg-yolk tellurite solution (Oxoid). Plates were incubated at 37 ℃ for 24 h. Catalase test was performed on positive colonies and confirmed with Staphylase test (Oxoid Ltd; Basingstoke, Hampshire, England).

Intestinal Enterococcus

About 100 ml of water samples was filtered through 0.45 µm membrane filter and placed on Slanetz and Bartley medium (Oxoid) mixed with 2, 3,5-triphenyltetrazolium chloride (Oxoid) after which plates were incubated at 37 ℃ for 44 h (ISO, 2000). Incubated 0.45 µm membrane filter that gave presumptive positive colonies was transferred to the surface of Bile Aesculin Azide Agar (Oxoid) and incubated at 44 ℃ for 2 h. Typical intestinal *Enterococci* colonies gave a tan to black colour.

Determination of physico-chemical parameters in surface water

The pH, temperature, turbidity, chemical oxygen demand (COD) of the irrigation water was determined concurrently with the microbiological analysis. The temperature of the surface water was measured with a Checktemp1

Portable digital thermometer (Hanna Instruments Inc. Woonsocket, R1, USA). The pH was measured with 211 Microprocessor pH meter (Hanna Instruments Inc. Woonsocket, R1, USA) while turbidity was determined with an H1 93703 Microprocessor turbidity meter (Hanna Instruments Inc. Woonsocket, R1, USA). Chemical Oxygen Demand (COD) was measured using the closed reflux colorimetric method, as described in standard methods (APHA, 2001). To a Teflon-coated tube, 2.5 ml of the sample was added, after which 1.5 ml of the digestion solution (10.2g/l K2Cr2O7, 170 ml/l concentrated H_2SO_4 and 33.3 g/l $HgSO_{4)}$ and 3.5 ml of concentrated H_2SO_4 were added. The tubes were placed in a COD reactor (HACD COD reactor) and refluxed for 2 h at 150 ℃. The tubes were allowed to cool and absorbance was read using a spectrophotometer (DR Lange Spectrophotometer, model CADAS 50S, Germany) at a wavelength of 600 nm. The absorbance of the samples was read along with potassium hydrogen phthalate standards that ranged from 0 to 1000mg-COD/l. The following formula was used to calculate the COD level of samples:

$$\text{COD (mg/l)} = \frac{\text{mg in final volume x 1000}}{\text{Sample volume}}$$

Statistical analysis

Analysis of variance (ANOVA), $p \leq 0.05$, (Tulsa, Oklohama, USA, 2003) was used to determine whether there were significant differences between the levels of turbidity, COD, aerobic plate count, aerobic sporeformer counts and anaerobic sporeformer 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).

3.1.3 Results

Physico-chemical properties of water from Loskop Canal, Olifants River and Wilge River

The turbidity of water samples differed significantly ($p \leq 0.05$) during the 12 sampling intervals (Table 8). During the sampling period, the Wilge River had the highest mean turbidity of 19.1 NTU followed by the Olifants River with 14.7 NTU and Loskop Canal with the lowest mean turbidity of 5.4 NTU (Figure 2). The mean turbidity level at all three sampling locations was higher than the international turbidity (1 NTU) standard for water (DWAF, 1996a). At some sampling intervals, there was a high variation between the NTU in both rivers and the canal. For example, the NTU for both rivers was very high at intervals 2, 5, 6, 7 and 12. However, no such trend was observed for the canal.

The COD of water samples also differed significantly ($p \leq 0.05$) during the 12 sampling intervals (Table 8). The Wilge River had the highest mean COD of 54.2 mg/l followed by the Olifants River with 53.5 mg/l and the Loskop Canal with the lowest COD of 50.4 mg/l. A similar trend to NTU was observed with COD higher at intervals 1 and 2, 4–7, for all 3 sites (Figure 3).

Table 8: Analysis of variance for turbidity, chemical oxygen demand (COD), aerobic colony count (ACC), aerobic sporeformers (ASF) and anaerobic sporeformers (AnSF) of water from Loskop Canal, Olifants River and Wilge River at 12 intervals for a period of twelve months

Effect	Degrees of freedom	Turbidity	COD	ACC	ASF	AnSF
Sampling interval	11	0.001	0.001	0.001	0.001	0.001
Location	2	0.001	0.010	0.001	0.001	0.001
Sampling interval x location	22	0.001	0.001	0.001	0.001	0.433

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

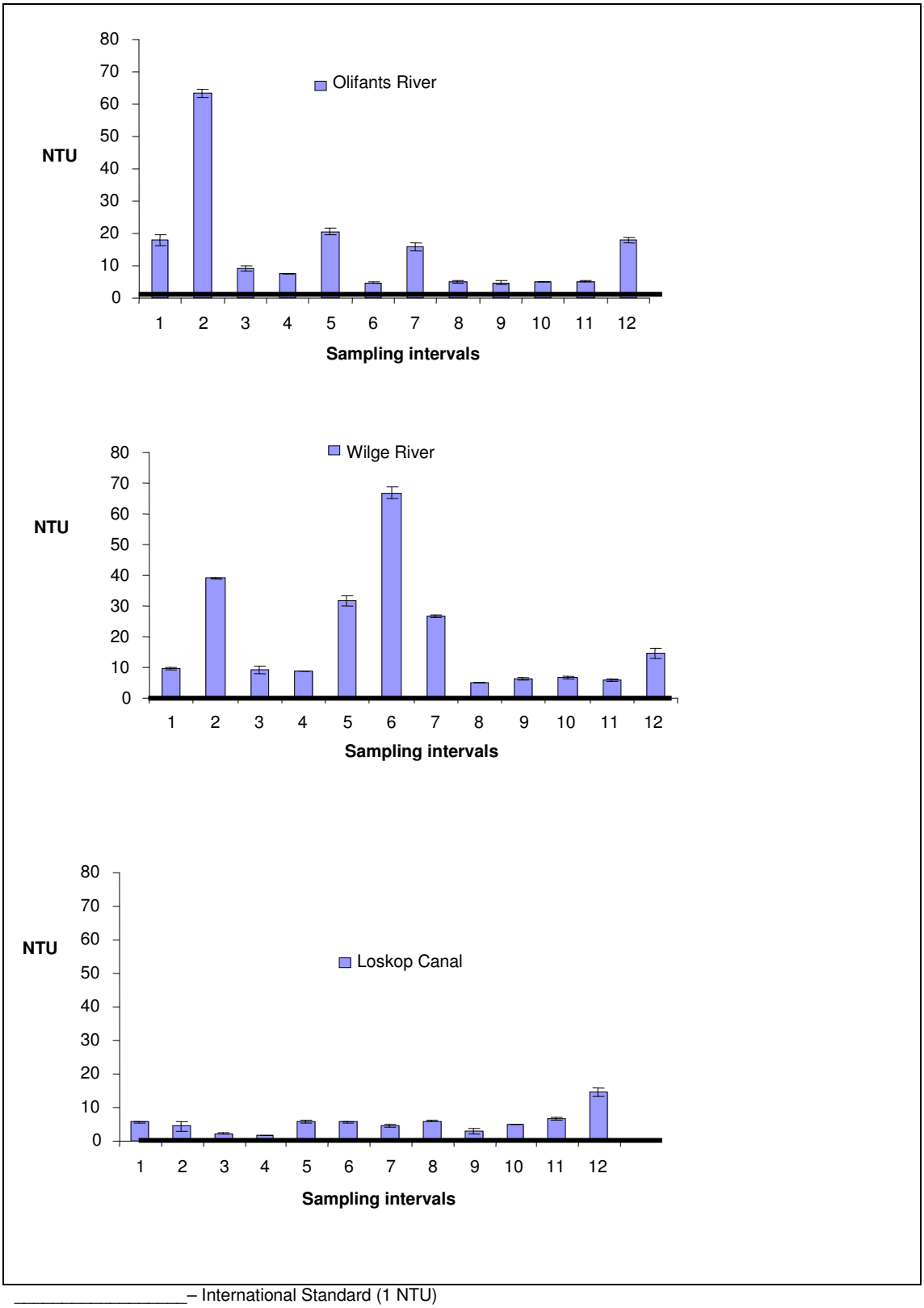
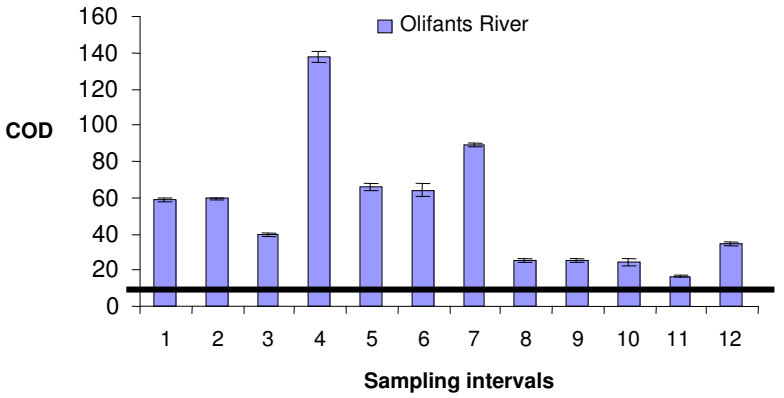
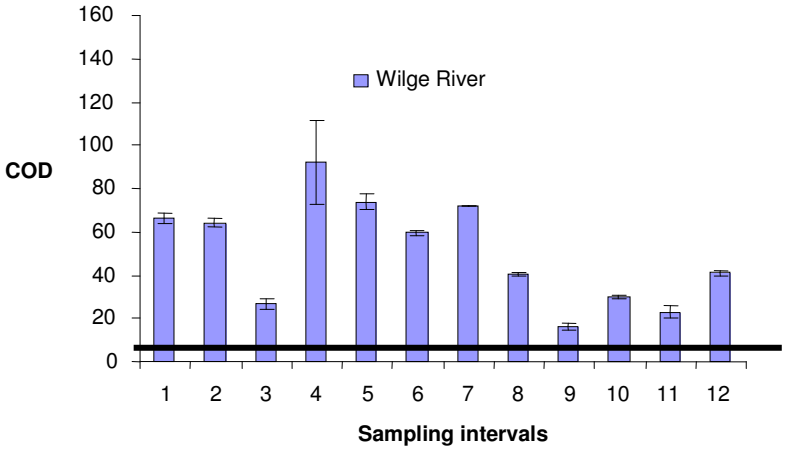
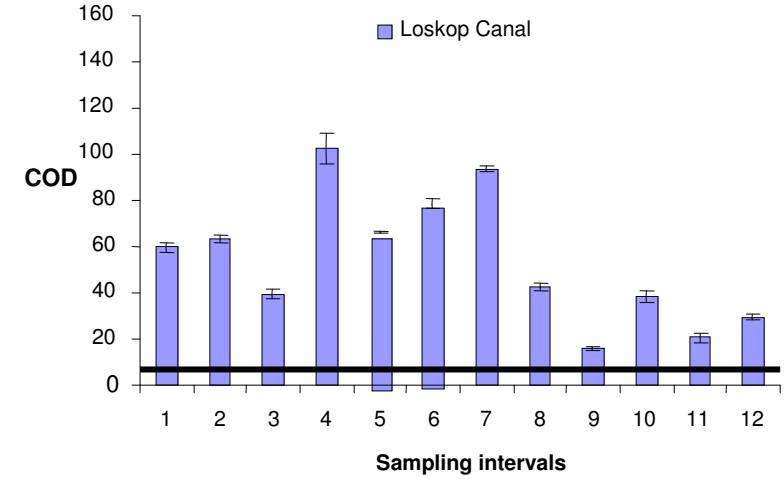


Figure 2: Turbidity of water from Loskop Canal, Olifants River and Wilge River during twelve sampling intervals







_____– International Standard (10 mg/l)

Figure 3: COD (mg/l) of water from Loskop Canal, Olifants River and Wilge River during twelve sampling intervals

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

Incidence of aerobic bacteria (APC), aerobic sporeformer bacteria (ASF) and anaerobic sporeformer bacteria (AnSF) in the Loskop Canal, Olifants River and Wilge River

The mean APC count of water samples ranged between 2.9–3.2 log $_{10}$ cfu/ml and differed significantly ($P \leq 0.05$) over time (Table 8). Similar to turbidity and COD, the Wilge River had the highest mean APC counts of 3.2 $\log_{10}$ cfu/ml followed by Olifants River with 3 $\log_{10}$ cfu/ml and Loskop Canal with the lowest APC counts of 2.9 $\log_{10}$ cfu/ml during the 12 sampling intervals (Figure 4). The APC counts of the two rivers and the canal during the sampling period followed the same trend with higher and lower counts noted at the same time at the three locations. Also, the lowest APC levels at interval 9 correspond with low COD and turbidity levels determined at interval 9.

ASF at the three locations differed significantly ($p \leq 0.05$) during the 12 sampling intervals (Table 8). The Wilge River had the highest mean ASF count of 2 $\log_{10}$ cfu/ml followed by the Olifants River with 1.66 $\log_{10}$ cfu/ml and the Loskop Canal's mean ASF was 1.23 log $_{10}$ cfu/ml (Figure 5). While ASF was detected in the water samples from the Wilge River during all the sampling intervals, it was not detected at sampling interval 8 in the Olifants River and intervals 8 and 11 in the Loskop Canal.

The mean AnSF count for both the Loskop Canal and the Olifants River was 1.23 log_{10} cfu/ml while the mean AnSF count for the Wilge River was 1.93 log_{10} cfu/ml.

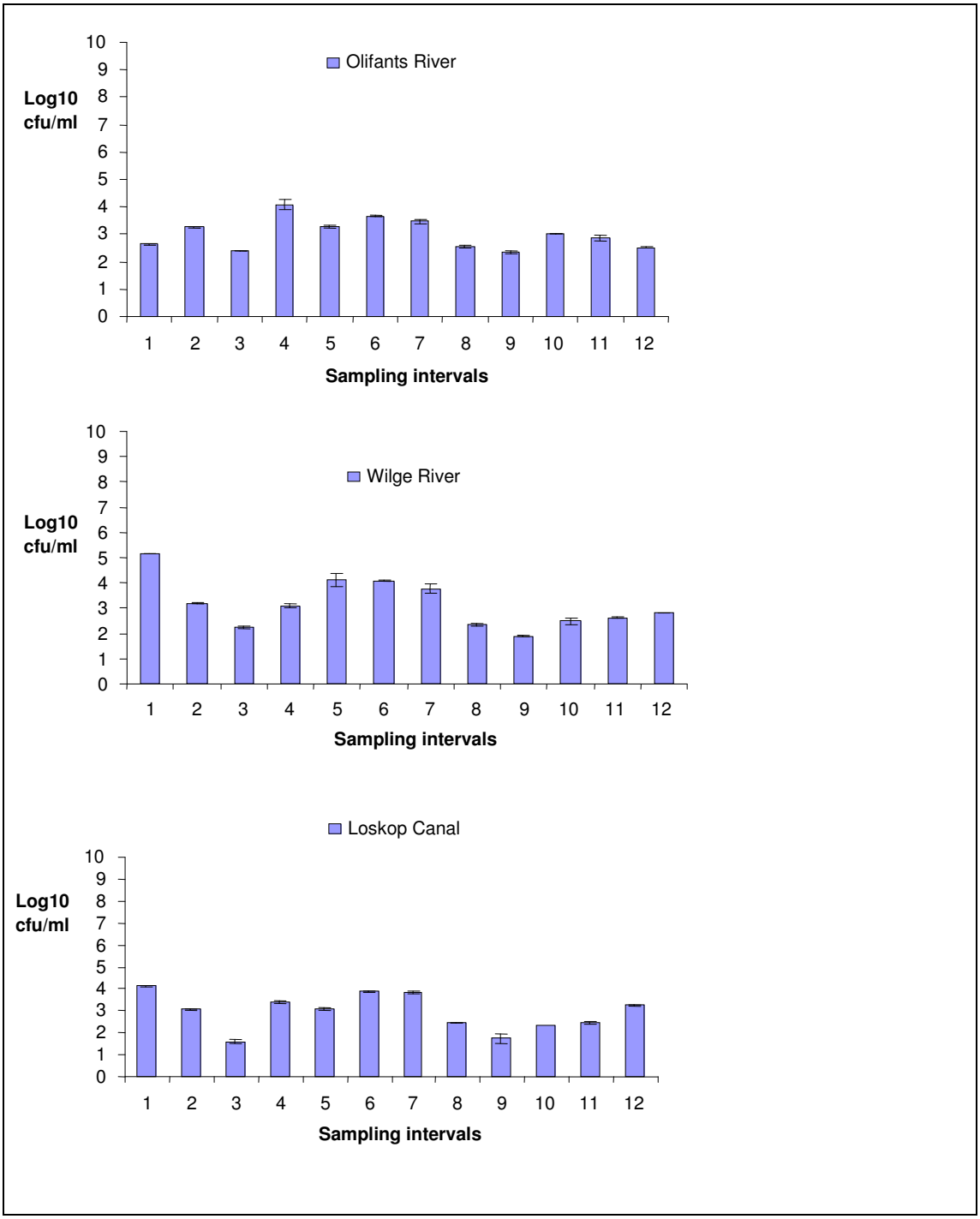


Figure 4: Aerobic colony counts (log 10cfu/ml) of water from Loskop Canal, Olifants River and Wilge River during twelve sampling intervals

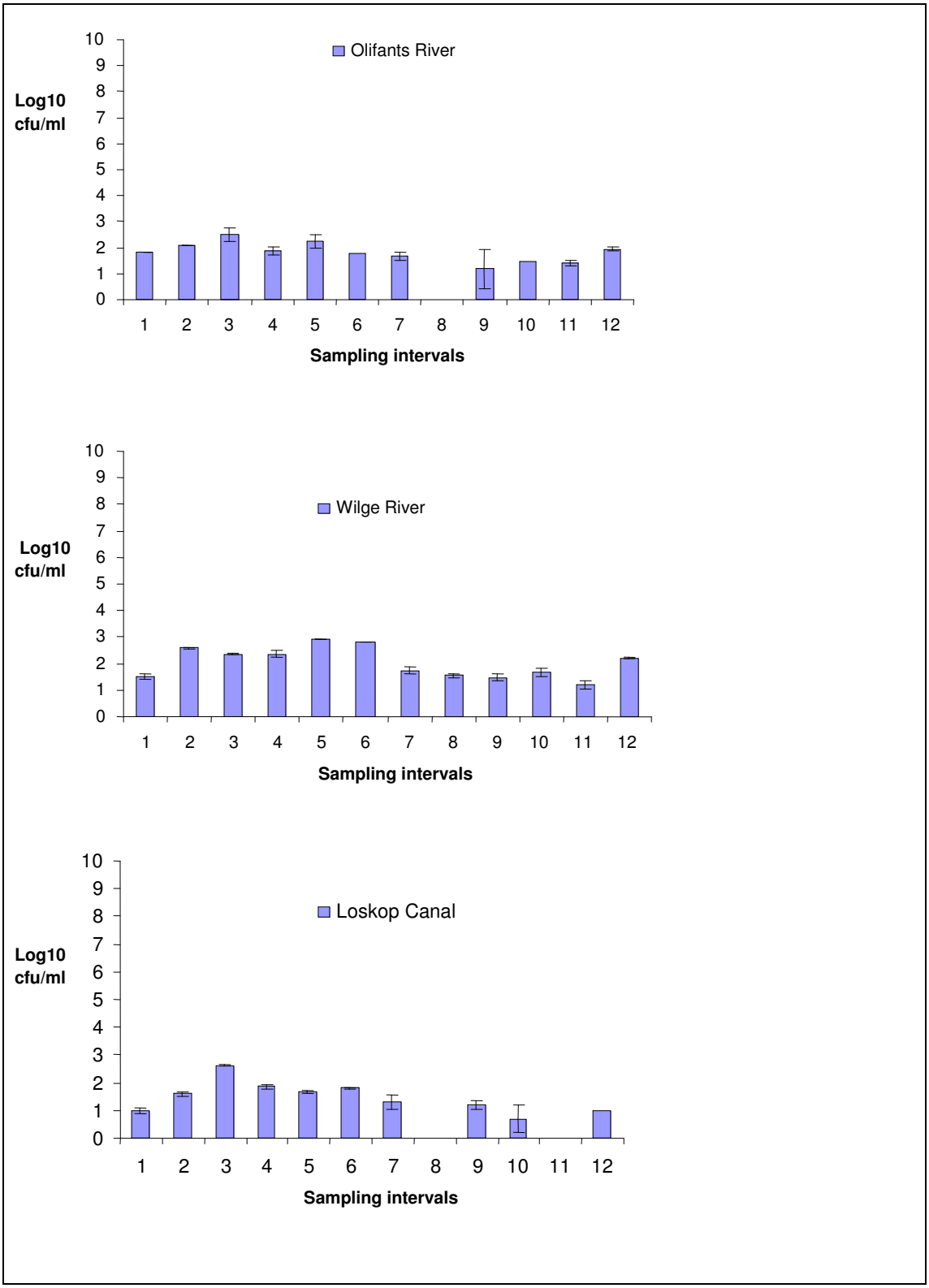
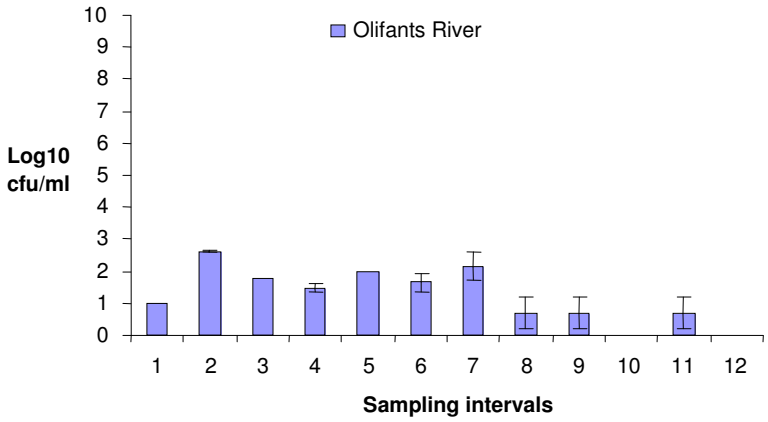
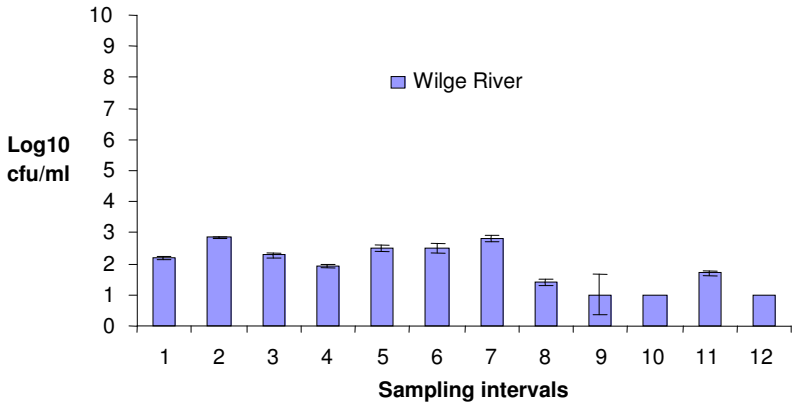


Figure 5: Aerobic sporeformer (log 10cfu/ml) counts of water from Loskop Canal, Olifants River and Wilge River during twelve sampling intervals

Similar to the ASF, AnSF was detected during all the sampling intervals in the Wilge River but it was not detected at sampling intervals 9, 11 and 12 in the Olifants River and at 10 and 12 in the Loskop Canal (Figure 6).





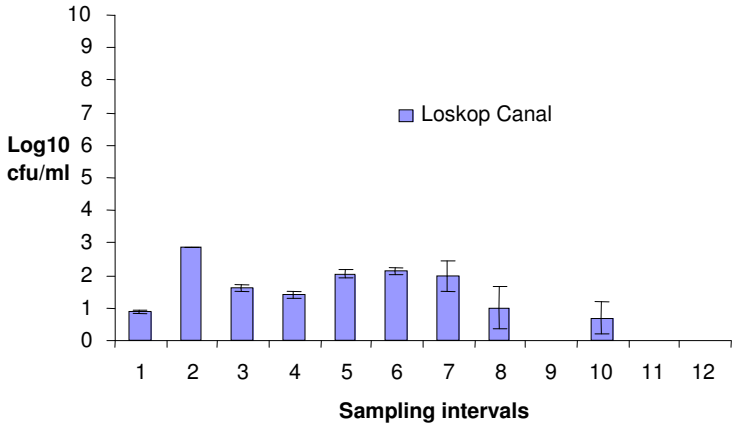


Figure 6: Anaerobic sporeformer (log10 cfu/ml) counts of water from Loskop Canal, Olifants River and Wilge River during twelve sampling intervals

Prevalence of S. aureus, E. coli, intestinal Enterococcus (IE), *Salmonella and L. monocytogenes in water from three surface water sites during the 12 sampling intervals*

Of the water samples collected during the 12 sampling intervals, 25% of the samples from the Olifants River, 33% from the Wilge River and 58% of the samples from the Loskop Canal were positive for *S. aureus* (Figure 7). However, the average *S. aureus* counts of water from the three surface water sampling sites were very low < 1 $\log_{10}$ cfu/ml. Incidence of *S. aureus* did not correspond between the sampling locations and only at interval 6 was *S. aureus* detected at all three locations (data not shown).

E. coli was recovered from the two rivers and the Loskop Canal during every sampling interval (Figure 7). Furthermore coliform and faecal coliform levels for the surface water met the international standard (1000 MPN/100ml) only once during the 12 sampling intervals in Loskop Canal water while at the Wilge River and Olifants River, the water samples met the standard during 25% and 30% of the 12 sampling intervals respectively.

IE was present in all the water samples collected from the Wilge River while incidence was lower in the Olifants River (67%) and the Loskop Canal (75%) (Figure 7). Incidence of *Salmonella* (50%) was higher in the Loskop Canal than in the Wilge River and the Olifants River (33% and 42% respectively). However, the incidence of *L. monocytogenes* (58%) in the Wilge River was higher than the 50% incidence observed in both the Loskop Canal and the Olifants River during the 12 sampling intervals (Figure 7).

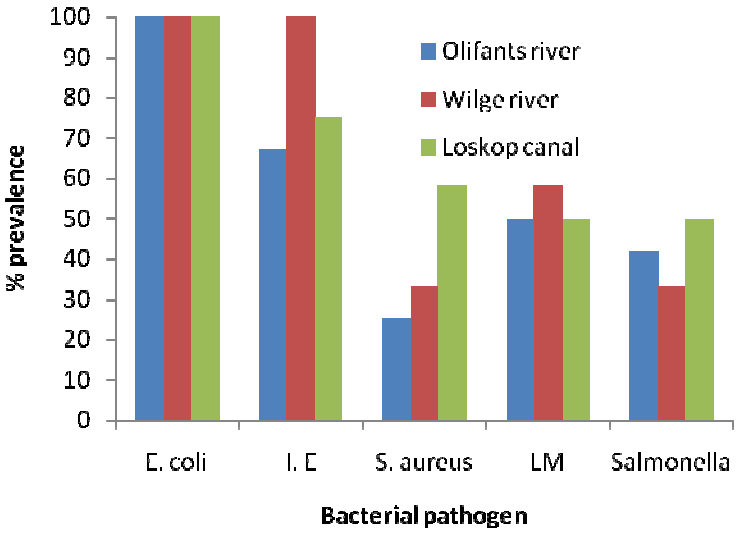


Figure 7: Prevalence of bacterial pathogens in the three water sources during twelve sampling intervals

Incidence of aerobic bacteria, aerobic spore bacteria and anaerobic spore bacteria on broccoli and cauliflower

The average APC, ASF and AnSF on the vegetables followed a similar trend. Although the numbers of the different groups of indigenous bacteria on broccoli were higher than on cauliflower during the three sampling intervals, the difference was less than 1 log (Figure 8).

The average APC on cauliflower was 3.8 $\log_{10}$ cfu/g while it was 4.1 $\log_{10}$ cfu/g on broccoli. Similarly, the average ASF and AnSF were also higher on broccoli. ASF on broccoli and cauliflower were 2 $\log_{10}$ cfu/g and 1.5 $\log_{10}$ cfu/g respectively while AnSF on broccoli and cauliflower were 1.6 $\log_{10}$ cfu/g and 1.4 $\log_{10}$ cfu/g 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 ($P \leq 0.05$) (Table 9). However, there was significant difference in aerobic colony count, aerobic spore counts and anaerobic spore counts in the two vegetables from the individual farms (Table 9).

The average APC in the three water samples from the Loskop Canal, Wilge River and Olifants River was lower than that on the two vegetables. However, the average ASF and AnSF were similar. Average APC, ASF and AnSF were 3.0, 1.6, 1.5 log_{10} cfu/ml while they were 3.9, 1.8, and 1.5 log_{10} cfu/g respectively on vegetables.

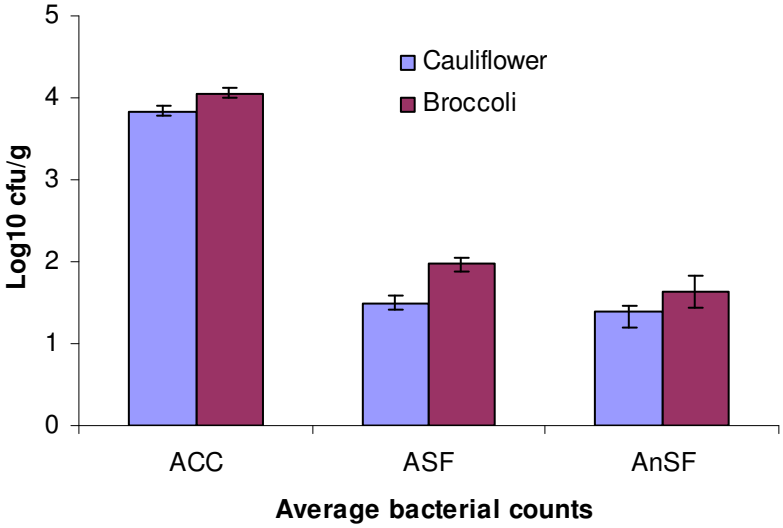


Figure 8: The average ACC, ASF, and AnSF on broccoli and cauliflower during three sampling intervals

Table 9: Analysis of variance for ACC, ASF, and AnSF of broccoli, cauliflower and irrigation water from the Loskop Canal during 3 sampling intervals

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: $p \leq 0.05$

Incidence of S. aureus, E. coli, intestinal Enteroccoci (I. E), Salmonella and L. monocytogenes (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 $\log_{10}$ cfu/ml (Figure 9).

E. coli was recovered from the Loskop Canal, in cauliflower and broccoli during the three sampling intervals. Incidence of intestinal *Enterococcus* on broccoli was higher than that on cauliflower. The incidence was 44% and 33% respectively. However, it was 67% in the Loskop Canal. Also, the incidence of *Salmonella* (33%) in the Loskop Canal was higher than the 11% incidence observed on broccoli and cauliflower (Figure 9). Only broccoli was positive for *L. monocytogenes* during the three sampling intervals. However, *L. monocytogenes* were recovered from the Loskop Canal at other sampling intervals when vegetables were not examined. Also, with the exception of *L. monocytogenes* that was not recovered from cauliflower, all the bacterial pathogens isolated from the three water sources were also isolated from the two vegetables.

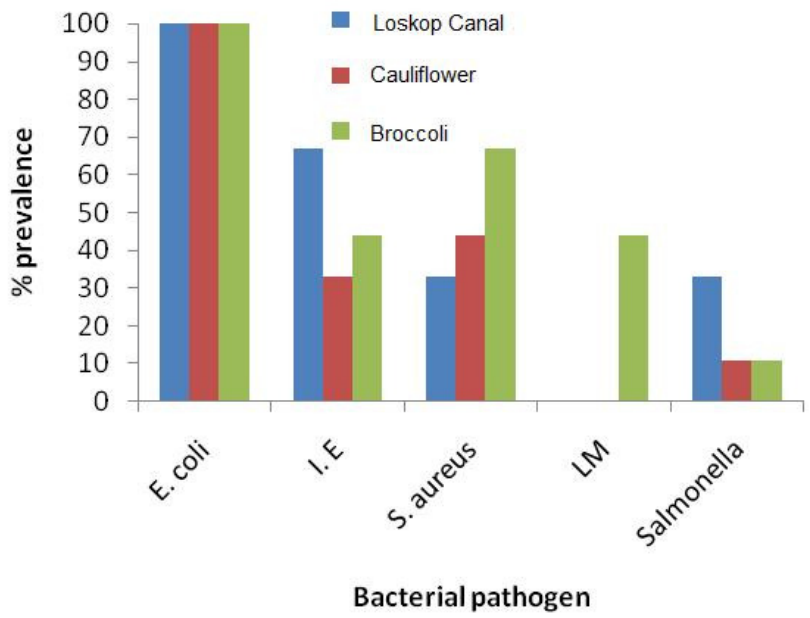


Figure 9: Prevalence of bacterial pathogens in the Loskop Canal and the two vegetables during three sampling intervals

3.1.4 Discussion

The temperature and pH values of the Loskop Canal and the two rivers that were conducive for bacterial growth may have influenced the survival of aerobic bacteria and bacterial pathogens in the water sources. According to Pautshwa *et al.* (2009), 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–d). The turbidity range for water of good quality should be between 0 to 1NTU. The high turbidity level of surface water in this work corresponds with the river turbidity results of Fatoki *et al.* (2003). Fatoki *et al.* (2003) also found high turbidity levels in surface water indicated that soil erosion and run-off could be a source of high turbidity in the water system. The soil erosion and run-off could have been caused by the informal settlements around the two rivers. The COD results for all three water samples from Loskop Dam, Olifants River and Wilge River also did not meet

the WHO standard of 10mg/litre. This shows that the surface water contains organic pollutants that may have originated from the informal settlements and mines around the region where rivers are located.

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

The recovery of aerobic sporeformers from the three water samples is similar to the work of Fournnelle (1967) who recovered them from Alaska water at the same low level. However, the level of anaerobic sporeformers observed in our water samples was lower than has been reported by Molongoski and Klug (1976). Molongoski and Klug (1976) recovered up to 6 log of anaerobic sporeformers from freshwater lakes. Although low level of aerobic sporeformers were observed in the water samples, it may be unsuitable for the irrigation of fresh produce because of the possibility of microbial growth and cell division after attachment and infiltration on the vegetables.

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

Although recovery of *S. aureus* from water samples is low, it may still pose a problem if such irrigation water is used for the production of produce that are

eaten raw (Khetarpaul, 2006). *S. aureus* was not expected to be recovered from the Loskop Canal, Wilge River or the Olifants River because its primary reservoir is the nasal cavity of humans (Jay, 2000). The presence of *S. aureus* in the two rivers and Loskop Canal also shows that the rivers may have contributed to the contamination level in the canal.

The result of heavy contamination of the three water sources, with *E. coli* and faecal coliforms corresponds to the work of Tshivhandekano (2006) on the Apies River, South Africa. This shows that the concern regarding contamination of surface water sources in SA may be valid and widespread. The two rivers may have been polluted with human faeces since *E. coli* and faecal coliforms are indicators of faecal pollution (Garcia & Servais, 2007). Human faeces contain higher faecal coliform counts, while animal faeces contain higher levels of faecal *Enterococci* (Gildreich & Kenner, 1969; Pautshwa *et al.,* 2009). The high incidence of *E. coli*, faecal coliforms and intestinal *Enterococcus* in the two rivers and the Loskop Canal indicate that the rivers are potential sources of contamination of the canal. In addition, the source of this contamination may be the informal settlements along the two rivers.

Contamination of water sources with other bacterial pathogens, namely, *L. monocytogenes* and *Salmonella* show that the two rivers and canal are of poor microbiological quality possibly as a result of faecal pollution. It also indicates that the two rivers are potential sources of contamination of the Loskop Canal. Other workers have reported the widespread contamination of faecal polluted surface water with these pathogens and this is a public health concern especially when water is used for agricultural purposes (Tymczyna *et al.,* 2000; Lyautey *et al.,* 2007; Garcia & Servais, 2007). According to Bhagwat (2006), the greatest concerns with human pathogens on fresh and minimally processed vegetables are *E. coli* 0157:H7, *Salmonella and L. monocytogenes.* The first two have low infective doses while *L. monocytogenes* grow very well under refrigeration storage conditions

(Bhagwat, 2006). Another safety concern with these pathogens is that they can form biofilms on the produce thereby making sanitizers ineffective (Somers *et al.,* 1994; Fonseca, 2006).

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

A lower incidence of *S. aureus*, *Salmonella*, intestinal *Enterococcus* and the absence of *L. monocytogenes* on cauliflower compared with broccoli show the possibility of differences in surface characteristics of the two produce affecting pathogen attachment and survival (Ukuku *et al.,* 2005; Fonseca, 2006). 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 study clearly indicates the potential effect of raw sewage spillage, informal settlements and wastewater from mines and industries on irrigation water sources and pre-harvest vegetables.

3.1.5 Conclusion

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

3.2 EFFECT OF ATTACHMENT TIME FOLLOWED BY CHLORINE WASHING ON THE SURVIVAL OF INOCULATED *LISTERIA MONOCYTOGENES* ON TOMATOES AND SPINACH

Abstract

The effect of attachment time (30 min, 24, 48 and 72 h) followed by chlorine washing (200 ppm) on the survival of inoculated *Listeria monocytogenes* on the surface and subsurface of tomatoes and spinach was studied. The work was done to determine the efficacy of chlorine to decontaminate surface and subsurface pathogens that may have come into contact with produce during pre-harvest. Tomatoes and spinach leaves were inoculated with a 6 log cfu/ml 18 h culture of *L. monocytogenes* ATCC 7644 (LM) on the surface and subsurface and incubated at 20 ℃ for either 30 min, 24, 48 or 72 h. LM attached and survived on the surface and subsurface structures of both control and chlorine-washed vegetables after each attachment time, up to 72 h. Higher levels of LM attachment and survival were however noticed on the subsurface structures. Chlorine had a greater effect on the LM on the surface structures compared with those in the subsurface structures, possibly because chlorine was not able to access the subsurface structures where the pathogens were located. Chlorine was not effective in totally inactivating the surface LM on spinach and tomato. This research indicated that LM could attach to both surface and subsurface structures of both tomatoes and spinach within 30 min, and that even after 72 h it still remained viable. It also indicated that chlorine treatment is more effective against surface LM compared to subsurface inoculated LM.

3.2.1 Introduction

A major pre-harvest source of contamination of produce is irrigation water (Beuchat & Ryu, 1997; Beuchat, 2002). Ibenyassine *et al.* (2006) reported that contaminated irrigation waters and surface run-off waters are the major

sources of pathogenic microorganisms that contaminate fruit and vegetables. Steele *et al.* (2005) carried out a survey on 500 irrigation water samples used for production of fruit and vegetables in Canada and found about 25% of the samples to be contaminated with faecal *E. coli* and faecal *Streptococci*. Surface water when used to irrigate produce poses a health risk of contamination with *Salmonella* (Johnson *et al.,* 1997). Most surface waters were also found to be contaminated with *Listeria.* Combarro *et al.* (1997) frequently isolated *Listeria* species from river water in Spain. Pathogens in irrigation water can attach to the surface of vegetables during pre-harvest (Ijabadeniyi, Minnaar & Buys 2008; Solomon *et al.,* 2006; Kenney & Beuchat, 2002; Ruiz Vargas & Garcia-Villanova, 1987).

Different researchers have shown that attachment of *Listeria monocytogenes* is possible through the release of an enzyme to the surrounding host tissue or produce to facilitate bacterial attachment and infiltration (Hall-Stoodley & Stoodley, 2005; Jedrzejas, 2001). It has been reported that extracellular fibrils and flagellin have also been used by *Listeria monocytogenes* to enhance attachment (Kalmokoff *et al*., 2008; Lemon *et al*., 2007). After attachment, they can gain access to the subsurface structures through natural openings and wounds on vegetable surfaces; a process called internalization (Warriner *et al.,* 2003; Bartz, 2006; Solomon *et al.,* 2006). Internalization is possible because of natural openings such as stem scars, stomata, lenticels, root systems and broken trichomes (Quadt-Hallman *et al.,* 1997; Allen *et al.,* 1990), as well as due to damage of the waxy cuticles on the plant tissues (Solomon *et al.,* 2006; Ukuku *et al.,* 2005).

Chlorine is routinely used as a sanitizer in wash, spray and flume waters in the fresh and minimally-processed fruit and vegetable industries (Fonseca, 2006; Bhagwat, 2006; Beuchat, 1999). Antimicrobial activity depends on the amount of sodium hypochlorite in water that comes into contact with microbial cells (Beuchat & Ryu, 1997; Beuchat *et al.,* 1998). The concentration normally used is between 50–200 ppm and the contact time is 1–2 min

(Beuchat, 1998). In South Africa, sodium hypochlorite is commonly used to sanitize fresh vegetables (Clasen & Edmondson, 2006).

Antimicrobial agents, such as chlorine, hydrogen peroxide and ozone are not effective in completely eliminating all the bacteria on the surface of plants or vegetables (Solomon *et al.,* 2006; Doyle & Erickson, 2008). Internalization is a major problem in the fresh-produce industry because pathogens that are present within the subsurface structures of plants or vegetables are protected from the sanitizing effect of antimicrobial agents such as chlorine, hydrogen peroxide and ozone (Solomon *et al.,* 2006; Doyle & Erickson, 2008).

Although much research has reported on the ability of pathogens such as *E. coli* O157:H7 and *Salmonella* spp. to attach and gain access to the subsurface structures of vegetables, not many reports have focused on *L. monocytogenes* (Beuchat, 1996). *L. monocytogenes* has the potential to cause human listeriosis after consumption of contaminated raw vegetables (Beuchat, 1996). *L. monocytogenes* has the ability to overcome food preservation and safety barriers such as refrigeration temperature, low pH and high salt concentration (Gandhi & Chikindas, 2007; Gorski, Palumbo & Nguyen, 2004; Brandl, 2006). Broccoli, cabbage, salad greens and other vegetables pose even a higher risk of being associated with listeriosis because of enhanced *L. monocytogenes* attachment (Ijabadeniyi *et al.,* 2009; Ukuku *et al.,* 2005; FDA/CFSAN, 2008). Attachment to and growth on some produce including spinach have been reported (Gorski *et al.,* 2004; Jablasone, Warriner & Griffiths, 2005).

The aim of this study was therefore to determine the effect of attachment time on the survival of *L. monocytogenes* on the surface and subsurface structures of tomatoes and spinach. Subsequently, the effect of chlorine on the subsurface and surface of *L. monocytogenes* on tomatoes and spinach after harvest was determined

3.2.2 Materials and methods

Reference strain

Listeria monocytogenes ATCC 7644 (LM) was obtained from the Agricultural Research Council, Irene, South Africa. The strain was cultured in Fraser Broth (FB) (Oxoid Ltd; Basingstoke, Hampshire, England) for 24 h at 37 °C and then stored at 4 °C. The working stock culture was subcultured into FB twice a month.

Tomatoes and spinach

Fresh tomatoes and spinach were purchased from a retail outlet on three separate occasions in Pretoria (South Africa). Tomatoes and spinach were examined and those with visual defects were not used. Tomatoes and spinach were washed with 70% alcohol and tested for the presence of LM.

Inoculation of surface and subsurface structures of tomatoes with L. monocytogenes ATCC 7644

A 6 log cfu/ml, 18 h culture of LM, determined using McFarland standards (Andrews, 2005), was used as inoculum for all the experiments. This method uses optical density to determine titer. Eight tomatoes were inoculated on the surface and eight within the subsurface per experimental repetition. The experiment was repeated three times. To inoculate the tomatoes within the subsurface structures, wounding was first simulated at five locations per tomato by using a sterile 1 ml plastic pipette tip, according to the method of Walderhaug *et al.* (1999). Five locations on the tomatoes were inoculated with 0.2 ml LM, to allow for even distribution of the inoculum into the tomato (Walderhaug *et al.,* 1999). To inoculate the surface of the tomatoes 1 ml of LM was released over the side of the surface of each tomato with a sterile

pipette. Tomatoes were brought into contact with roll-off liquid on the sterile inoculating dish, using sterile tweezers, to ensure that roll-off liquid was absorbed onto the tomato surface.

Inoculation of surface and subsurface structures of spinach with L. monocytogenes ATCC 7644

Eight spinach leaves were inoculated on the surface and eight within the subsurface per experimental repetition. To inoculate the spinach on the subsurface structures, a sterile needle was used to make a thin line in-between the leaf petiole (stem of a leaf) and 1 ml of the LM culture was introduced across the thin line (Walderhaug *et al.,* 1999). To inoculate the surface of spinach leaves, a sterile pipette was used to release 1 ml of the LM culture over its surface while the leaves were lying flat. After inoculation, they were allowed to attach and the extent of attachment of LM was studied.

Chlorine washing of inoculated vegetables

After attachment of LM for 30 min, both surface-inoculated and subsurface inoculated tomatoes were washed by dipping into 200 ppm of chlorine for 1 min (Beuchat, 1998). The control was washed by dipping into distilled water. To disallow tomatoes from floating during washing, sterile tweezers were used to submerge the tomatoes in the chlorine water. The procedure was repeated for the treated and control samples after attachment of LM for 24, 48, and 72 h respectively.

After attachment of LM for 30 min, both surface-inoculated and subsurface inoculated spinach leaves were washed by dipping into 200 ppm of chlorine for 1 min (Beuchat, 1998). The control was washed by dipping into distilled water. The procedure was repeated for the treated sample and control after attachment of LM for 24, 48, and 72 h respectively.

Enumeration of L. monocytogenes ATCC 7644 on the surface and subsurface structures of vegetables

To enumerate the number of LM on tomatoes, at each attachment time interval, on the surface and within the subsurface, about 100 g (one whole tomato) of tomato was added to 900 ml of distilled water after which maceration in a Stomacher lab-blender 400 (Fisher Scientific, Mississauga, Canada) and plating on Palcam Agar (Oxoid Ltd; Basingstoke, Hampshire, England) were done. Enumeration of LM was done with the pour-plate method.

To enumerate the number of LM on spinach leaves at each attachment time interval on the surface and within the subsurface, about 10 g of spinach leaf was added to 90 ml of distilled water after which maceration in a Stomacher lab-blender 400 (Fisher Scientific, Mississauga, Canada) and plating on Palcam Agar (Oxoid Ltd; Basingstoke, Hampshire, England) were done. Enumeration of LM was done with the pour-plate method.

Preparation and observation of specimens for SEM

Pieces of tomato/spinach (about 2 by 2 mm area and 0.5 mm thickness) were gently cut off the inoculated surface of each tomato/spinach sample using a sterile blade. The cut pieces were fixed overnight in 4% glutaraldehyde and rinsed twice with 0.1 M sodium phosphate buffer pH 7.0. The samples were further fixed in 2% osmium tetroxide for 1 h and rinsed twice with 0.1 M sodium phosphate buffer. Fixed samples were dehydrated in a graded ethanol series (30%, 50%, 70% and 100%). All procedures through to dehydration were carried out at about 48 ℃. The samples were dried in a LADD Critical-Point Drier (LADD Research Industries, Inc., Burlington, Vermont, USA) with CO_2 as the transition gas. They were then mounted on specimen stubs and coated with approximate 30 nm layer of gold-palladium using a Hummer I sputter coater (ANATECH, LTD, Springfield, Virginia, USA).

The samples were examined with a JEOL JSM-840 scanning electron microscope (JEOLUSA Inc., Peabody, Massachusetts, USA) at an accelerating voltage of 5 KV. Digital micrographs were collected at a resolution of 1280 x 960 and dwell time of 160 s. The digital images were adjusted using Adobe PhotoShop 5.0 and printed with a Codonics 1660 dye sublimation/thermal printer (Codonics, Inc., Middleburg Heights, Ohio, USA) using the thermal method.

Statistical analysis

Analysis of variance (ANOVA) was used to determine whether there was a significant difference between the following factors: inoculation site (surface vs. subsurface), chlorine and attachment time. The experiment was repeated three times (n=3). ANOVA was performed using Statistica from Windows, version 7 (Tulsa, Oklohama, USA, 2003).

3.2.3 Results

Effect of attachment time followed by chlorine washing on the survival of inoculated Listeria monocytogenes on tomatoes

Effect of attachment time

Attachment time, significantly ($p \leq 0.05$) affected the LM count on the surface and subsurface structures of tomatoes (Table 10). LM attached and survived on the tomato after each attachment time. The level of LM that survived and attached to the surface of tomato was lowest after 24 h (3.81 log cfu per tomato) and highest after 72 h (4.78 log cfu per tomato) (Fig 10). The level of LM that survived and attached to the subsurface of tomato was at similar levels after 30 min, 24 and 48 h, but increased significantly after 72 h of attachment time, to reach 5.39 log cfu per tomato (Fig 10). The greatest effect of attachment time was therefore observed after 72 h of attachment to

both surface and subsurfaces of tomatoes. The ability of LM to attach to the surface of tomato after 24 h of attachment was illustrated using a scanning electron microscope (Figure 11).

Table 10: P values of effect of chlorine, site and attachment time on survival of inoculated *Listeria monocytogenes* on tomatoes and spinach

Treatment effect	P value for tomato	P value for spinach
Chlorine	0.001*	0.001*
Site	0.001*	0.001*
Attachment time	0.001*	0.246
Chlorine x Site	0.722	0.528
Chlorine x Attachment time	0.031*	0.021*
Site x Time	0.542	0.821
Chlorine x Site x Time	0.496	0.649

* Denotes statistical significant of treatment at $p \leq 0.05$

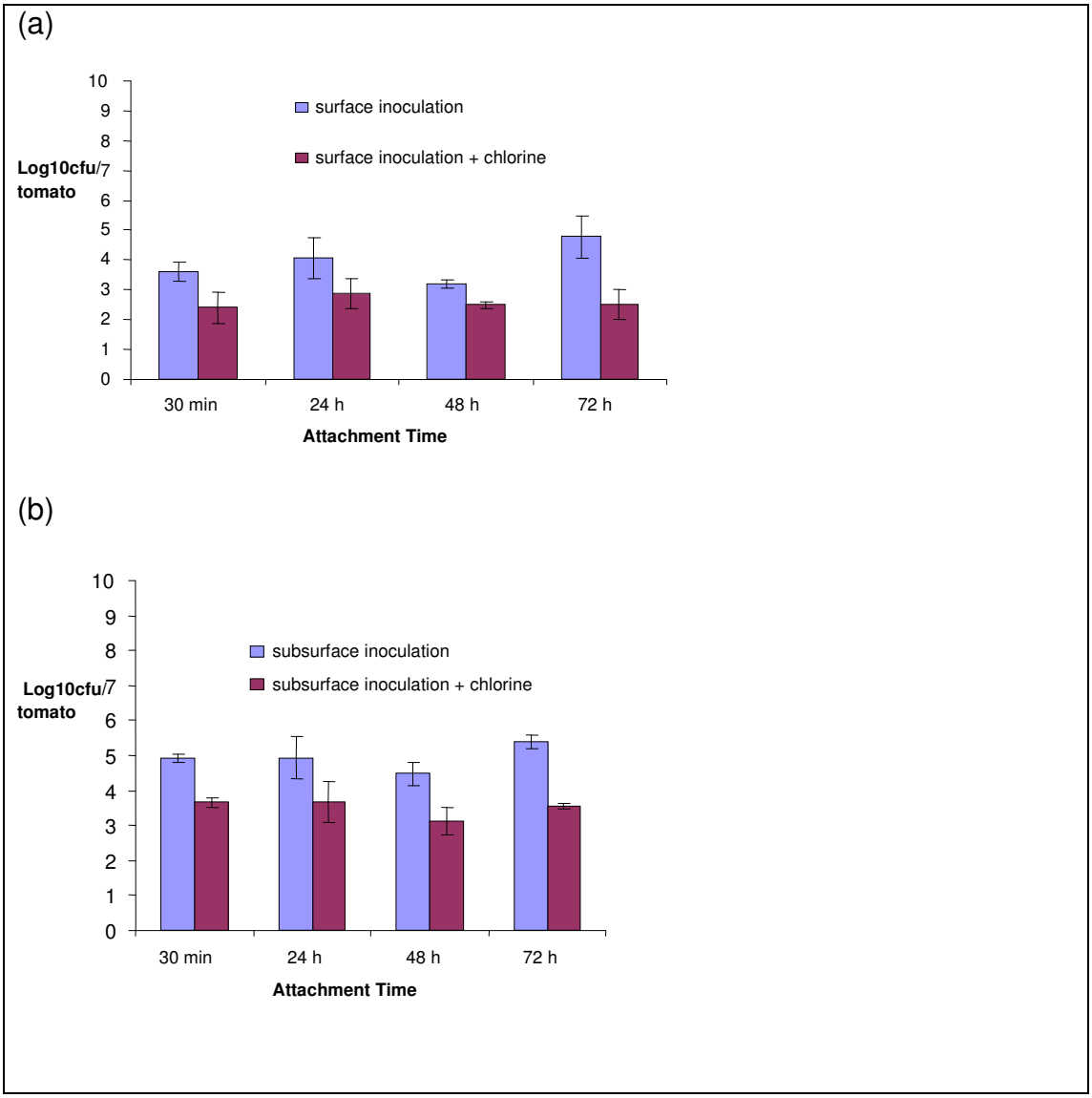


Figure 10: Attachment and survival of *L. monocytogenes* on the surface (a) and subsurface (b) of tomatoes with or without chlorine washing

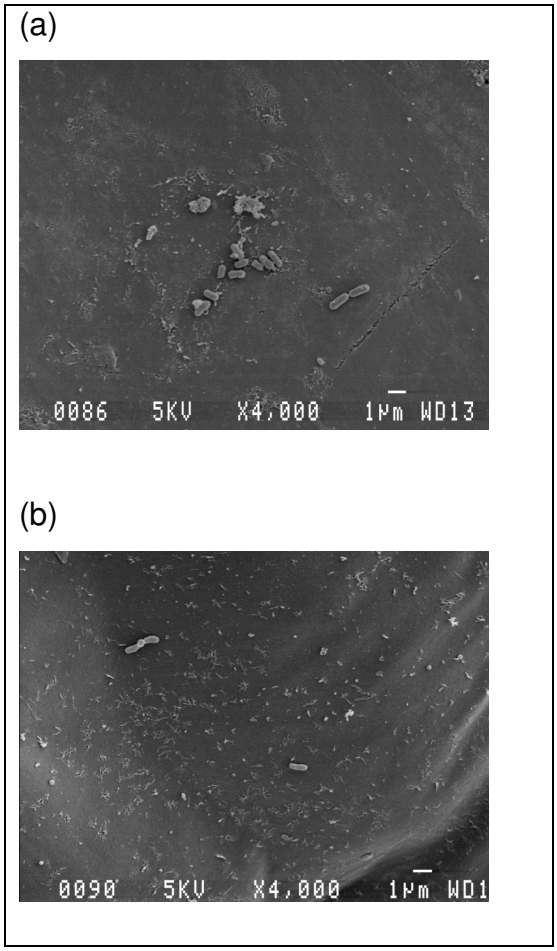


Figure 11: Scanning Electron Microscopy (SEM) of attachment of LM to the surface of tomato (a) and spinach (b) after 24 h

Effect of chlorine

Overall, chlorine affected the LM counts significantly ($p \leq 0.05$) (Table 10). There was a significant difference ($p \leq 0.05$) between the LM counts for the control (washed with distilled water) and the inoculated tomatoes washed with chlorine in both surface and subsurface inoculated samples and after each attachment time (Figure 10, Table 10). The ability of LM to survive the sanitizing effect of chlorine after attachment to tomatoes for 24 h was illustrated using a scanning electron microscope (Figure 12).

After all attachment times, the LM levels for the control samples were higher than those for the chlorine-washed samples. After 30 min of attachment time

for the surface-inoculated tomatoes, there was a 1.21 log cfu per tomato difference in LM levels between the control and the chlorine-washed tomatoes. After 72 h attachment time, the difference between the surface-inoculated control and the chlorine-washed tomatoes was significantly higher than for the other attachment times, i.e., 2.26 log cfu per tomato (Figure 10).

The LM levels for the subsurface-inoculated tomatoes followed the same trend, i.e., LM levels for the control higher than LM levels for the chlorine washed at different attachment times (Fig 10). The differences in LM on the subsurface of control tomatoes and the treated ones followed the same trend as the surface-inoculated samples. However, the effect after 72 h was not as pronounced as that between the two treatments.

Effect of inoculation site

There was a significant difference ($p \leq 0.05$) between the subsurface-inoculated LM and surface-inoculated LM in tomatoes at different attachment times (Table 10). The LM levels for the subsurface-inoculated tomatoes were higher for both control and chlorine-washed samples at each attachment time, than those of the surface-inoculated tomatoes. The differences in LM between subsurface-inoculated and surface-inoculated control samples, decreased as the attachment time increased, i.e., 1.3 log cfu per tomato after 30 min and 0.6 log cfu per tomato after 72 h of attachment (Fig 10). For the chlorine-washed tomatoes the differences in LM, subsurface-inoculated and surface-inoculated did not follow a similar trend, with the greatest difference in LM counts between the treatments after 30 min and 72 h of attachment, namely, 1.26 and 1.04 log cfu per tomato respectively.

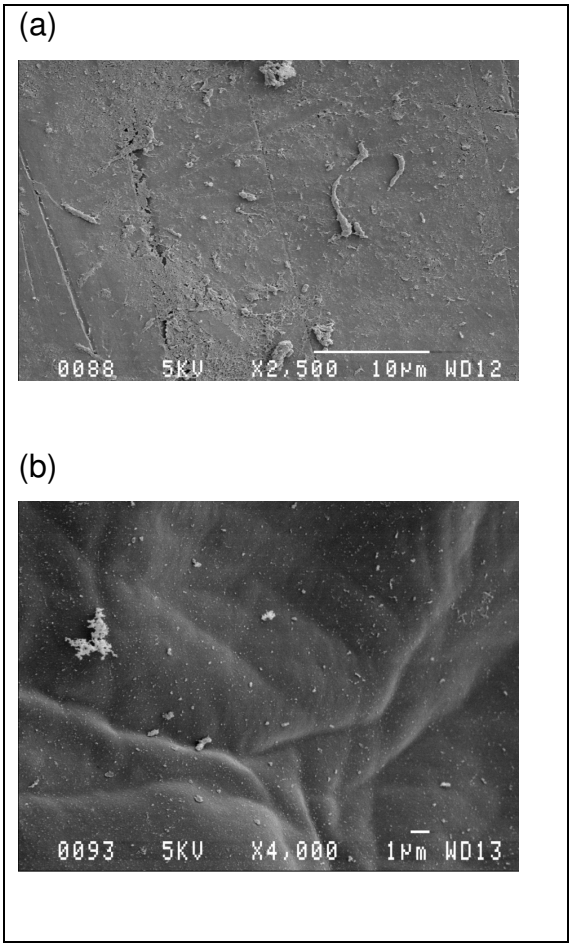


Figure 12: Scanning Electron Microscopy (SEM) of attachment of LM to the surface of tomato (a) and spinach (b) after 24 h followed by chlorine washing

Effect of attachment time and chlorine washing on the survival of inoculated Listeria monocytogenes on spinach

Effect of attachment time

Attachment time did not significantly ($p \geq 0.05$) affect the LM count on the surface and subsurface structures of (Table 10). LM attached and survived on the spinach after each attachment time as observed for tomato. The level of LM that survived and attached to the surface of spinach reduced as attachment time increased, 4.86 log cfu per leaf after 30 min and 3.41 log cfu per leaf after 72 h (Figure 13). The level of LM that survived and attached to the subsurface of spinach followed the same trend, reducing with increased

attachment time, 5.17 log cfu per leaf after 30 min and 4.18 log cfu per leaf after 72 h (Figure 13). The ability of LM to attach to the surface of spinach after 24 h of attachment was shown with a scanning electron microscope (Figure 11).

Effect of chlorine

As for tomato, overall, chlorine affected the LM counts significantly ($p \leq 0.05$) (Table 1). There was a significant difference ($p \leq 0.05$) between the LM counts for the control (washed with distilled water) and the inoculated spinach washed with chlorine in both surface-inoculated and subsurface-inoculated samples and after each attachment time (Table 10). The ability of LM after attachment to spinach for 24 h to survive the sanitizing effect of chlorine was illustrated using a scanning electron microscope (Figure 12).

At all attachment times the LM levels for the control samples were higher than for those of the chlorine-washed samples. After 30 min of attachment time for the surface-inoculated spinach, there was a 3.01 log cfu per leaf difference in LM levels between the control and the chlorine-washed spinach. After 24, 48 and 72 h attachment time intervals, the differences between the surface-inoculated control and the chlorine-washed spinach reduced with increasing attachment time, i.e., 2.55, 1.38 and 1.54 log cfu per leaf respectively (Figure 13).

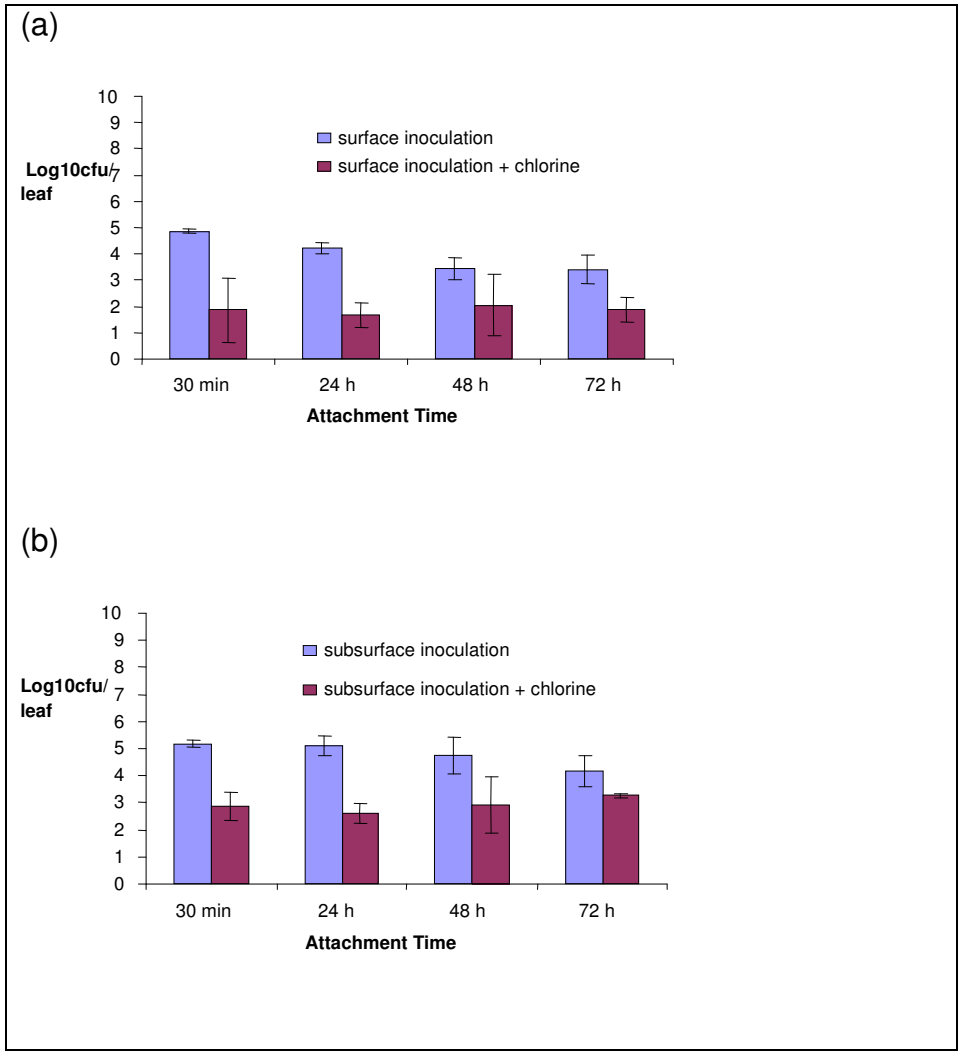


Figure 13: Attachment and survival of *L. monocytogenes* on the surface (a) and subsurface (b) of spinach leaves with or without chlorine washing

The LM levels for the subsurface-inoculated spinach followed the same trend, i.e. LM levels for the control were higher than LM levels for the chlorine-washed at different attachment times (Figure 13). The differences in LM on the subsurface of control spinach followed a similar trend as noted for the surface-inoculated samples. More than a two log difference was found after 30 min of attachment time with only a 0.91 log cfu per leaf reduction after 72 h of attachment time.

Effect of inoculation site

There was a significant difference ($p \leq 0.05$) between the subsurface-inoculated LM and surface-inoculated LM in spinach at different attachment times (Table 10). The LM levels for the subsurface-inoculated spinach were higher for both control and chlorine-washed samples at each attachment time than for those of the surface-inoculated tomatoes. The differences in LM between subsurface-inoculated and surface-inoculated control samples increased with an increase in attachment time, i.e., 0.3, 0.88, 1.31 and 0.77 log cfu/g after 30 min, 24, 48 and 72 h of attachment, respectively (Figure 13). For the chlorine-washed spinach the differences in LM, subsurface-inoculated and surface-inoculated, were comparable between attachment times. Differences in LM ranged between 0.86 and 1.4 log cfu/g (Figure 13).

3.2.4 Discussion

It was evident from the results that LM was able to attach to both the surface and subsurface structures of both spinach and tomatoes. This observation signifies that LM will attach to vegetables within 30 min of coming into contact with it in irrigation water or other sources. Although a shorter attachment time was not determined in this work, Ells and Hansen (2006) reported that LM could attach to intact and cut cabbage within 5 min of exposure to intact and cut cabbage. Other workers reported the same time range of attachment of LM to lettuce, cantaloupe and *Arabidopsis thuliana* (Li, Brackett & Beuchat, 2002; Ukuku & Fett, 2002; Milillo *et al.,* 2008; Solomon *et al.,* 2006). It is evident that attachment of pathogenic bacteria to produce occurs in a rapid manner (Fonseca, 2006; Liao & Cooke, 2001).

LM survived on the subsurface and surface of spinach and tomato up to 72 h. It has been found that pathogens could survive on tomatoes for a longer time. Elif, Gurakan and Bayindirli (2006) showed that *Salmonella enteritidis* could survive and grow during storage of tomatoes for 220 h.

The significant difference between subsurface-inoculated LM and surface-inoculated LM in both vegetables at each time interval indicates that LM attaches in higher numbers to wounds or subsurface structures than to undamaged surfaces (Takeuchi *et al.,* 2000). Timothy and Hansen (2006) showed that LM has a preference to attach to cut or wounded tissues compared to intact leaf surfaces. This may be because surface structures of vegetables constitute a harsh environment with fluctuations in temperature unlike subsurface structures (Solomon *et al.,* 2006). The subsurface structures or cut surfaces also have a significant amount of liquid containing nutrients that is utilized by the attached microorganisms (Bhagwat, 2006). Furthermore, pathogens are able to create a more hospitable microenvironment in the subsurface structures than on the surface structures (Sapers, 2001).

In this study chlorine was relatively ineffective to decontaminate the surface inoculated LM on tomatoes and spinach. This observation was not different from several reports emphasizing that vigorous washing and treatment with chlorine does not remove all bacterial pathogens on fruit and vegetables (Solomon *et al.,* 2006; Doyle & Erickson, 2008). Ineffectiveness of chlorine may be due to the low concentration (200 ppm) used and the ability of LM to form biofilms (Ukuku *et al.,* 2005). According to Kim, Yousef and Chism (1998), low levels of chlorine may not be effective against certain bacteria. A higher concentration (more than 200 ppm) is not used in the produce industry because it can generate residual by-products such as trihalomethanes in the wastewater (Simpson *et al.,* 2000; Moriyama *et al.,* 2004). It may also lead to a reaction with organic residues resulting in the formation of potentially mutagenic or carcinogenic reaction products (Moriyama *et al.,* 2004; Nakano *et al.,* 2000; Nukaya *et al.,* 2001; Rodgers *et al.,* 2004; Velazquez *et al.,* 2009).

Chlorine was more effective on the surface LM than on the subsurface LM, probably because it was not able to access the subsurface structures

effectively, where the pathogens were located (Doyle & Erickson, 2008; Fonseca, 2006; Sapers *et al.,* 1990). This is in line with the observation of Liao & Cooke (2001) who found that *Salmonella* Chester survived chlorine washing to a much greater extent when attached to the subsurface structures of green pepper disks than on surface structures. According to Seymour *et al.* (2002), entrapped or internalized pathogens are not readily accessible to chlorine because of the components, i.e., organic matter coming from the tissue exudates. The organic matter is able to neutralize some of the chlorine before it reaches the microbial cells (Bhagwat, 2006).

Chlorine was more effective on surface-inoculated LM after 30 min attachment time compared to 72 h attachment time in spinach. This is in agreement with the work of Ukuku and Sapers (2001) who confirmed that *Salmonella* serovar Stanley populations in cantaloupes was reduced by 3 log cfu/ml after a sanitizer was applied immediately after inoculation but there was reduction by less than 1 log when sanitizer was applied 72 h post-inoculation. The effectiveness of chlorine at an earlier attachment time was expected because sanitizer will easily remove a pathogen that has just attached to the surface of produce compared to the one that has attached over a longer period of time (Sapers *et al.,* 1990). However, this was not the case in tomatoes in which chlorine was more effective on the surface-inoculated LM after 72 h attachment time compared to an attachment time after 30 min. This is because the effectiveness of sanitizer on microbial reduction is dependent on the type of vegetable at any given attachment time (Abadias *et al.,* 2008; Ukuku *et al.,* 2005). The difference may also be as a result of pathogen attachment, infiltration, internalization and biofilm formation which affect sanitizer effectiveness and vary from one produce to another (Ukuku *et al.,* 2005). Also according to Fonseca (2006), differences in surface characteristics of the produce, the physiological state of a pathogen, and environmental stress conditions interact to influence the activity and efficiency of the sanitizer. It may therefore be necessary to customise sanitizing

treatments for different types of produce because of this complexity (Bhagwat, 2006).

3.2.5 Conclusion

This work shows that *Listeria monocytogenes* will attach to spinach and tomato within 30 min and it will remain viable after attachment even up to 72 h. Other authors have reported a shorter attachment time of LM on other vegetables. Also, there is a difference in the attachment and survival of LM in both vegetables, showing that attachment and survival of LM vary from one vegetable to another. The present study also confirms that chlorine is more effective on the pathogens on the surface of vegetables than on the subsurface, as it could reduce only ≤ 3 logs inoculated and attached LM both on the surface and subsurface structures.

3.3 BACTERIAL PATHOGENS IN IRRIGATION WATER AND ON PRODUCE ARE AFFECTED BY CERTAIN PREDICTOR VARIABLES

Abstract

The possibility of predicting the presence of pathogens in irrigation water and on vegetables was determined. Logistic regression analysis was used to determine whether various predictor variables could be used to predict the occurrence of *L. monocytogenes*, *Salmonella* spp and intestinal *Enterococcus* in irrigation water and vegetables (cauliflower and broccoli). It was evident that COD was statistically reliable to predict *L monocytogenes*, turbidity, reliable to predict intestinal *Enterococcus* and faecal coliforms and coliforms, and reliable to predict *Salmonella* in irrigation water. Also, while the regression analysis showed that the aerobic colony count (ACC) and aerobic sporeformer count (AnSF) could be used to predict *Salmonella* and intestinal *Enteroccus* in vegetables, *S. aureus* and ACC were indicated to be significant parameters in predicting *L monocytogenes* on vegetables. This work showed

that in addition to the common indicators, i.e., *E. coli,* faecal coliforms, and faecal *Streptococci*, the microbiological quality of irrigation water and vegetables might be indicated after physico-chemical properties and ACC.

3.3.1 Introduction

The rate of foodborne disease outbreaks caused by produce contamination increased from 0.7% in the 1970s to 13% between 1990 and 2005 (Ailes *et al.,* 2008). There are ample avenues for produce to become contaminated during production and afterwards (Beuchat & Ryu, 1997; Beuchat, 2002; Beuchat, 2006). According to Johnston *et al.* (2006), contamination takes place at different stages of the growth, harvest, packing and distribution of produce. Contaminated irrigation water sources have been reported as a major way by which fruits and vegetables become contaminated with bacteria pathogens (Ibenyassine *et al.*, 2006).

According to Ailes *et al.* (2008), improved diagnostic methods and enhancements to foodborne disease surveillance systems have helped in produce safety and vegetable recall. Another thing that may lead to improved produce safety is the use of other indicator organisms different from the common ones, i.e., faecal coliforms, faecal *Streptoccoci* and *E.coli.* Physico-chemical properties may also be used for monitoring the microbiological safety of water (Horman *et al.* 2004). Horman *et al.* (2004) found that together with *E. coli* and faecal coliform, *C. perfrigens* could be used as an indicator of water safety. Furthermore, a combination of suitable indicators such as coliform and acid-fast bacteria, coliphages, the standard plate count, and fecal *Streptococci* has been recommended for adequate monitoring (Grabow *et al.,* 1983). In fact, Harwood *et al.* (2005) believed that public health cannot be adequately protected through simple monitoring schemes based on the use of *E. coli* alone but suggested that additional parameters should be used as indicators. Scott *et al.* (2002) also confirmed that the use

of other pathogens, chemical methods, genotypic and phenotypic methods are fundamental to microbial source tracking.

Our goal was to use logistic regression analysis and some predictor variables to predict the presence of selected bacterial pathogens, i.e., *Salmonella* spp, *L. monocytogenes* and intestinal *Enterococcus* in irrigation water and vegetables. Determination of the presence of all these pathogens in irrigation water and vegetables could be costly and also time consuming. Although the use of logistic regression analysis for prediction in irrigation water and fresh produce is uncommon, Ailes *et al.* (2008) used this model to confirm that microbial concentrations on fresh produce are predicted by post-harvest processing, importation and the season. Also, the absence of some indicators in water was significant to predict its safety through the logistic regression model (Horman *et al.,* 2004).

3.3.2 Materials and methods

For the selection of rivers and vegetables, bacterial and physical analyses of samples, refer to Ssection 3.1.2.

Statistical analyses

All statistical analyses were completed by using SAS version 9.2 (SAS Institute, Inc., Cary, NC). ACC, ASF, AnSF, and S. aureus were log-transformed to satisfy the assumption of normality. The associations of the occurrence of *L. 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, *L. 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 (coliforms, faecal coliforms, COD and turbidiy)

were taken into the model. On the other hand, ACC, *S. aureus*, location, ASF, AnSF, coliforms and faecal coliforms 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.

3.3.3 Results and discussion

Predictive relationships between predictors

A pooled data set from the Loskop Canal, Olifants River and Wilge River were analysed to determine if the concentrations of any of the indicators, total coliforms, faecal coliforms, *S. aureus*, aerobic sporeformers, anaerobic sporeformers 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 faecal coliforms and total coliforms ($r = 0.999$, p-value < 0.0001), aerobic sporeformers and anaerobic sporeformers ($r = 0.535$, p-value < 0.0001), *S. aureus*, aerobic sporeformers ($r = 0.498$, p-value < 0.0001), aerobic colony counts and anaerobic sporeformers ($r = 0.354$, p-value $= 0.0002$), aerobic colony counts and *S. aureus* ($r = 0.345$, p-value $= 0.0003$); and a significant correlation was observed between anaerobic sporeformers and *S. aureus* ($r = 0.203$, p-value $= 0.0354$). Except between turbidity and *S. aureus*, chemical oxygen demand and total coliforms, chemical oxygen demand and faecal coliforms, 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 faecal coliform, location, COD and turbidity were predictive of the presence of *L. monocytogenes, Salmonella* sp and intestinal *Enterococcus* in irrigation water.

Binary logistic regression was also used to test the hypothesis that ACC, ASF, AnSF, *S. aureus*, faecal coliform and coliform were predictive of the presence of *L. monocytogenes, Salmonella* sp and intestinal *Enterococcus* on vegetables.

Prediction of L. monocytogenes, Salmonella and intestinal *Enterococcus in water samples from Loskop Canal, Wilge River and Olifants River*

Results of the logistic regression indicated that only one predictor, COD, was statistically reliable ($p \leq 0.05$) to predict the presence *L. monocytogenes.* The estimates of regression coefficients of the predictors $\hat{\beta}$, Wald statistic and p-values are presented in Table 11.

Table 11: Prediction of *L. monocytogenes* in irrigation water

Predictors	$\hat{\beta}$	**Wald**	**p-value**
Faecal coliforms	-0.0014	0.5785	0.4469
Coliforms	0.0001	0.5194	0.4711
Turbidity	-0.0199	0.6958	0.4042
COD	-0.0399	9.4825	0.0021

A p-value of ≤ 0.05 was considered statistically significant

Like the result of the prediction of *L. monocytogenes* in irrigation water samples in which only one predictor was associated with it, only one predictor, turbidity was found to be statistically significant ($p \leq 0.05$) to predict the presence of intestinal *Enterococcus* in the water samples from three sources (Table 12).

Table 12: Prediction of intestinal *Enterococcus* in irrigation water

Predictors	$\hat{\beta}$	Wald	p-value
Faecal coliforms	0.0013	0.4224	0.5157
Coliforms	-0.0001	0.3564	0.5505
Turbidity	-0.0544	5.7643	0.0164
COD	0.0264	2.4581	0.1169

A p-value of ≤ 0.05 was considered statistically significant

Faecal coliforms and coliforms however were found to be significant ($p \leq 0.05$) to predict the presence of *Salmonella* sp (Table 13).

Table 13: Prediction of *Salmonella* sp in irrigation water

Predictors	$\hat{\beta}$	Wald	p-value
Faecal coliforms	0.0048	3.8008	0.0500
Coliforms	-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

Prediction of L. monocytogenes, Salmonella sp *and* intestinal *Enterococcus on vegetables*

The result of logistic regression analysis showed that two predictors, ACC and *S. aureus*, were statistically significant (both p-values are ≤ 0.05) to predict the presence of *L. monocytogenes* on vegetables. The estimates of regression coefficients of the predictors $\hat{\beta}$, Wald statistic and p-values are shown in Table 14.

Table 14: Prediction of *L. monocytogenes* on vegetables

Predictors	$\hat{\beta}$	Wald	p-value
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 coliforms	-0.0004	0.0855	0.7700
Coliforms	0.0001	0.0830	0.7733

A p-value of ≤ 0.05 was considered statistically significant

Also, from the result of the logistic regression analysis, ACC and AnSF were observed to be significant ($p \leq 0.05$) to predict the presence of intestinal *Enteroccocus* and *Salmonella* sp respectively (Table 15 and Table 16).

Table 15: Prediction of intestinal *Enterococcus* on vegetables

Predictors	$\hat{\beta}$	Wald	p-value
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 coliforms	-0.0020	3.1176	0.0775
Coliforms	0.0002	3.3093	0.0689

A p-value of ≤ 0.05 was considered statistically significant

Table 16: Prediction of *Salmonella* sp on vegetables

Predictors	$\hat{\beta}$	Wald	p-value
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 coliforms	0.0007	0.3020	0.5827
Coliforms	-0.0001	0.2633	0.6079

A p-value of ≤ 0.05 was considered statistically significant

The result of the prediction of *L. monocytogenes* in irrigation water signifies that there may be a direct relationship between *L. monocytogenes* and COD in irrigation water. Higher COD results in water may result in a high concentration of *L. monocytogenes* in irrigation water. The reason why other predictors, i.e., faecal coliform, coliform and turbidity were not associated with *L. monocytogenes* in irrigation water is not clear. The result also signifies that there is a direct relationship between intestinal *Enteroccocus* and turbidity. Faecal coliforms and coliforms have long been known as indicators of enteric bacteria in water (Jay, 2000). The logistic regression result proved that faecal coliforms and coliforms can be used to predict the presence of *Salmonella* sp in water and that there is relationship between faecal coliform and *Salmonella* sp. This is similar to the observation of Polo *et al.* (1998) who showed that there is a direct relationship between the presence of *Salmonella* sp and indicators of faecal pollution, i.e., coliforms and faecal coliforms in rivers, freshwater reservoirs and seawater. Ferguson *et al.* (1996) also observed that the higher the concentration of faecal coliform, the higher the recovery of *Salmonella* sp in an aquatic habitat.

The reason why faecal coliforms and coliforms were not significantly associated with *L. monocytogenes* and intestinal *Enterococcus* may be because they are not usually found in human faeces, unlike *Salmonella* sp. According to Gildreich and Kenner (1969) and Pautshwa *et al.* (2009), human faeces contain higher faecal coliform 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 prediction of *L. monocytogenes*, intestinal *Enterococcus* and *Salmonella* in irrigation with the aerobic colony count (ACC) shows that it may be an important parameter to indicate the presence or absence of these pathogens. The relationship between the three bacterial pathogens and ACC may be an

indirect one, i.e., low aerobic colony count was associated with the prevalence of *Salmonella*, intestinal *Enterococcus* and *L. monocytogenes* on vegetables.

Several workers have reported that there is an indirect relationship between indigenous bacteria and foodborne pathogens (Johnston *et al.,* 2006; Ruiz *et al.,* 1987; Ukuku *et al.,* 2005). It was also observed from our study that there was a prevalence of these bacterial pathogens in irrigation water and vegetable samples while low aerobic colony counts were observed.

The logistic regression analysis may therefore be used as a tool for a 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, Dalgaard & Tienungoon, 2000).

3.3.4 Conclusion

Faecal coliforms and coliforms indicate a high probability of *Salmonella* presence in water and they may be used as risk parameters. There is a relationship between the physiochemical properties of water i.e., COD and turbidity and certain bacterial pathogens i.e., *L. monocytogenes* and intestinal *Enterococcus*.

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

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.

CHAPTER 6: REFERENCES

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