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.