ASSESSMENT OF MICROBIAL LOADS OF THE PLANKENBURG AND BERG RIVERS AND THE SURVIVAL OF ESCHERICHIA COLI ON RAW VEGETABLES UNDER LABORATORY CONDITIONS

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Declaration

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Abstract

Over the last decade, studies on the quality of the water in many of South Africa's rivers revealed an increase in pollution levels (DWAF, 1996; DWAF, 2000). In agricultural areas of the Western Cape many farmers draw water for irrigation from nearby rivers without knowing what the microbial pollution level is. In order to investigate the current quality of river water in some of the Western Cape's major rivers and to explore the possibility of pathogen carryover from water onto produce, this research project was initiated.

In an exploratory study over a five month period, the microbiological and water chemistry of three selected sites from the upper Berg and two from the Plankenbrug Rivers were assessed. Studied organisms included *Salmonella*, Staphylococcus, *Listeria*, endosporeformers, coliforms, *Escherichia coli* and intestinal *Enterococci*, while the chemical analysis consisted of pH, alkalinity, conductivity and chemical oxygen demand (COD). Faecal coliform counts ranging from 540 to 1 700 000 cfu.100ml^{-1} and 490 to 160 000 cfu.100ml^{-1} were found for the Berg and Plankenbrug Rivers, respectively. The water temperature ranged from 12° to 21°C with COD values always below 100 mg.L^{-1}. Potential human pathogens such as *Salmonella*, Staphylococcus, *Listeria*, endosporeformers, *E. coli* and intestinal *Enterococci* were frequently isolated from all five sites that were sampled. These results are of great concern to farmers, fresh produce retailers and consumers alike as the river water is regularly drawn for irrigation of produce that is later consumed raw or after a minimal processing step. From the exploratory study it was concluded that the water from all the sites were not suitable for use in irrigation practices as they regularly exceeded the guidelines for faecal coliforms and *E.coli* as set out by South African authorities.

Irrigation with faecally polluted river water is one way that fruit and vegetables can become contaminated with foodborne pathogens. The risk of disease transmission from potential pathogens present in the irrigation water is influenced by the microbial load present and the numbers carried over to the produce. In this study the carry-over and survival of *Escherichia coli* on green beans, sugar-snap peas and cocktail tomatoes was assessed under controlled laboratory conditions. The produce was exposed to *E.coli* under different combinations of exposure times (5, 15 or 30 min), drying times (30 or 120 min) and different inoculum concentration ranges (10^7, 10^5, 10^4, 10^3 and 10^2). In all cases a reduction of at least one log value in original inoculum number was found with the 10^2 inoculums showing no survivors. Follow-up studies with an exposure time of 60 min and

increased drying times of 6 or 12 h. Neither of these parameter changes affected the variation in numbers for the same inoculum or the *E.coli* survivors. Similar *E.coli* loads to those detected on the green beans were detected on the sugar-snap peas. The number of survivors on the cocktail tomatoes was much lower than found for the beans and peas. The number of survivors on the cocktail tomatoes was much lower than found for the beans and peas. This was attributed the 'smooth' surface of the tomato skin probably making attachment of the *E.coli* bacteria difficult. With the exception of the 10^2 inoculum range, all *E.coli* survivors detected on the three types of produce studied exceeded the guideline numbers set for fresh produce. If similar survival patterns are to be found in the environment then results from this study should serve as a warning that the Plankenburg river water is unsafe for use in the irrigation of fresh produce. Some farmers are already treating river water with chlorine prior to irrigation to eliminate the chance of pathogen transfer onto produce. However, this is not a feasible solution for most farmers due to the high cost of implementing a system such as this.

Opsomming

Oor die afgelope tien jaar het studies oor die gehalte van die water in baie van Suid-Afrika se riviere 'n toename in besoedelingsvlakke getoon. Baie boere in landbou gebiede van die Wes-Kaap wat watertrek vir besproeiing uit die nabygeleë riviere is onbewus van die mikrobiese besoedelingsvlak van die rivier. Hierdie navorsingsprojek is geïnisieer ten einde die huidige gehalte van die water in sommige van die Wes-Kaap se riviere te monitor, asook die moontlikheid van patogeen oordrag van die water na vars produkte te ondersoek.

Met behulp van 'n verkennende studie oor 'n vyf maande tydperk, is die mikrobiologiese en water chemie kenmerke van drie geselekteerde moniteringspunte uit die boonste Bergrivier, en twee uit die Plankenburgrivier bemonster. Die bestudeerde organismes het ingesluit: *Salmonella, Staphylococcus, Listeria*, endosporeformers, fekale kolivorme, *Escherichia coli* en intestinale *Enterococci*. Die chemiese parameters het bestaan uit pH, alkaliniteit, geleiding, temperatuur en chemiese suurstof behoeftes (CSB). Fekale kolivorme tellings het gewissel van 540 - 1 700 000 cfu.100ml^{-1} en 490 tot 160 000 cfu.100ml^{-1} is gevind vir die Berg- en Plankenburgriviere, onderskeidelik. Die water temperatuur het gewissel van 12° tot 21°C met die CSB-waarde altyd onder 100 mg.L^{-1}. Potensiële menslike patogene soos *Salmonella, Staphylococcus, Listeria*, endosporeformers, *E. coli* en intestinale *Enterococci* is dikwels geïsoleer uit al vyf die moniteringspunte. Hierdie resultate is van groot belang vir boere, kleinhandelaars en varsprodukte verbruikers omdat die riviere se water gereeld onttrek word vir besproeiing van produkte wat later gebruik word in rou of in minimale geprosesseerde vorm. Vanuit die verkennende en uit die langer termyn studie (12 maande) is die gevolgtrekking gemaak dat die water vanuit die riviere nie geskik is vir gebruik in besproeiingspraktyke waar die riglyne vir fekale kolivorme en *E. coli*, soos uiteengesit deur die Suid-Afrikaanse owerhede, gereeld oorskry word nie.

Besproeiing met fekale besoedelde rivierwater is een manier waarop groente en vrugte besmet kan word met voedsel patogene. Die risiko vir die oordrag van moontlike patogene teenwoordig in die besproeiingswater word beïnvloed deur die mikrobiese lading teenwoordig en die getalle wat oorgedra word na die produkte. In hierdie studie is die oordrag en oorlewing van *E. coli* op groenboontjies, ertjies en tamaties onder beheerde laboratoriumtoestande beoordeel. Die produkte is blootgestel aan *E. coli* onder verskillende kombinasies van blootstellingstydperke (5, 15 of 30 min), drogingstye (30 of

120 min) en verskillende inokulum konsentrasies (10^7, 10^5, 10^4, 10^3 en $10^{2)}$. In elke geval is 'n afname van ten minste een log waarde van die oorspronklike inokulum konsentrasie gevind. Die 10^2 inokulum reeks het geen oorlewende selle getoon nie. Opvolg studies is gedoen met 'n blootstellingstyd van 60 min en verlengde drogingstye van 6 of 12 uur. Daar is gevind dat hierdie parameter veranderings die variasie in getalle vir dieselfde inokulum of die oorlewende *E. coli* nie beïnvloed het nie. Soortgelyke *E. coli* ladings wat op die groenbone aangetref is, is ook op die ertjies aangetref. Die aantal oorlewendes op die tamaties was heelwat laer as die resultate vir die boontjies en ertjies. Dit word toegeskryf aan die "gladde" oppervlak van die tamatie-vel wat waarskynlik die aanhegting van die *E. coli* selle bemoeilik. Met die uitsondering van die 10^2 inokulum reeks, het al die *E. coli* oorlewende selle gevind op die drie tipes produkte, die riglyn getalle oorskry wat vir vars produkte gestel is. As soortgelyke oorlewingspatrone gevind word in die omgewing, behoort die resultate van hierdie studie as waarskuwing te dien dat die water van die Plankenburgrivier ongeskik is vir die besproeiing van vars produkte. Sommige boere is reeds besig met chloor behandeling van die rivierwater voor besproeiing, om die kans van patogeenoordrag na die produkte uit te skakel. Dit is egter nie 'n haalbare oplossing vir die meeste boere nie as gevolg van die hoë kostes verbonde aan die implementering van' n stelsel soos hierdie.

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CONTENT

The language and style used in this thesis is in accordance with the International Journal off Food Science and Technology. This thesis represents a compilation of manuscripts where each chapter is an individual entity and some repetition between chapters has, therefore, been unavoidable.

CHAPTER 1

INTRODUCTION

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

Over the last decade, studies on the quality of the water in many of South Africa's rivers revealed an increase in pollution levels (DWAF, 1996; DWAF, 2000; Bezuidenhout *et al*., 2002; DWAF, 2002; Griesel & Jagals, 2002; Barnes, 2003; Barnes & Taylor, 2004; Dalvie *et al*., 2004; DWAF, 2004; Germs *et al*., 2004; DWAF, 2005). The microbiological pollution levels have, in more recent years, reached unacceptable and dangerous levels (Barnes & Taylor, 2004). There have also been widespread public discussions over the last years with media headlines such as: "*Kyk voor jy swem*; *Beware of badly polluted river;* and *Groundwater badly polluted with faecal matter*" (Barnard, 2008; Davids, 2008; Gosling, 2008), occurring nearly every week.

Consumption of fresh fruit and vegetables is integral to a healthy diet supplying essential vitamins, minerals and fibre. Worldwide the consumer is encouraged to include five to nine daily servings of fresh fruits and vegetables in their diet (Matthews, 2006). The health aspects of fresh produce are now widely acknowledged by the general consumer and it is thus essential to ensuring availability of a safe product for the consumer. Consumers are also becoming more aware that produce consumed raw can be sources of disease-causing microbes.

Changes in consumer trends, consumer health awareness, population movements, increases in distance that food is transported, a growing immunocompromised population and increased microbial resistance to anti-microbial compounds are impacting the incidence of foodborne diseases. The occurrence of food-related illness outbreaks have increased globally (Johnston *et al*., 2006; Matthews, 2006) and this has stimulated research into food-related outbreaks and resulted in food safety becoming the fast growing and ground-breaking field of study that it is today. There has also been an increased awareness of the illnesses associated with foodborne pathogens as well as the carriers of

these pathogens and the environmental conditions that lead to their survival and proliferation. The increase in both pollution levels and the frequency of food-related illnesses has led researchers to re-examine the link between polluted irrigation water and food safety (Backeberg & Odendaal, 1998; Johnston *et al*., 2006; Backeberg, G. 2007. Water Research Commission, personal communication).

It has often been shown that poor quality water used for irrigation can serve as a source of foodborne pathogens on fruit and vegetables that are consumed fresh or even after undergoing a minimal processing step (Francis *et al*., 1999; Steel & Odumeru, 2004; Hamilton *et al*., 2006; Johnston *et al*., 2006). Since this type of produce is consumed raw and no intervention practices are employed that will effectively control or eliminate potential pathogens prior to consumption it is a potential source of foodborne illness.

This places the responsibility of washing and disinfecting the produce on the retailers and consumers (Bruhn, 2006). Thus, negligence of food safety – particularly in more rural areas – can result in unsafe produce being sold to consumers. In other cases, even though retailers do have safety systems in place, the microbial loads on products may be too high, resulting in insufficient removal during the cleaning processes. The responsibility of cleaning the produce sufficiently cannot be placed on consumers as they are legally entitled to the provision of safe food from retailers (Anon., 1997). In addition to this, not all consumers are equally aware of a potential health risk associated with fresh produce, nor are many of them in a position to be educated. The ability to educate consumers depends on their level of formal education, the available resources for spreading this knowledge, the geographic location of the consumers and the availability of funds for this purpose (Bruhn, 2006). It must also be kept in mind that not all consumers are in a position (financially or geographically) to choose where they purchase their produce from and simply obtain produce from the local supplier. These suppliers may have different safety/hygiene requirements and therefore the safety of the consumer is left in the hands of the respective supplier. One way to increase the assurance of food safety is to improve the quality of the raw materials used in agriculture, including irrigation water (Johnston *et al*., 2006).

Having said this, it has already been mentioned that the state of many South African rivers is currently unacceptable (Bezuidenhout *et al*., 2002; Germs *et al*., 2004; Barnes & Taylor, 2004). The levels of pathogens that were reported in previous studies indicated that the use of river water for the irrigation of minimally processed foods posed a potential health risk to the consumer if direct carryover from the water to the produce takes place.

The microbial quality of fresh fruit and vegetables is essential to ensure a safe product for the consumer but preventing contact with microorganisms is nearly impossible as produce grown in a natural environment are exposed to a wide range of microbes. The carry-over of potential pathogens from irrigation water is also influenced by many environmental factors including the microbial load present in the water, survival and attachment characteristics of specific species, type of produce, water retainment on produce, and a host of other single or interacting factors. While much research has been done on pathogenic survival on produce during post-harvest conditions, pre-harvest microbial carry-over and survival is often overlooked. It was with is this in mind that academic bodies and research institutions began to realise the importance of the quality of river water to the entire agricultural sector, but especially to the producers of produce that is going to be consumed raw. It was for this reason that the Water Research Commission (Backeberg, G. 2007, Water Research Commission, personal communication) decided to initialise and support a study on the "quantitative investigation into the link between irrigation water quality and food safety". Concurrent studies will investigate the possibility of pre-harvest pathogen transfer from water to produce through irrigation and the survival of these pathogens. Should the results confirm the direct link between river water polluted with potential pathogens and the presence of these potential pathogens on produce irrigated with this water, the safety of irrigation water will have to be addressed very seriously and stringently. If this does not happen, the possibility of an increased occurrence of food-related outbreaks in the future can be anticipated. Irrespective of the findings, the state of many South African rivers (DWAF, 1996; DWAF, 2000; Bezuidenhout *et al*., 2002; DWAF, 2002; Griesel & Jagals, 2002; Barnes & Taylor, 2004; DWAF, 2004; Germs *et al*., 2004; DWAF, 2005) currently poses a health risk to all who come into contact with the water. The pollution situation therefore requires immediate attention and actions need to be taken if further deterioration of the rivers is to be prevented.

The overall objective of this study was to do an exploratory study to get an indication of the level of microbial pollution in selected river and irrigation waters from the Berg and Plankenburg Rivers. The first aim was to do an exploratory study of the types and quantities of indicator and index microbes present in selected river and irrigation waters from the Plankenburg and Berg Rivers over five months. A further study over a longer period of 12 months for the Plankenburg-1 site will also done in order to observe microbial level variations in the water from this sampling site. The second aim will be to evaluate the survival of a surrogate *Escherichia coli strain* on selected produce after exposure to a range of *E. coli* inoculums at known concentrations under controlled laboratory conditions.

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CHAPTER 2

LITERATURE REVIEW

Introduction

Fresh and minimally processed foods (MPFs) provide most of our daily requirements for vitamins, minerals and fibre. 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. In order to benefit significantly from these health properties, the World Health Organisation (WHO) recommends an intake of 400 g, or five to nine portions, of fresh fruits and vegetables per day (Matthews, 2006). The World Health Organisation has issued reports claiming that correct fresh produce intake alone could save 2.7 million lives a year and that 31% of heart disease cases are due to an insufficient intake of such foods (Johnston *et al*., 2006). As a result of the WHO recommendations (WHO, 2006a), fruit and vegetable consumption increased by at least 29% per capita in the United States between 1980 and 2000 (Matthews, 2006).

An increase in salad bars and a trend towards healthier living has resulted in a much wider consumption of fresh salad products and healthier foods, and consumer demand is forcing shops to stock fresh produce that is prepared to a ready-to-eat level and also low in – or completely free of – preservatives (Johnston *et al*., 2006). A concern related to this increase in fresh produce consumption is the increased exposure to potentially pathogenic bacteria as well as an increase in the total number of bacteria that are ingested, both of these increasing the chance of infection (Anon., 1998a; Harris *et al*., 2003).

Competition amongst producers, as a result of an increased demand for fresh produce, has led to a wide variety and availability as well as a generally high quality. Developments are constantly being made to prolong the shelf-life of the produce by better refrigeration, packaging materials as well as modified atmosphere packaging. The minimal processing that the produce is exposed to means that the pathogens transferred to the produce in the field remain and survive any washing, processing or packaging that the produce is exposed to. These microbes may even multiply if the storage conditions are within the growth range of those that are present (Anon., 1998a; Francis *et al*., 1999).

It has been shown in the literature that many South African rivers that are drawn from for agricultural irrigation purposes are heavily polluted and have high pathogenic loads (Barnes & Taylor, 2004). In several cases fresh produce is irrigated using this water (Bezuidenhout *et al*., 2002; Germs *et al*., 2002; Anon., 2003; Mikize, 2006). Concern has arisen that there could be a carryover of pathogens from the polluted river water to the fresh produce during irrigation and that should the bacteria survive on this produce, the risk of infection for the consumer could be high. The increase in consumption of contaminated produce can only increase the infection rate if carryover of pathogens takes place (Suslow *et al*., 2003; Bihn & Gravani, 2006). While posing a threat to the health of consumers, outbreaks of associated illnesses would damage the trust of the public, thereby affecting the credibility as well as the sales of all similar producers (Johnston *et al*., 2006). Outbreaks could also result in legal battles which could potentially lead to producers losing their export licences as well as possible rejection by the local market (Suslow *et al*., 2003). For South Africa such outbreaks could be disastrous considering that this agricultural sector is one of great economic importance and would therefore not welcome such a setback.

Consumer awareness is a slow process and the public cannot be relied on to wash or cook fruits and vegetables sufficiently to destroy any pathogens that may be present (Bruhn, 2006). With a growing fresh produce market the food and agricultural industries are facing new challenges that require attention especially in terms of protecting the consumer against microbiological hazards (Anon., 1998a; Garrett *et al*., 2003).

Water Quality

Water quality

When assessing the safety of produce the term ‘water quality’ is based on the pathogenic load of the water as a measure of quality (WHO, 1989). This term is more generally used when determining the efficacy of a treatment process on a water sample (Ayres & Mara, 1996). In the case of produce safety it is the pathogenic load that is determined, rather than measuring chemical parameters (Carr, 2005). Water quality as described above is important as it dictates for what purposes the water is suitable (WHO, 1989). There are five different categories into which pathogens are classed according to their survival characteristics. Categories 1, 3, 4 and 5 include the nematodes, helminths and viruses while Category 2 contains the bacteria. These bacteria are consider those that are

infective immediately upon excretion but can still multiply outside of the host and generally have a higher median infective dose than the other four pathogen categories (WHO, 1989; Ayres & Mara, 1996; Carr, 2005). More recently thermotolerant *E. coli* evaluation has become one of the major tools used world-wide to determine the microbial quality of water (WHO, 1989; DWAF, 1996a; WHO, 2006b).

In South Africa there is a high demand for fresh water in agriculture and >60% of the nation's fresh water is used for agricultural purposes with at least a third being used for the crop irrigation (Rahman *et al*., 2002). The high usage of fresh water could create problems if this water was to become heavily microbially contaminated especially as there are no alternative water resources available. It is therefore of utmost importance that the microbial quality of South Africa's fresh water resources be maintained.

Use of river water for irrigation purposes

The global demand on water resources has, over the past few decades, forced farmers into a position where they have had to accept lower quality for irrigation purposes (Mena, 2006). This has mainly been due to a general decrease in water availability due to changing weather as well as the rapidly-increasing global population (Toze, 2006) putting more pressure on the use of natural water resources. One example of continuous and over-extraction from rivers has resulted in a decrease in groundwater levels of up to 200 m in countries such as Australia and Saudi Arabia (Toze, 2006). The reuse of wastewater has therefore almost become a necessity in order to alleviate the pressure on natural water systems and the use of it for irrigation purposes (Mena, 2006; Toze, 2006). The term wastewater includes water from stormwater runoff, overflows from sewage works and wastewater from animal-feeding plants as well as from an increasing number of other industries (Mena, 2006). Contaminants that may be expected in wastewater are, among others, human or animal pathogens, pharmaceuticals, chemicals and endocrine disruptors (Toze, 2006). There has also been an increasing number of foodborne pathogens found responsible for fresh produce related outbreaks, some of which have been attributed to the quality of the irrigation water (Garrett et al., 2003; Mena, 2006).

of foodborne pathogens found responsible for fresh produce related outbreaks, some of which have been attributed to the quality of the irrigation water (Garrett et al., 2003; Mena, 2006).

Despite a lot of negativity surrounding the use of wastewater from those concerned with food safety, not all of it contains dangerously high levels of contaminants. In some

instances it is seen as beneficial to use, due to it often having a high nitrate and phosphate content, which have a fertilizing effect on the crops (Toze, 2006). Another advantage of wastewater is that it is plentiful as it is consistently being generated through the constant use of water by society.

Despite research and monitoring, there are still unknowns regarding the effects of wastewater irrigation on the safety of the harvested crops, as well as on the health of the consumers. For example, until 15 years ago, *Escherichia coli* had never been linked to food-related disease outbreaks, while today it is considered to be a major pathogen associated with foodborne illnesses (Suslow *et el*., 2003; Matthews, 2006).

From a South African agricultural perspective, rivers are an essential source of irrigation water (Backeberg & Odendaal, 1998). It has been shown (Griesel & Jagals, 2002; Barnes & Taylor, 2004; Dalvie *et al*., 2004; Mikize, 2006) that many rivers in South Africa are heavily polluted with faecal organisms and when these rivers are used for irrigation of fresh produce they will pose a potential threat to human health. This is not only in terms of irrigation but exposure to the river water can occur during recreation and during daily household purposes including drinking, cooking, washing or sanitation. This places those who are exposed to the water at risk of contracting disease from any potential pathogens present in the river (Hamilton *et al*., 2006).

Another aspect that has contributed to the pollution of river water is malfunctioning sewage systems and population relocation and the rapid increase in the size and number of informal settlements without the necessary sanitation and waste disposal infrastructure (Barnes & Taylor, 2004). The disposal of waste and wastewater into rivers has led to the rivers effectively becoming transport systems for the wastewater. In many cases this is carried downstream to where the same water is extracted for irrigation purposes (Matthews, 2006).

Water Standards

In order to be able to ensure that water will be sufficiently safe for its intended use, it has been necessary to construct a set of guidelines for a variety of uses and all water should comply with the regulations and guidelines pertaining to its intended use. The chemical oxygen demand (COD) is a measure of the amount of organic pollution present (Clescerl *et al*., 2006). In South Africa, wastewater is not allowed to be released into water sources (which include local rivers) if the COD exceeds 75 $mg.L^{-1}$. Even at this low COD level, under specific conditions, a daily maximum of 2 000 m^3 of water can be released (Anon.,

1999; DWAF, 2004). However, for the use of wastewater for irrigation the COD cannot exceed 400 $mg.L^{-1}$ if less than 500 m^3 of wastewater is used. In contrast if 50 m^3 or less of wastewater is used for irrigation, the COD should be lower than 5 000 $mg.L^{-1}$ (DWAF, 2004). Thus it can also be concluded that the more water used to irrigate a particular crop, the greater the total amount of organic pollution that enters the field system will be. Therefore, as the total amount of wastewater used for irrigation increases, the permissible limit for COD (as well as for all other parameters that are monitored) should decrease in order to compensate for this. For irrigating with volumes greater than 2 000 m^3, the COD should be less than 75 $mg.L^{-1}$. Water for agricultural use is grouped into three categories of quality. Category 1 is of superior quality and has a COD value of 0 - 10 $mg.L^{-1}$. Category 2 allows a value up to 15 $mg.L^{-1}$; Category 3 up to 30 $mg.L^{-1}$ and Category 4 a maximum COD of 75 $mg.L^{-1}$ (DWAF, 1996a). Throughout the period that Barnes & Taylor (2004) monitored the Plankenburg River, the COD exceeded the given cut-off value of 30 $mg.L^{-1}$ 52% of the time.

pH is a measurement of the available hydrogen ion concentration in a solution and is used to determine the acidity or alkalinity of a solution (Clescerl *et al*., 2006). The acceptable pH for irrigation water is between 6 and 9, while wastewater being released into rivers can vary between pH 5.5 and 9.5 (DWAF, 2004).

In terms of the microbiological quality of water, guidelines for faecal coliforms are given, depending on the method used, as the maximum permissible number of colony forming units per 100 mL water (cfu.100 mL^{-1}) (WHO, 1989). Both the World Health Organisation (WHO) and the South African Department of Water Affairs and Forestry (DWAF) have guidelines for the quality of irrigation water. They both recommend that water used for the irrigation of fresh produce should have a faecal coliform load of less than 1 000 cfu.100 mL^{-1} (WHO, 1989; DWAF, 2004). This applies to all water being used for the irrigation of crops, irrespective of its source. The current guidelines for *E.coli* in irrigation water are not more than 1 000 organisms.100 mL^{-1} (DWAF, 1996c; WHO, 1989; WHO, 2006b). From the literature there is no clear indication as to how the value of <1 000 *E.coli* per 100 mL was reached but it is considered as a very conservative maximum. However, it is yet to be tested at what point and in what quantity carryover of pathogens from irrigation water to fresh produce takes place. It is interesting to note that the permissible load of *E. coli* on raw fruits and vegetables is zero per g product (DoH, 2006). Therefore if *E. coli* present in irrigation water is carried over onto produce, the produce should be considered as suspect.

There are not many published limits or guidelines available for the total number of microorganisms and where limits do exist, the values vary greatly. The total number of microorganisms is determined by performing an aerobic colony count (ACC) on a water sample. Since the organisms detected are not necessarily harmful to the produce or to the consumer, the value obtained from this test is used to indicate number of possible pathogens or spoilage bacteria. Further tests should be performed if more specific information is required regarding the different microorganisms present. However, if the ACCs are high, there is a greater chance that there are corresponding high levels of spoilage organisms or pathogens. Similarly, a low total load usually reflects very low levels of spoilage organisms or pathogens, if there are any. The result for the ACC can, therefore, in broad terms serve as an indication of contamination. In literature, recommended limits for ACCs range from 2.9 to 7.3 log $cfu.g^{-1}$. In Japan a limit of 5 log $cfu.g^{-1}$ has been drawn as the maximum ACC acceptable level (Korsten & Zagory, 2006).

When assessing the safety of food, the lag time, exponential growth phase and the decay/survival rates of the organisms present on the products are important. In terms of food spoilage, a product is considered to be spoiled when its microbial load exceeds 1 x 10^{5-7} organisms per g (Geldreich, 1996; Stine *et al*., 2005). This limit does not make an allowance for the presence of pathogens. The infective doses of pathogens can be as low as 1-10^3 organisms, so a product can appear to be unspoiled while it is actually carrying dangerous levels of pathogens. The infectivity of the *Escherichia coli* pathogenic strains is substantially higher than that of the other strains. As few as 100 EHEC organisms can cause infection (WHO, 2006b). For this reason, it is important to test the quality of irrigation water and fresh produce regularly; although in practice it is not always possible (WHO, 2006b). Clearly, the microbially contaminated water can pose a big threat to food safety if produce is unknowingly being infected with pathogens while being sold as within ‘safe’ bacterial levels (Wijtzes *et al.*, 1998). For this reason, the South African government has drawn up a set of standards that dictate the legal maximum loads for different pathogens that can be present on a product (DWAF, 1996d). While national and international standards and regulations are published and enforced by, or should be, official authorities, there are also certain standards that are set by the industry itself.

The benefit of such industry standards is that they are usually manageable and deal with food safety problems faced in the specific industry through the sharing of information. The role of inspections and certifications by third party groups is a further means of insurance and assurance for retail food companies. It also provides a guarantee of a

certain level of quality to the client and prevents each client from independently needing to inspect the supplier prior to purchase (Michaels & Todd, 2006).

Sources of Contamination

Contaminants - entry into water and soil systems

The two main categories of contaminants are chemical and microbiological with the latter being the focus of this particular study. For this reason, further discussions will only include aspects of microbiological contamination. However, chemical contamination is equally important and the health risks – both short-term and chronic – are serious and not to be neglected. The origin of chemical contamination and methods of detection differ greatly from microbiological contaminants and are thus generally studied separately.

Most of the microbiological contaminants posing a threat to the health of consumers originate from humans or animals, with the majority of these being of faecal origin or transferred through faeces (Harris *et al*., 2003). According to Jamieson and co-workers (2004) and Maciorowski *et al.* (2007) contamination of water can be divided into two major mechanisms namely point-source and non-point-source contamination. Point-source contamination emanates from a clearly identifiable point such as animal feedlots as well as from runoff from storage facilities. Non-point-source contamination can be from different sources or even many points. For example this type of contamination can occur at: the site of manure application at a surface level; at the site where the manure is actively combined with the soil; at the site where the origin of the manure is (livestock); and sites where manure is not the causative factor (Jamieson *et al.*, 2004; Maciorowski *et al.*, 2007).

The use of manure as a fertiliser has increased in popularity as consumers are seeking fresh produce that has been produced without harmful or chemically-loaded pesticides and fertilisers (Suslow *et al*., 2003). The alternative to fertiliser is manure and its use in the field is increasing mainly as a result of consumers “organic” trends to enhance health. Ironically it is only for conventional fertilisers that the microbiological safety can be assured and the use of them is, in fact, much less risky than manure. Manure is likely to be loaded with bacteria present in animal faeces and the risk of contamination of irrigation water is high. Thus a potential hazard does exit for the carry-over of potential pathogens to fresh produce directly or indirectly through contaminated irrigation water. In South Africa, the use of manure as a fertiliser is governed by the Fertilizers, Farm Feeds, Agricultural

Remedies and Stock Remedies Act, 1947 (Act 36 of 1947) which requires disinfection and stabilisation of the sludge prior to it being used (Anon., 2004).

Another pathway for faecal contamination is through direct contact with human sewage. Biosolids, or sewage sludge are what remains after the liquid phase of sewage has been removed for treatment. This has been used as a fertiliser or added to nutritious slurries for crops (Minhas *et al*., 2006). However, this has been recognised to be potentially heavily loaded with pathogens and has been outlawed by the British Retail Consortium (Coetzer, 2006). While animal manure is a problematic contaminant that is very difficult to control, contamination of rivers and water systems with human faeces is an enormous problem that is on the increase. Human faeces is entering river systems through failing sewage pipes and treatment plants, illegal release of untreated sewage and the close proximity of informal settlements (with no sanitation facilities) to river resources which become the obvious dumping ground for the generated waste (Barnes & Taylor, 2004). Until these informal settlements are provided with functioning sanitation facilities and are trained to use them properly, little change for the better can be expected and these communities have no option but to continue dumping their waste into nearby river systems. While informal settlements are responsible for some faecal pollution and even waste dumping, they are, by far not the only guilty party. For example, it was reported by Barnes (2003) that a winery downstream of the Kayamandi settlement was dumping cellar and production effluent into the rivers. Depending on the fermentable carbon load of this type of pollution it might result in increased fermentation in the rivers. The acidity and survival conditions of the water would therefore also be changed, thereby allowing organisms which would not normally be able to survive in river water to grow and multiply.

In South Africa, the Department of Water Affairs and Forestry (DWAF) is the government control body that aims to “to ensure the integrated sustainable management of the water quality of the water resources of South Africa” (Anon., 1998b). When considering the increasing pollution of South African rivers and the downward trend in water quality, it is clear that the situation will hardly improve unless a control body or regulatory agency takes charge and enforces the quality standards of river water from which are drawn from for irrigation.

While fruits and vegetables are in the field, polluted water is one of the major threats for product contamination. The produce can be exposed to water during both irrigation and application of pesticides, and the water used for these purposes can be drawn from rivers, streams, open ditches or canals, dams or ponds, or reservoirs. Alternatively, if available,

municipal water can be used but the quality of this water can not always be relied on (Johnston *et al*., 2006).

Another source of contamination is the land on which the produce is grown. In some cases, farms have been acquired without knowledge of its previous purpose and if it was used for animals, or was even loaded heavily with manure, then the reservoir that has built up in the soil can potentially contaminate the produce (Coetzer, 2006). In the case of farms positioned near rivers, the land use upstream is also important for the safety of the plot. For example, during times of flooding, contaminants that are carried by the river from various sources upstream can be washed onto land that the river does not usually reach and result in unexpected and irreversible contamination. It is ideal that land should have previously also been used for the production of produce for human consumption (Coetzer, 2006).

Contamination of fresh produce can also take place post-harvest (Harris *et al*., 2003). In a food safety review, Harris and co-workers (2003) reported that numerous microbial pathogens have been isolated from fresh fruits and vegetables but not all were linked to produce associated illnesses. Many of the isolated organisms have the potential to under the right conditions to cause illnesses. Vehicles of post-harvest microbial transmission include harvesting equipment, packing house conditions, unhygienic workers, processing plants and even pests (in the field or post-harvest) (Matthews, 2006; WHO, 2006b). In the packing house, transmission of pathogens through practices such as washing can occur if the water is not properly disinfected, filtered or replaced on a regular basis. It is thus important that producers acknowledge the role that the origin and pollution level of the irrigation water can play in the final safety of the end-product (Legnani & Leoni, 2004).

Precautionary measures against contamination

While it is recognised that there is practically no way to prevent a certain degree of contamination in raw or minimally processed produce, the chance of microbial contamination can be reduced by taking precautionary measures (Hutchison *et al*., 2007). Firstly, irrigation water should be tested regularly for microbial levels, and secondly, physical barriers such as fences can be installed in order to prevent animals from entering fields where they potentially contaminate the crops with their faeces (Coetzer, 2006). Thirdly, fertilisation with manure should only take place long enough prior to harvest to allow pathogens to die off. According to Islam *et al*. (2004b), this window period should be at least 120 days before harvest. Fourthly, all farm equipment should be cleaned on a

regular basis to prevent any transmission of the pathogens from equipment onto crops. Lastly, it is recommended that where possible surface or drip irrigation be used instead of overhead sprayers to keep contact between the contaminants and the edible portion of the produce at a minimum (Matthews, 2006).

With regards to humans as a vehicle of transmission at the post-harvest stage, thorough hand washing should be enforced and these facilities should be easy to operate and conveniently placed for workers to use. If available hot water should be plentiful and a good sanitising soap should also be provided (Bruhn, 2006).

Determination of the source of product contamination

By studying the presence and type of microbes found in contaminated irrigation water and on produce, a good indication of the possible source of contamination can be calculated. Some pathogens, for instance, are naturally present in soil (*Bacillus cereus* and *Clostridium botulinum*) and could be carried over to water making it impossible to predict the original contamination source. In other cases like faecal contamination the presence of species like *Escherichia coli, Salmonella* and *Shigella* can be correlated using microbial source tracking techniques (Stoeckel & Harwood, 2007). Both the qualitative and quantitative assessment of the microbes present can also be used to indicate the source of the contamination. A thorough investigation of the crop environment in addition to monitoring activities upstream of the farm (or the point that the water is drawn from) will give a better indication with regards to how exactly the contaminant entered the river system. Observation of harvest activities and treatment of the produce post-harvest can also indicate whether the contaminants are being introduced to the produce by workers, wash water or machinery post-harvest (Michaels & Todd, 2006).

It is still, however, the bacteria of faecal origin which should give rise to the greatest concern as it is very difficult to control or prevent their entry into the river systems. Many of these bacteria are pathogenic and therefore should produce become contaminated with them it will pose a health risk to the consumers. Due to the nature of river systems, very little can be done to improve the quality of river water once the contamination has taken place and therefore products irrigated with faecally-contaminated water should preferably not be used to irrigate fresh produce that later will be available for human consumption.

Indicator Organisms

Indicator, Index and surrogate organisms

In the analysis of soil, water, produce and minimally processed foods (MPF) for pathogenic loads, it is impractical as well as uneconomical to always perform a whole range of tests for the presence of different organisms (WHO, 2006a). The results from the test for the particular organism or group should give a fair indication of the type of pathogens that could be present and in what concentration they can be expected to be found. Therefore, the practice of monitoring the number of a specific organism or of a group is used. For this purpose such specific marker organisms or groups have been defined as either indicator, index or surrogate microbes. In terms of food safety, "indicators" are used as markers whose presence in given numbers is an indication of inadequate processing (Jay, 1997). Indicators are also widely used as a water quality parameter (Leclerc *et al*., 2001). In this case the indicators are defined (Savichtcheva & Okabe, 2006) as organisms from faecal origin and their presence in water may indicate faecal pollution and possible association with enteric pathogens. Similarly, the detection of an index organism provides evidence that a related pathogen may also occur (Busta *et al*., 2003). Thus index organisms are markers whose presence in numbers exceeding given numerical limits indicates the possible occurrence of ecologically similar pathogens. While indicator organisms are useful in giving an indication of the type of contamination that took place, an index organism can provide vital insight into other pathogens that will most likely also be present when it is detected (WHO, 2001). Index organisms also behave in a similar manner to those that they are associated with and therefore can serve as a model for the behaviour of the other accompanying pathogens. These organisms are especially useful when contamination is detected in water or on produce as it can be assumed with a degree of certainty that the relevant associated organisms will also be present (WHO, 2001).

Surrogate organisms are more specific to the indicator system and are chosen to evaluate the effects or responses to selected treatments (Busta *et al*., 2003).

In terms of water quality faecal coliforms, and more specifically *E.coli,* are the most commonly used bacterial indicators of faecal pollution. This indicator group is used to evaluate the quality of wastewater effluents, river water, sea water at bathing beaches, raw water for drinking water supply, treated drinking water, water used for irrigation and aquaculture and recreational waters (DWAF, 1996b). The presence of *E. coli* is used to confirm the presence of faecal pollution by warm-blooded animals or often interpreted as

human faecal pollution. Some organisms detected as faecal coliforms may not be of human faecal origin but are also from warm-blooded animals. The requirements of an indicator is that it should have a broad survival criteria and should be able to survive for at least as long as the pathogens, it should be more numerous than the pathogens themselves, unable to multiply in an aquatic environment, have a moderate to long survival time, correlate in number to the degree of pollution of the water and be absent in the absence of pollution and relatively safe to work with in the laboratory (DWAF, 1996d; NHMRC & ARMCANZ, 1996; Busta *et al*., 2003). The inability of indicator organisms to multiply in water means that the numbers detected when the indicator is tested for will resemble that of the original inoculum and should give a good reflection of the pathogenic load.

Total coliforms

Coliforms are defined as being Gram-negative, non-spore forming, rod-shaped facultative anaerobes that are part of the family *Enterobacteriaceae* (Bergey & Holt, 1994; Leclerc *et al*., 2001). The coliforms are also characterised by their ability to ferment lactose at 35°C, resulting in gas formation. Approximately 10% of all intestinal microorganisms (including *E.coli*) fall into the coliform group. However, this group is not exclusive to intestinal bacteria and it has thus been broken down into smaller sub-groups in order for the intestinal bacteria to be able to be classified separately. This sub-group is known as the faecal coliforms (Bergey & Holt, 1994; Leclerc *et al*., 2001). There are also several other genera not part of the coliforms that can ferment lactose and possess beta-galactosidase and can yield false total coliform reactions. A major limitation of using the coliforms as indicator is the classification which presents major problems as a result of the high degree of character variation extending from the lactose positive/negative variations to the highly reactive *Enterobacter* genus.

Faecal (thermotolerant) coliforms

The faecal coliforms are considered a sub-group of the total coliforms. Many of them are mesophiles and capable of growing and producing acid from lactose at 44.5°C. These are generally considered to be the thermotolerant. This temperature tolerance is specific to those coliforms many of which are adapted to survive within the intestine of a warm-blooded host. Beside *E.coli* several species of the genera *Klebsiella, Enterobacter, Citrobacter, Hafnia, Pantoea, Raoultella* and *Serratia* also fall into the faecal coliform group

and many are thermotolerant (Leclerc *et al*., 2001). However, members of these genera are also present in the environment (Beauchamp *et al*., 2006) and their presence in water and produce is not necessarily related to faecal contamination (Alonso *et al*., 1999). Thus the specificity of faecal coliforms as indicators of faecal pollution varies considerably depending on environmental conditions. While the presence of faecal coliforms is often indicative of faecal pollution, more specific tests have been developed to detect which coliforms are present.

Escherichia coli

The *Escherichia coli* group is one of the most common indicator organisms and are used particularly for the detection of faecal contamination, especially in drinking water. The presence of *E. coli* is never beneficial to a consumer and always points to the possibility of faecal contamination. Its presence, therefore, should not be ignored if it is detected in a sample.

Escherichia coli is of the family *Enterobacteriaceae* and most strains are normal inhabitants of the intestinal tract and are practically always present in faeces and thus also in faecally contaminated water. This has resulted in the almost universal use of *E. coli* as the standard indicator for faecal contamination (Francis *et al*., 1999). There are also several reports in the literature confirming the presence of *E.coli* and other thermotolerant coliform bacteria in the environment (Beauchamp *et al*., 2006). Not all strains are harmless and major pathogenic strains like *E. coli O*157:H7, have been identified in several MPF-related food outbreaks. According to Francis *et al*. (1999), if ingested, this strain can result in haemorrhagic colitis, gastroenteritis and kidney failure, while it less commonly results in thrombocytopenic purpura and haemolytic uremic syndrome (Gil & Selma, 2006). Serious cases can even result in death. The monitoring of faecal matter in rivers and on the MPFs is therefore of great importance since there is very little control possible over animal faeces entering the river (Francis *et al*., 1999).

E.coli has been reported to be the most sensitive thermotolerant coliform to environmental stresses and does not usually grow outside the human or animal gut (Geldreich, 1996). In contrast, it has also been reported that the general survival ability of *E. coli* increases upon exposure to one environmental stress which indicates that it is able to activate survival mechanisms when it is threatened (Maciorowski *et al*., 2007). *Escherichia coli* are known to be able to withstand very highly acidic environments and can survive at pH ranges as low as 3.3 - 4.2. The number of *E. coli* present in an environment

was found to increase logarithmically with an increase in oxygen, indicating that *E. coli* requires high levels of oxygen for metabolism and therefore grows better under conditions of high atmosphere (Maciorowski *et al*., 2007).

E.coli is the preferred indicator of faecal contamination in water sources and on produce (APHA, 1998). Unfortunately, the methods used to determine the presence of *E.coli* for use as indicator of faecal contamination also have there limitations. There are several other coliform species that can ferment lactose even at 44.5°C, grow on McConkey agar, are glucuronidase-positive and are indole-positive making a positive identification of *E.coli* difficult.

Intestinal Enterococci (Streptococcus)

This group of bacteria form part of the genus *Enterococcus* and are also of faecal origin. Intestinal enterococci are also recommended as indicators of faecal contamination but generally they are found in much lower numbers than *E. coli* and are not as easy to detect (Jagals & Grabow, 1996; Hagedorn *et al*., 2003). This group is, however, more resistant than *E. coli* to environmental changes and therefore have a higher survival rate and a slower decay rate. While the low numbers do not make it a suitable substitute test, it is a good test to be performed in addition to the indicator test for the confirmation of faecal contamination and as an indication of the levels present. The two species that are most frequently detected in faecally contaminated systems are *Enterococcus faecalis* and *Enterococcus faecium* (Bergey & Holt, 1994).

Coliphages

Another indicator for faecal contamination is coliphages (Savichtcheva & Okabe, 2006) which attack and infect the *E. coli* host. This would mean that these coliphages would most likely be present when *E. coli* is present. According to the APHA (1998) correlations between coliphages and coliform bacteria in fresh water generally show that coliphages may be used to indicate the sanitary quality of water. The test for coliphages is a simple one but their survival curves follow more closely those of the human viruses than of *E. coli* and other bacteria of faecal origin (Griesel & Jagals, 2002). Therefore, despite the ease of testing, coliphages would not give the most accurate indication of the number of *E. coli* present.

Clostridia

Members of the genus *Clostridium* have successfully been used as faecal index marker for sewage contaminated river water (Savichtcheva & Okabe, 2006) and as indicators of contamination of produce (Jay, 1997). The two members of the genus *Clostridia* that are of major pathogenic concern in produce are *Cl. perfringens* and *Cl. botulinum* while *Cl. perfringens* is of value for water. *Clostridium perfringens* is commonly found in the faeces of both humans and animals (Johnston *et al*., 2006), both of which are found abundantly in local river systems. *Clostridium perfringens* is also found in soil and dust, but faeces is the most common source. Thus far, findings of *Cl. perfringens* in the food industry have been fairly limited to mixed raw vegetables (Johnston *et al*., 2006).

All members of this genus are extremely resistant to environmental stress and persist for longer time than any other indicator bacteria. They are all Gram-positive, rod-shaped, endospore-forming obligate anaerobes (Francis *et al*., 1999) and need a temperature above 5°C to grow (Gil & Selma, 2006). *Cl. Botulinum* is the bacteria responsible for botulism and while some strains need external proteases to activate the neurotoxin; others can activate the neurotoxin themselves, increasing their pathogenicity further. In the food industry it is known for its' acid-tolerant properties and is usually associated with sewage, fermenting silage, wheat and hay but it is also commonly found in soil and thus can be housed in the sediment of a river or in soil after irrigation (Johnston *et al*., 2006). Contamination of produce with *Cl. botulinum* is thought to happen when soil is disturbed or moved in such a way that it lands on the produce (Maciorowski *et al.*, 2007). Members of the Clostridia are also associated with the intestinal tracts of fish and the gills and intestines of shellfish (Johnston *et al*., 2006). It is commonly only seen as a threat in canned foods but the increase in popularity to store MPFs under modified atmospheric packaging has created ideal growth and survival conditions for this pathogen and thus a rise in cases related to MPFs has occurred. Fresh produce that have been carriers of the toxin are cabbage, asparagus, broccoli, tomatoes, lettuce (Francis *et al*., 1999) and melons (Larson & Johnson, 1999). One criticism of using members of the genus Clostridium as indicator or index markers is that they have an extended viability and very wide environmental distribution in both aquatic and soil environments making it impossible to positively source track (Savichtcheva & Okabe, 2006).

Listeria

Listeria monocytogenes is commonly found in the environment is considered as indicative of recent human or animal faecal contamination (Steele & Odumeru, 2004). *Listeria monocytogenes* is a very invasive, Gram-positive, non-spore forming pathogen (Maciorowski *et al.*, 2007). It has the ability to survive in a wide range of environmental conditions including high moisture concentrations and low oxygen concentrations. It can also become facultatively anaerobic and grow at low temperatures (0.5°C) (Francis *et al*., 1999; Johnston *et al*., 2006) which makes it an ideal waterborne pathogen indicator and index organism (Maciorowski *et al*., 2007). The only condition under which it cannot cope is osmotic stress, which is experienced during desiccation. An unusually long incubation time makes an infection caused by *L. monocytogenes* very difficult to trace back to a specific product (Johnston *et al*., 2006).

Listeria monocytogenes occurs abundantly in the natural environment (Francis *et al*., 1999), it is mostly found on decaying vegetable matter as well as in faeces, soil and on water surfaces (Johnston *et al*., 2006). Soil containing *L. monocytogenes* can contaminate plants during fertilisation as well as by water droplets splashing from the soil onto the plants (Johnston *et al*., 2006). *Listeria monocytogenes* can survive for extremely long periods of time and has been reported to remain viable for 10-12 years (Maciorowski *et al*., 2007).

Infection caused by *L. monocytogenes* is extremely serious and contact with the eye can cause eye-infections while infections in humans and ruminants can result in meningitis, septicaemia, still-births and abortions (Francis *et al*., 1999; Maciorowski *et al*., 2007). Should *L. monocytogenes* manage to penetrate the central nervous system once inside the body; the infection can be fatal (Gil & Selma, 2006). The seriousness of the infections caused by exposure to *L. monocytogenes* has resulted in strict legislation regarding its presence on produce. France and Germany permit levels of up to 10^2 cfu.g^{-1} while in America and England complete absence of the organism is required in a 25 g sample and any sample found to contain colonies is rejected (Francis *et al*., 1999; Gil & Selma, 2006).

Bacillus cereus

The only member of the genus *Bacillus* that has been considered as an index organism in terms of food safety is *B. cereus.* This species is also a Gram-positive rod-shaped bacterium which forms endospores making it resistant to most unfavourable environmental variations (Bergey & Holt, 1994; Jay, 1997). It is found widely as it occurs naturally in the soil as well as on plants. As *B. cereus* is an endospore-former 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 state (Johnston *et al*., 2006). *B. cereus* has been detected on alfalfa, mung bean, wheat sprouts and broccoli that had been irrigated with polluted water (Johnston *et al*., 2006).

Salmonella

An index organism is a microbe or group of microorganisms that are indicative of a specific pathogen (Busta *et al*., 2003). For this reason *Salmonella* are mostly considered index organisms by the food industry but for the same reason have also been used as indicators of pathogens on fresh produce (Jablasone *et al*., 2004; Maciorowski *et al*., 2007). Other examples of the use of *Salmonella* as an indicator have been summarised by Geldreich (1966) who reported that there is a clear correlation between the frequency of *Salmonella* presence and the range of faecal coliform density in fresh and river water.

Like *E. coli, Salmonella* is also a pathogen of the family *Enterobacteriaceae*. These bacteria are Gram-negative, non-spore forming and rod-shaped. The genus comprises five pathogenic strains namely *S. typhimurium, S. enteritidis, S. heidelberg, S. saintpaul and S. montevideo.* (Francis *et al*., 1999). Members of the genus *Salmonella* are very resistant pathogens and it have a wide survival range. The group are well adapted to survive outside the intestine, particularly at water activity values (a_w) between 0.43 and 0.52 (Maciorowski *et al*., 2007) and at low oxygen concentrations (Francis *et al*., 1999) making it a very effective human pathogen (Maciorowski *et al*., 2007). *Salmonella* grows optimally in warm temperatures between 35°C and 43°C while its growth is substantially retarded at 15°C, and generally absent at 7°C. It was assumed for a long time that the low pH of fruit juices had a preventative effect on *Salmonella* until an outbreak was traced back to unpasteurised orange juice (Johnston *et al*., 2006). Members of this pathogen group alone has been responsible for 1.3 million outbreaks of foodborne illnesses annually, as well as for being the second biggest bacterial cause of diarrhoea, both in the United States and in the United Kingdom (Mena, 2006).

Salmonella has always been associated with dairy herds, pigs, poultry and even insects and is passed on to humans when meats, eggs or milk from these animals are consumed undercooked (Johnston *et al*., 2006). It has also now been shown that produce that has in contact with faeces and polluted irrigation water can also carry *Salmonella* (Jablasone *et al*., 2004; Maciorowski *et al*., 2007).

Since *Salmonella* is of animal and human origin there is a wide range of fresh products on which it has been found. Fresh produce that have tested positive for *Salmonella* include bean sprouts, leafy vegetables (Francis *et al*., 1999), carrots (Islam *et al*., 2004b), melons, strawberries, mung bean sprouts, alfalfa seeds, lettuce (Francis *et al*., 1999; Johnston *et al*., 2006), salad greens, mixed raw vegetables, chilli, cilantro, parsley, artichoke, cabbage, cardoon, cauliflower, celery, aubergine, endive, fennel (Francis *et al*., 1999; Johnston *et al*., 2006), green onions, spinach, beet leaves and tomatoes (Jablasone *et al*., 2004; Johnston *et al*., 2006; Korsten & Zagory, 2006). In several of the cases it was postulated that irrigation water was the medium of transfer. Pre-cut melon in salad bars has also been associated with several major *Salmonella* outbreaks, of which one outbreak was reported to have affected up to 25 000 people (Johnston *et al*., 2006).

Shigella

Shigella is a widespread foodborne pathogen of which all four known species (*S. sonnei, S. boydii, S. dysenteriae* and *S. flexneri)* are pathogenic. Several diseases outbreaks have been linked to contaminated fruit and vegetables (Steele & Odumera, 2004) and the presence of *Shigella* on irrigated produce considered as an indicator of faecal contamination. Only some stains are capable of survival below pH 6 and *S. sonnei* can survive at low temperatures as well as at low oxygen concentrations (Gil & Selma, 2006). *Shigella* has a very low infective dose, making even a low level of contamination dangerous for the consumer (Gil & Selma, 2006).

Shigella does not occur naturally in soil, water or on produce but is transmitted via the faecal-oral route or via flies. Its faecal origin makes it an indicator of faecal contamination, and this contamination could have come from faecally contaminated water or bad worker hygiene (Johnston *et al.*, 2006). It was estimated to have caused more than 166 million illnesses throughout the world in 1999 alone (Johnston *et al.*, 2006).

Symptoms of *Shigella* infections can be relatively serious and include abdominal cramping and bloody diarrhoea (Gil & Selma, 2006). Certain cases of *Shigella* outbreaks to have been traced back to contamination of raw baby corns, green onions (Gil & Selma, 2006; Johnston *et al.*, 2006), lettuce (Islam *et al*., 2004a; Gil & Selma, 2006; Johnston *et al.*, 2006), melon, celery and parsley (Islam *et al*., 2004a; Johnston *et al.*, 2006).

Foodborne Pathogens Associated With Fresh Produce

Background

A foodborne illness is defined as an illness that results from consumption of or exposure to food. This definition is very broad and includes many aspects of a product that could cause illness in a consumer including bacterial, viral, parasitic and even chemical contamination (Korsten & Zagory, 2006). The increased consumption of fresh and minimally processed fruit and vegetables has led to a significant number of outbreaks of foodborne illnesses (Aruscavage *et al.*, 2006). It was also reported that most minimal processing steps required for fresh produce will result in the produce carrying some form of microbes and varying microbial load levels. Most minimally processing practices lead to a reduction of the microbial load but do not eliminate contaminating pathogens from the produce surface (Aruscavage *et al.*, 2006). Some of these microbes could potentially be a hazard to human health. Thus, minimizing and controlling contamination of agricultural commodities such as fresh produce presents a serious challenge to the agricultural and food industries.

Outbreaks associated with consumption of fresh produce

The number of foodborne illness outbreaks linked to the consumption of fresh produce has increased over the last years (Harris *et al*., 2003; CDC 2006). This increase could be as a result of a number of factors and a large variety of bacteria, viruses and parasites have been directly linked to outbreaks associated fresh produce. A list of bacterial genera that have been associated with fresh produce is given in Table 1 and a summary of fresh produce linked to foodborne illness outbreaks is given in Table 2. This highlights just how far-reaching the problem has become and how many consumers could potentially be affected by a foodborne outbreak.

Table 1. Bacterial genera associated with fresh produce (Stine, 2004; Matthews, 2006).

Acinetobacter *Aeromonas* *Bacillus* *Bacteroides* *Bifidobacterium* *Brucella* *Campylobacter* *Citrobacter* *Clostridium* *Edwardsiella* *Escherichia*	*Enterobacter* *Enterococcus* *Franciella* *Hafnia* *Klebsiella* *Listeria* *Leptospira* *Mycobacterium* *Pasteurella* *Plesiomonas* *Proteus*	*Providencia* *Pseudomonas* *Salmonella* *Serratia* *Shigella* *Staphylococcus* *Streptococci* *Vibrio* *Yersinia*

Table 2 Sources of pathogens and fresh produce with which they have been linked (taken from Harris *et al*., 2003)

Pathogen	**Soil**	**Water**	**Faecal**	**Produce**
Bacillus cereus	yes	no	no	Alfalfa, mungbean, sprouts, broccoli
Campylobacter jejuni	no	no	yes	Lettuce, parsley, spring onion, potato, mushroom, spinach, radish
Clostridium ssp.	yes	no	yes	Mixed raw vegetables, cabbage, melons, broccoli, asparagus, tomatoes, lettuce
Escherichia coli	no	no	yes	Celery, coriander, lettuce, radishes, peas, parsley, vegetable products, sprouts
Listeria monocytogenes	no	no	yes	Cabbage, potato, bean sprout, raw salads, lettuce, radishes, broccoli, cress, tomatoes, mushroom, green pepper, cucumber
Salmonella ssp.	yes	yes	yes	Sprouts, leafy vegetables, carrots, fennel, tomatoes, melons, strawberries, lettuce, chilli, spinach, parsley, artichoke, cabbage, cauliflower, celery, aubergine,
Shigella ssp.	no	no	yes	Raw baby corn, lettuce, celery, melon, parsley, green onion,

Over the last 25 years, *E. coli O*157:H7 has turned from being basically unheard of as a fresh produce pathogen to being responsible for 34% of all the *E.coli* outbreaks, of which none were a result of contamination during preparation. According to Matthews (2006) this correlates directly to the increasing levels being found in river systems. Produce on which it has been more regularly detected are celery, coriander, cilantro, mixed raw vegetable products (Johnston *et al.*, 2006), lettuce (Islam *et al*., 2004a; Gil & Selma, 2006; Mena, 2006), radishes (Mena, 2006), parsley (Islam *et al*., 2004a) and alfalfa sprouts (Gil & Selma, 2006; Mena, 2006). Gil & Selma, (2006) also reported no effect of modified atmospheric packaging on the growth of *E. coli.*

In one of the largest reported cases of an *E. coli* O157:H7 outbreak in Japan, it was linked to the consumption of contaminated sprouts resulting in about 6 000 cases of infections including 17 deaths (Johnston *et al*., 2006; Matthews, 2006). It was thought that these seeds were infected with the pathogen prior to sprouting.

From there being no recorded cases prior to 1990 of *E. coli* O157:H7 outbreaks, several cases started emerging after that year with at least six related to unpasteurised apple cider (Johnston *et al*., 2006). Research began to show that *E. coli* could survive for relatively long periods at low pHs which was contrary to what had been expected. As a result of this, laws were drafted to force producers and retailers of unpasteurised or treated products to state clearly on the label if a product had been treated or was unpasteurised. By doing this, consumers are given the choice of whether they wish to purchase such a product or not. This law was followed up by others which stated that production procedures should follow HACCP steps in order to bring about a microbial reduction of at least 100 000 cfu.mL^{-1} in an attempt towards a more widespread and guaranteed food safety system (Johnston *et al*., 2006).

Listeria monocytogenes has been found in wheat as well as in other vegetables such as cabbage (Francis *et al*., 1999), bean sprouts, lettuce, potatoes, radish, broccoli, cucumber, leafy vegetables, green pepper, mushrooms, field cress and tomatoes. It has also been found in ready-to-use products such as salad packs, pre-mixed vegetable packs and coleslaw (Francis *et al*., 1999; Johnston *et al*., 2006). This wide selection of fresh produce on which *L. monocytogenes* is found makes it a very real pathogen threat in river-irrigated MPFs (Maciorowski *et al*., 2007).

One of the first cases of *L. monocytogenes infection* was registered in Canada when coleslaw caused infections amongst consumers. The cabbage that had been used tested positive for the specific *L. monocytogenes* strain and it was also isolated from the patients'

blood. The cabbage was found to have been contaminated in the field by sheep manure that had been used as an organic fertiliser. This was later correlated with two sheep from the same farm that died of listeriosis and so the link between the contaminant and its associated foodborne illness was proven (Johnston *et al*., 2006).

Salmonella has over time been found to be responsible for a very wide range of outbreaks. In specific cases, carrots and radishes were carriers of *S. typhimurium* (Islam *et al*., 2004b; Islam *et al*., 2005) while tomatoes were a carrier of *S. enteritidis* (Jablasone *et al*., 2004), both resulting in outbreaks. *Salmonella saintpaul* and *S. virchow* were detected on bean sprouts after they were found responsible for an outbreak (Francis *et al*., 1999). Another fruit involved in many cases of *Salmonella* infection has been melon. Several outbreaks have been reported after sale in a pre-cut form and in a specific outbreak it is thought to have affected up to 25 000 people with two reported deaths (Johnston *et al*., 2006). There have been a relatively high number of *Salmonella* outbreaks on tomatoes which causes a lot of concern amongst producers since large volumes are consumed. Five billion pounds of tomatoes are consumed annually in the United States alone which, if they all carried a high pathogenic load, could pose a great disease threat.

There have also been outbreaks where the source could not be identified, even after a thorough environmental study. While this will sometimes be the case, it is also important to realise that the more certain one is of the quality of the water used for irrigation, the easier it will be to isolate the sources of these outbreaks and to bring them under control (Matthews, 2006).

In 1999 (James, 2006), the United States Food and Drug Administration (FDA) conducted a survey where 1 000 various food samples imported from 21 different countries were analysed. The samples included broccoli, celery, melon, cilantro, lettuce, parsley, green onions, strawberries and tomatoes and were analysed for *Salmonella, E. coli* 0157:H7 and *Shigella*. Of the samples, 4% were contaminated with one of the three tested pathogens and but none were positive for *E. coli* 0157:H7. *Salmonella* was found on 80% of the contaminated samples with *Shigella* only present on 20% of the samples. Samples that tested positive for *Shigella* were awarded the status DWPE (detention without physical examination) after only one positive result while the products testing positive for *Salmonella* were placed on this status if two positive results were obtained. The only way that a producer could be removed from this list was if it could prove that the source of contamination had been identified and removed. When the same set of tests was repeated a year later only 1 percent of the total samples were found to be contaminated, indicating a significant improvement in the safety of the produce (Harris *et al*., 2003; James, 2006).

In 2007, a recall due to the possible presence of *Salmonella* was made in the UK on all basil even though the nature of basil being a fresh herb meant that products carrying the earlier expiry dates were likely to have already been sold and consumed (Anon., 2007). Similarly, in 2008, in the USA recalls were made due to the presence of *Salmonella saintpaul* on tomatoes (Anon., 2008). Later 40 confirmed cases were found to have been caused after tomatoes contaminated with *Salmonella saintpaul* had been consumed (Anon., 2008). While there were no deaths, at least 17 people were hospitalised.

Sprouts are often implicated in food-related outbreaks, with *Salmonella* and *E. coli* being the most common contaminants. The sprouts are small with a large surface area to volume ratio which can result in extremely high superficial loads (Britz, 2005). The wrinkled anatomy of a dry seed makes it an ideal host for potential pathogens especially during storage. The bacterial contaminants become dormant under the dry conditions. In certain cases they were present but undetected when dry seeds were tested. They resumed viability upon sprouting when the moisture levels had been increased (Robertson *et al*., 2002). This form of dormancy under drier conditions means that potential pathogens can easily be transmitted from seed to seed during sprouting which in turn leads to the contamination becoming more widespread and thus to an increase in resulting illness.

Irrigation Modes As A Carry-over Mechanism

The microbial quality of irrigation water is of importance as poor quality water can lead to the introduction of pathogens onto produce during pre- and post-harvest activities. Because of this problem, indirect or direct contamination of produce from water of persistent pathogens on harvested vegetables has been long recognized as a potential hazard (Busta *et al*., 2003; WHO, 2005). Though reports on direct evidence of foodborne illness due to contamination of fresh produce during “commercial” production are more limited, many of these crops have been implicated in foodborne illnesses. Already in 1987 Garcia *et al.* (1987), showed that under commercial conditions of 181 irrigation samples and 859 vegetables irrigated with the same water source in Spain were contaminated with *Salmonella typhimurium; S. kapemba; S. london* and *S. blockey* serotypes. Similarly, Steel *et al.* (2004) carried out a survey on 500 irrigation water samples used for production of fruit and vegetables in Canada and found that 25 % of the samples were contaminated with faecal *E. coli* and faecal Streptococci.

Different irrigation methods have been found to correlate with the level of microorganisms present on produce (FDA, 1998). It has also been reported that the transfer of microorganisms from irrigation water to produce is dependent on the nature of the produce (Beuchat & Ryu, 1997). Spray irrigation could be expected to increase the risk of contamination in comparison to drip irrigation or flooding because leafy vegetables provide large contact surfaces for water and for the attachment of microorganism (Sadovski *et al*., 1978; Ibenyassine *et al*., 2006).

Other popular methods are drip irrigation, subsurface drip irrigation or furrow irrigation; all of which result in minimal splashing of water and thus minimal exposure of the edible produce to potentially-contaminated water (Johnston *et al*., 2006). These methods could then be used in cases where contamination of produce is a real threat and contact between the water and the produce is preferred to be kept to a minimum.

Irrigation choices should also take factors such as water quantity, cost, soil type, slope of the field and the type of crop rotation system into account. These factors must be weighed up against the likelihood of pathogen contamination and a decision must be made for each specific situation (Mena, 2006).

Different researchers have evaluated the presence or persistence of pathogens conveyed to crops by spray irrigation, irrigation by sewage effluent (Teltsch & Katznelson, 1978; Garcia *et al.,* 1987) or drip irrigation (Sadovski *et al.*, 1978). It was found that carry-over varied and was depended upon the level and nature of environmental stress. Carry-over was correlated to target population densities in the source water and spatial orientation relative to the point source. The level of organic matter in the water also impacted the survival of pathogens.

Irrigation water polluted with manure has also been implicated in the outbreaks of enterohaemorrhagic *E. coli* 0157:H7 infections (Kim *et al*., 2006). The infections were associated with lettuce and other leaf crops and they are occurring with increasing frequency (Mahmoud *et al*., 2007). However, it has been found that *Salmonella* became undetectable on effluent-irrigated lettuce five days after irrigation was terminated, but *E. coli* indicator strains persisted (Mukherjee *et al.*, 2004).

It was reported by Matthews (2006) that in the USA spray/overhead irrigation resulted in a greater number of lettuce plants' testing positive for *E. coli* O157:H7 at harvest following a singe exposure to the pathogen. Similarly in Nigeria lettuce and carrots were positive for *Salmonella*, *Vibrio* spp. and *E. coli* following irrigation with water that tested positive with the same pathogens (Stine *et al*., 2005; Matthews, 2006). Mukherjee and co-

workers (2004) and Mahmoud and co-workers (2007) reported that strawberries tested positive for the presence of *E. coli* after both irrigation by drip and overhead methods.

Contaminated irrigation and surface run-off waters and the use of sewage as a fertilizer can also be sources of pathogenic microbes that contaminate fruits and vegetables in the field (Beuchat & Ryu 1997; Ibenyassine *et al.,* 2006). It was also found that with sewage contamination between 84 and 100% samples were contaminated with either *L. monocytogenes* or *L. innocua* during a two year sampling period. *Salmonella* was also present in more than 50% of irrigation water samples contaminated with raw sewage or primary treated chlorinated effluents (Wang *et al.*, 1996).

It has been found that cholera and typhoid microbes can also be transferred during the irrigation of vegetables with untreated wastewater. Therefore, in areas where rivers are known to test positive for such pathogens, the method of irrigation as well as the option of water treatment should be critically considered. If a producer intends on continuing to provide produce for a MPF market, the money expended on a treatment system could protect the integrity of the producer and thus ensure economic stability (WHO, 1989; Assadian *et al*., 2005).

Farming conditions and practices play a critical role in the contamination of produce and it is usual for the level of contamination to have dropped substantially from when it is harvested to the time of consumption (Francis *et al*., 1999). Temperature is one of the more important factors that influences the growth, survival or decay of bacteria on produce post-harvest. Each group of bacteria has its own growth criteria and therefore different bacteria will react differently under the presiding conditions (Peleg, 2000). In contrast to the usual decay patterns, *Listeria monocytogenes* as well as non-proteolytic strains of *Cl. botulinum* and *Aeromonas* are psychrotrophic and *Aeromonas* have been found to increase by 1 log value after 7 days at 3-4°C (Francis *et al*., 1999). This means that there is still a chance that some of contamination pathogens can multiply on produce after harvest.

Growth Kinetics of Potential Pathogens

General aspects on growth, survival and decay rates

A combination of factors including changes in food consumption trends for health reasons have led to numerous foodborne outbreaks being associated with fresh produce (Stine, 2004). Several outbreaks have been directly shown to have been caused by the contamination of fresh produce and this not only brought the problem to the attention of the

public but also a realization that very little is known about the contamination carry-over and survival of pathogens on fresh produce (Matthews, 2006). There are many environmental factors that would affect microbial survival on plants (Islam *et al*., 2005; Matthews, 2006) but relatively few studies have investigated carry-over loads from irrigation water to plants and the impact of environmental factors on survival (Lang *et al*., 2004; Steele & Odumeru, 2004; Aruscavage *et al*., 2006). Three question that have continuously been asked is what is the microbial load necessary in polluted water to be sure of carry-over to fresh produce, which factors influence survival and what does the survival kinetics look like?

The growth of pathogens is affected by the same environmental factors that affect the spoilage microorganisms inherently present on the produce. These include temperature, pH, available oxygen, carbon sources and concentrations, presence of other nutrients, redox potential, ultraviolet light, water activity (a_w), type of organism, cell concentration, lag time and metabolic activity (Wijtzes *et al*., 1998). It must always be taken into account that in the environment these parameters can impact the growth and survival of a microorganism singly or in combination making kinetic predictions very difficult. When determining the growth and decay rates of organisms, it is important to consider the optimum growth condition for each growth parameter as well as the combined interaction before calculating the maximum specific growth rate (Wijtzes *et al*., 1998). When combining the optimum conditions for the respective growth parameters, the effects can be multiplied to provide a single dimensionless growth rate (Wijtzes *et al*., 1998).

Another aspect that can affect the survival or growth of organisms is that of river flow dynamics such as speed of flow, turbulence, amount of sediment and vegetation. Any form of disturbance to the water can result in a disturbance to the sediment which in turn re-suspends the bacteria that had settled on or amongst it. Faecal coliform counts have been found to correlate strongly with the amount of suspended solids in water which indicates that a disturbance of the sediments could cause a rise in faecal coliforms counts. This has lead to the acceptance that faecal coliforms commonly settle and are housed in the sediment; however the rise in coliforms could possibly rather be due to an increase in available nutrients from the disturbed sediments (Jamieson *et al*., 2005).

Besides the factors directly impacting the microbe, there is the role of the irrigation water (Buchanan & Doyle, 1997). Normally trace back analysis after outbreaks is a historical analysis that takes place weeks after the crops were irrigated. In free flowing rivers water conditions change rapidly and assessment of the quality of the water that had been used for irrigation is very difficult so long after an outbreak had taken place.

When microbes die off over time, it is generally assumed that they follow a function of first-order decay which is represented mathematically as $N_t = N_o e^{-kt}$ where the *t* refers to time, *k* is the inactivation constant, N_o is the initial organism count and N_t is the organism count after time, *t* (Jamieson *et al.*, 2004). When decay-rate experiments are performed in the laboratory, the curve profiles are documented and these can then be used under standardised conditions in combination with the equation to predict the size of a specific culture. However, this is kinetic data generated under controlled laboratory conditions. Even if it gives valuable indications of growth and survival trends it must be remembered that under environmental conditions other aspects will play a role.

The continual reduction in the availability of free water on the outer surface of a product after irrigation leads to growth conditions that become less ideal for most organisms over time. According to Mena (2006) this explains the general trend for organisms on produce to die off over time rather than to multiply (Mena, 2006).

Another factor that should also be considered is that a post-harvest washing process could remove the natural bacteria from produce while leaving the resistant and strongly-attached pathogens behind. The absence of competing bacteria can result in the pathogens growing and proliferating at a higher rate than if the product had been left untreated (Francis *et al*., 1999). *E. coli O*157:H7 is one of the most resistant pathogens and competition with natural bacteria is thought to have no hindering effect on its growth (Francis *et al*., 1999).

While pathogens have different inherent survival rates and growth curves when they are present on fresh produce, it must be remembered that these can change completely when the atmosphere is altered as in the case of modified atmosphere packaging (Wijtzes *et al*., 1998). When conditions such as these are created to enable a longer shelf-life, they can also encourage the proliferation of pathogens that would not normally be able to survive and replicate under conventional storage conditions (Francis *et al*., 1999).

Another factor that must be taken into consideration is environmental stress on viability of a strain. Studies have revealed that bacteria can take on several different states during times of environmental stress and that not all of these are detectable by traditional detection methods. In conditions that are unfavourable for growth but do not result in cell death, some of these bacteria enter a viable but nonculturable state (VBNC) in which they are unable to multiply (del Mar Lleò *et al*., 2005). The transition of cells to a VBNC state could result in contamination being underestimated when it is used as an indicator and false ‘safe’ results could be dangerous for potential users of the water or products.

There are, therefore, many factors influencing the growth, decay and survival of organisms with different intrinsic and extrinsic factors affecting and influencing the different organisms in different ways. Each product should be looked at specifically in order to identify the pathogens and spoilage bacteria that it could be carrying and steps should be taken to treat the product in such a way that the threat of these bacteria surviving or proliferating is minimised. If these factors are not taken into consideration and care is not taken to remove or contradict ideal growth conditions, contamination on produce could lead to outbreaks.

Growth and decay rates in water and on produce

To determine the survival rates of faecal coliforms, *Salmonella* and *Vibrio cholera* studies were done (WHO, 1989) to determine the length of survival in water and soil at holding temperatures between 20°C and 30°C. This temperature range was chosen to represent the temperature for soil and water reached during summer months. The time periods for which the bacteria survived in both the soil and water is given in Table 3. The survival rates obtained gives an indication of the time for which water and soil can probably pose a risk to the safety of the produce being grown (WHO, 1989).

Table 3 Survival times (days) of faecal coliforms, *Salmonella* and *Vibrio* in soil at 20-30°C (WHO, 1989).

Organism	Soil	Water
Faecal coliforms	<70, but normally <20	<30, but normally <15
Salmonella	<20, but normally <10	<5, but normally <2
Vibrio cholera	<70, but normally <20	<30, but normally <15

The data in Table 3 shows that the survival times of faecal coliforms and *Vibrio cholera* would exceed the normal growing time for small produce such as beans, cocktail tomatoes and sprouts. This leads to concern as it can be predicted from the data that soil and water organisms can remain viable on produce up to the point of sale.

Another factor to be taken into consideration when calculating decay rates is that enteric bacteria are known to have a good survival rate in estuarine waters. This is an additional factor that should be taken into consideration when river pollution levels are

being monitored. For example, *E. coli* strains that were isolated from a hospital in Senegal were inoculated into river water and were found to die off within 96 h when they were exposed to direct sunlight. However, when they were deprived of sunlight, the bacteria were still present at a concentration of 100 000 cfu.mL^{-1} after the same time period (Troussellier *et al*., 2004). Therefore, if the latter environmental conditions were to prevail in a contaminated river, the microbial counts might indeed remain high rather than decrease or die off, which might have initially been expected.

In a study to determine the decay rate of *E. coli* O157:H7 in an equal soil and compost mixture and also in contaminated irrigation water. Three different types of composts were used namely: dairy cow manure, poultry manure and alkaline-buffered compost (Islam *et al*., 2004a; Islam *et al*., 2005). The composts were inoculated with *E. coli O*157:H7 at a concentration of 10^7 cfu.g^{-1} before being blended with soil. The irrigation water was inoculated with 10^5 cfu.mL^{-1} *E. coli O*157:H7. In field plots, carrot and onion seeds were planted and irrigated as necessary with distilled water to supplement rainfall. Three weeks after the seeds were sowed; the plots containing un-inoculated compost were treated with a once-off, 2 L dose of the inoculated water. Carrots and onion were then sampled daily. *E. coli* was found up to day 154 in the soil and up until day 168 on the carrots (USDA, 2000; Islam *et al*., 2004a). The counts on the onions were only found up until day 74. This shorter survival time was attributed to antimicrobial phenolic compounds present in onion flesh (Islam *et al*., 2004b; Islam *et al*., 2005). It was thus concluded that not only environmental conditions affect the survival of pathogens on produce, but the nature of each product and its specific compounds affect pathogen survival.

In studies on sheep and cow manure that was kept under controlled conditions, it was found that *E. coli O*157:H7 survived for up to 21 months in the sheep manure and for 47 days in cow manure. It has also been reported that *Salmonella* in manure can survive for between two days and six weeks (Matthews, 2006), which is longer than the growth period of smaller produce. In such cases, viable *Salmonella* could be transferred in the field from manure to the soil or irrigation water and in turn from the soil or water to the produce. The produce could be purchased while the *Salmonella* is still viable, posing a threat to the health of the consumer.

In a very comprehensive study (Minhas *et al.,* 2005) on cabbage, okra and ridge gourd, the crops were irrigated with water with a faecal coliform load of 1.5x10^8 cfu.100 mL^{-1}. The vegetables were harvested and analysed for *Salmonella*, *Shigella*, aerobic bacterial counts, faecal coliforms and fungi. All three crops were analysed whole and the cabbage additionally analysed after the removal of the outer leafs in order to determine whether the

outer leaves carried a higher load than the inner leaves. It was anticipated that the removal of a critical number of outer cabbage leaves would lead to a lower load and possibly a load acceptable as a safe level for consumption. In addition to monitoring the effects of the time of harvest on the bacterial load, the ridge gourd was harvested at two-hour intervals throughout the day and night. This allowed the effect of the full range of temperatures throughout the day on the pathogenic load to be observed. Of the samples that were monitored during the day, half of the samples were left in the sun while the other half were stored under shade. A portion of the samples were also given a vigorous washing in an attempt to reduce the pathogenic load on the vegetables. The data obtained showed that while the average faecal coliform counts on the cabbage samples was 2.3×10^2 MPN.100g^{-1} the loads on the ridge gourd were on average 9×10^5 MPN.100 g^{-1}. It was found that the removal of the leaves showed a rapid decrease in pathogenic load (Table 4).

Table 4 Coliform survivors on irrigated cabbage (n=8). Sampling was done after removal of the outer leaves (Minhas *et al*., 2005). The inoculum load was 1.5×10^8 cfu per g

Number of leaves removed	Faecal coliforms per 100 g	Faecal coliforms per 100 g
	Range	Mean
0	2 - 3400	180
1	2 - 2600	29
2	<2	<2
3	<2	<2
4	<2	<2

The data presented in Table 4 clearly shows that the highest counts present on the parts of the plant which came into direct contact with the irrigation water. After as few as two leaf removals, the coliform counts dropped to acceptable limits. It was also found that the coliform counts of the vegetables left in the sun decreased to within acceptable levels within 4 h of harvesting. Washing was found to reduce the number of coliforms on the

produce but as a result of the extremely wide variation in counts it was concluded that the reduction was not consistent enough to be able to guarantee levels to be within acceptable limits. The same study was repeated with the water being administered by drip irrigation and no infection was found on any product growing at a height of 10 cm or more above the ground (Minhas *et al*., 2005). It was concluded that drip irrigation does offer a solution to minimise contamination of produce when irrigation water is used that carries high pathogen levels and the produce is of such a nature that the edible portion is carried more than 10 cm above the ground.

In a study on the impact of types of irrigation on the survival of *E.coli* on lettuce Solomon and co-workers (2002) spray irrigated with a microbial load of 10^7 cfu per mL and found that 90% of the plants tested became contaminated in contrast to 19% when surface irrigation was used. They did report a large variation in specific survivor numbers. They also reported that in a separate study when lettuce was spray irrigated daily over a period of several days with a load of 102 cfu mL-1 the population of the pathogen on the lettuce increased with each watering. Again no specific load values were given.

The fate of five serotypes of salmonellae applied (10^6 cfu mL^{-1}) to tomato plants was investigated by Guo *et al*. (2001). 37% were found to be positive for *Salmonella* at harvest. Of the serotypes in the inoculum, Montevideo was the most persistent, being isolated from tomatoes 49 days after inoculation, and Poona was the most dominant, being present in 5 of 11 *Salmonella*-positive tomatoes. Results confirm that *Salmonella* survives on tomatoes from the time of inoculation through fruit ripening. Tomato stems and flowers are possible sites at which *Salmonella* may attach and remain viable during fruit development, thus serving as routes or reservoirs for contaminating ripened fruit.

Lang *et al*. (2004) studied the impact of the method for applying microbial inoculum (dipping, spotting and spraying) and of drying times (2 and 24 hours at 22°C and at 4°C) on the survival of *E. coli*, *Salmonella* and *Listeria monocytogenes* on lettuce and parsley. The inoculum size was not standardised for each test and varied from 20 000 to 1 479 100 $cfu.mL^{-1}$ making comparison log reduction difficult. It was however found that significantly higher survivors were present on both produce types and for both produce types when applying the dipping method compared to the spray and spotting methods. Survivors recovered from lettuce and parsley after drying for 2 h at 22°C were significantly higher than those recovered after drying for 22 h and at 4°C clearly showing that longer drying times and drying at lower temperatures led to large reductions in numbers surviving.

Gorski *et al*. (2004) reported that the attachment of *L. monocytogenes* to alfalfa sprouts is dependent on the strain. They also found that when contamination took place with a good attaching strain it proved difficult to remove cells through washing. This resulted in wrong conclusions being reached in terms of survivors remaining on the sprouts. They also found that the original isolation source of a strain also impacts survival rates.

Johannessen *et al*. (2005) investigated the transfer of *E.coli* O157:H7 from soil contaminated with 10^4 cfu. g^{-1} to lettuce. They reported that the pathogen persisted in the soil for at least 8 weeks but was not detected after 12 weeks. The pathogen was only detected sporadically on the lettuce at harvest and never from the roots of the plants. They concluded that transmission of the pathogen from manure-soil to the lettuce did not take place in high numbers.

Another characteristic of microbes that influences the survival of pathogens during flood, drip and overhead irrigation, is internalization of produce. Solomon *et al*. (2002) demonstrated that pathogenic strains of *E. coli* can enter lettuce plants through the root system and migrate throughout the edible portion of the plant. This characteristic of *E.coli* will complicate the detection of the pathogens in/on fresh produce.

Recently, Warner *et al*. (2008) showed that entry into the plant can also be accomplished by entry through plant stomata's making food safety control even more difficult. Keevil *et al.* (2008) confirmed this type of colonization of produce and additionally showed that biofilms can also play an important role in as a major source of organisms during internalization. This route of contamination is particularly problematic as many pathogens can now survive despite the associated environmental stresses that would lead to higher decay rates. This ability of microbes to be able to integrate into surface exposed biofilms and then translocate into stomata compromises detection and the ability to disinfect. Such drawbacks have significant implications in terms of food safety for quality monitoring and outbreak prevention.

Economic Impact of Fresh Produce-Related Outbreaks

Consumption of fresh fruit and vegetables has substantially increased over the last few years mostly due to the nutritional evidence that consumption of at least five fruits and vegetables helps prevent many degenerative diseases. In addition there is also an

increased demand for low-caloric food products with fresh-like characteristics. However, as a result of inappropriate pre- and post-harvest practices both pathogenic and spoilage microbes may contaminate produce and lead to an increased risk of both microbial diseases and product spoilage. In fact the number of foodborne outbreaks associated with the consumption of fresh produce and fresh-cut fruit and vegetables has increased (Harris *et al*., 2003). Additionally quality losses of fresh produce have also increased. Sources of contamination of fresh produce at the pre-harvest stage include faecal sources, inadequately composted manure, contaminated soils, animals and humans and polluted irrigation water.

South Africa is the major exporter of fresh fruit and vegetables to Africa. Ndiame & Jaffee (2005) reported that 73% of all fruit and vegetables exported to the USA under the AGOA preference from Africa (African Growth and Opportunity Act) were from SA. SA is now the largest third world supplier of fruit and vegetables to the Europe and has 31% of total EU imported fruit market share. Several countries in Sub-Saharan Africa export vegetables but three, Côte d'Ivoire, Kenya and the SA, account for nearly 90% of the region's trade to international market with South Africa the leading exporter (Ndiame & Jaffee, 2005). For some produce, an especially fruit, SA is one of the leading world fresh produce exporting countries in terms of monetary value (WESGRO, 2006).

Nationally and internationally one of the major sources of microbial contamination has been shown to be polluted rivers that are used for irrigation purposes (Islam *et al*., 2005). In South Africa there is a high demand for water in agriculture and >60% of the nation's fresh water is used by farmers with 33% being utilised for the irrigation of crops with some 35% of all domestic foodstuffs and 85% of all agricultural exports being derived from irrigated lands (Backeberg, 1996). Such a high usage of fresh water could create problems if this water was to become microbially contaminated to dangerous levels since the water use is so widespread that no alternative resource would be possible. It is therefore of the utmost importance that the quality and safety of South Africa's fresh water resources be improved and maintained.

In order for the problem of contaminated river water being used for irrigation to be tackled on a global scale, a concerted effort needs to be made from all parties concerned to put measures in place to reduce - and ideally to prevent – unnecessary pathogenic contamination. It is difficult for less-developed countries to have the same level of surveillance systems in place as in more technologically-advanced countries such as the United States and Europe, but a global effort (Calvin *et al*., 2006) should be made to support and implement these systems in the poorer countries if expectations of a certain

quality are going to be placed on their indigenous produce that is in such high demand (James, 2006).

It is clear from the previous sections that fresh fruit and vegetables are important to the SA economy. A foodborne outbreak can have a severe impact on the economics of the fresh produce market. A typical example, as given by Calvin *et al*. (2006), of how a foodborne outbreak can impact an industry is the following: in 2005, leafy greens, head lettuce, leaf lettuce, romaine and spinach, were the most important of the top five fresh-market vegetables in the USA with a farm production value of 2 140 million US$. After an FDA announcement regarding the *E. coli* O157:H7 – spinach outbreak, the price, sales and shipments of spinach, were affected severely (Calvin, 2006).

Conclusions

A foodborne illness is defined as an illness that results from consumption of or exposure to food. This definition is very broad and includes many aspects of a product that could cause illness in a consumer. The increased consumption of fresh and minimally processed fruit and vegetables has led to a significant number of outbreaks of foodborne illnesses. Several outbreaks have been directly shown to have been caused by the contamination of fresh produce and this not only brought the problem to the attention of the public but also a realization that very little is known about the contamination carry-over and survival of pathogens on fresh produce. There are many environmental factors that affect microbial survival on produce but relatively few studies have investigated carry-over loads from irrigation water to plants and the impact of environmental factors on survival making kinetic predictions nearly impossible.

It is imperative that such research be conducted as the condition of South African river water has been shown to be deteriorating rapidly. Should things continue in the current manner, the rivers might reach a point where pollution prevents the water from being used for irrigation at all? If the condition of the rivers is unable to be improved upon, South Africa will not only face a food safety crisis, but if the rivers are declared unsafe for irrigation we will be faced with an agricultural crisis as well.

In the meantime, however, if contaminated river water is used for irrigation of fresh produce, an effort should be made to minimise contact between products and the contaminated water by controlling the way in which it is administered and an effort should

be made to educate consumers on how to prepare food to reduce the risk of infection or illness.

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CHAPTER 3

MICROBIAL AND CHEMICAL LEVELS IN SAMPLES FROM THE BERG AND PLANKENBURG RIVERS: QUANTIFICATION, CHARACTERISATION AND IDENTIFICATION

Summary

In agricultural areas of the Western Cape many farmers draw water for irrigation from nearby rivers without knowing what the microbial pollution level is. In an exploratory study over a five month period, the microbiological and water chemistry of three selected sites from the upper Berg and two from the Plankenbrug Rivers were assessed. Studied organisms included *Salmonella*, Staphylococcus, *Listeria*, endosporeformers, coliforms, *Escherichia coli* and intestinal *Enterococci*, while the chemical analysis consisted of pH, alkalinity, conductivity and chemical oxygen demand (COD). Faecal coliform counts ranging from 540 to 1 700 000 cfu.100ml^{-1} and 490 to 160 000 cfu.100ml^{-1} were found for the Berg and Plankenbrug Rivers, respectively. The water temperature ranged from 12° to 21°C with COD values always below 100 mg.L^{-1}. Potential human pathogens such as *Salmonella*, Staphylococcus, *Listeria*, endosporeformers, *E. coli* and intestinal *Enterococci* were frequently isolated from all five sites sampled. From the exploratory study it was concluded that the water from all the sites were not suitable for use in irrigation practices as they regularly exceeded the guidelines for faecal coliforms and *E.coli* as set out by South African authorities.

A further study over a longer period of 12 months was done on a site on the Plankenburg River in order to observe microbial level variations over a longer period. Over the period faecal coliforms and *E.coli* counts were found to be present in consistently high loads with only one of results from the ten samples being lower than the international guideline of <1 000 organisms.100mL^{-1} set by the WHO. Several other potential pathogens were also detected in most samples analysed during the longer study period. These results are of great concern as lower down the river water is regularly drawn for irrigation of produce that is later consumed raw or after a minimal processing step. The close proximity of this site to an informal settlement was used to explain most of the high pathogen levels detected in the water samples. The COD values were slightly higher than those detected at the other sites but still fell within DWAF Category 4 (<75 mg.L^{-1}). The pH ranged between

5.64 and 6.84, showing only a slight fluctuation in pH and indicating that it is relatively stable shows a degree of consistency in the water. The temperature of the water at this site changed with the seasons with the lowest in August 2008 as 10.2°C and the highest as 20.8°C in February 2008. While this temperature range might appear wide, it covers the lower growth range for mesophilic and the higher for the psychrophilic bacteria and thus provides favourable conditions for psychrophilic growth. The ACC levels were high, reflecting the presence of a large number of heterotrophic microorganisms in the river water which, in turn, suggests likelihood that disease causing organisms being present. The presence of *Listeria* was observed in eight of the 10 water samples and were identified as *L. grayi, L. ivanovii, L. innocua* and *L. monocytogenes. Salmonella,* intestinal enterococci and *Staphylococcus* were present in most of the samples and taken and are an additional indication of faecal contamination. From the data obtained it can be seen that the levels of bacterial contamination of the water from the five river sites are high and exceed all limits for irrigation as well as product consumption. These levels are in excess of safe levels and could pose a risk to consumers if levels as high as these were to be ingested.

Introduction

The population demographics of the Western Cape Province have changed dramatically as urbanisation has increased (United Nations, 2004). This is evident in the rapid growth of informal settlements such as Kayamandi in Stellenbosch. Informal settlements often develop before the necessary services and foundations can be established in order to support them. One of these vital aspects that is missing is sanitation facilities and therefore most household and personal waste ends up either directly in local rivers, or on nearby land from which runoff into the rivers occurs (United Nations, 2004). In addition to this, the areas in which urban infrastructure does exist, they are not being serviced properly, due to either negligence or lack of funding. In turn, some of these facilities are malfunctioning which is also resulting in raw sewage effluent entering rivers or groundwater systems (Barnes, 2003).

This scenario, in addition to other pathways that can possibly introduce contaminants into rivers – such as industrial effluent, storm-water, sewage overflow and animals – has been shown to cause of increased pollution levels in rivers. It has been shown by Barnes & Taylor (2004) who monitored specific rivers in the Western Cape that

as a result of the current polluted state, the rivers are not suitable to be used for irrigation (Backeberg, G. 2007. Water Research Commission, personal communication).

In agricultural areas of the Western Cape, South Africa, many farmers draw water for irrigation from nearby rivers. On most farms, the water does not undergo any treatment to improve the water quality before it is administered to the crops (Kirsten, F. 2007. Goedvertrou Farm, Stellenbosch, personal communication). On some farms the water is held in storage dams prior to irrigation which can result in a reduction of microbes in the water but this is not a standard treatment and most farms do not have sufficient space for such dams (Kirsten, F. 2007. Goedvertrou Farm, Stellenbosch, personal communication). The quality of the water in rivers used for irrigation is, therefore, very important as it comes into contact with produce that is ultimately going to be eaten by consumers.

A combination of all of the factors presented above led to this investigation into the quality of water that is used for the irrigation of produce (Harris *et al*., 2003). In order to assess the state of pollution and the possible presence of pathogens in the rivers, it was necessary to conduct a study in which water chemical and microbiological levels will be evaluated and monitored over a period of time.

The objective of this research was to do a study to get an indication of the level of microbial pollution in selected river and irrigation waters from the Berg and Plankenburg Rivers. This will be done by firstly doing an exploratory study of the types and quantities of indicator and index microbes present in selected river and irrigation waters from the Plankenburg and Berg Rivers over five months. Secondly, a further study will be done over a longer period of 12 months for the Plankenburg 1 site in order to observe microbial level variations in the water from this sampling site.

Materials and Methods

Site Selection

Five different sites were carefully chosen and monitored over five months with a total of four samples taken at each site during this period. One of these sites was further monitored over a total of 11 months in order for the exploratory study to span a more representative period.

The sampling sites used in this study were selected to represent both river water and sites from which irrigation water is drawn. Two of the sites were chosen in places

where water was known to be drawn regularly from the rivers regularly for irrigation. All of the sites were chosen on two Western Cape rivers. Three of the chosen sites were at different positions along the Berg River with the first being downstream of Franschhoek (Berg 1), the second site between Franschhoek and Paarl (Berg 2) and the third site downstream of Wellington (Berg 3). The remaining two sites were along the Plankenburg (Plank 1 and Plank 2). It should be noted that the Plankenburg River is in close proximity to the Eerste River and these rivers converge at Plank 2, resulting in that sample being a combination of water from both rivers. Sampling at Plank 2 was specifically done at the confluence from where irrigation water flows into an irrigation furrow.

In as many cases as possible, sites were selected in conjunction with farmers who irrigated produce that would be eaten in a raw or minimally processed state, with river water from that site.

After monitoring all five sites on the Plankenburg and Berg Rivers for the initial sampling period, it was decided that Plank 1 would continue to be monitored for a further seven months, until September 2008. It was chosen as the site was practical to sample from and had been found to carry consistently high pollution loads over the first five months of sampling. High microbial loads have also been reported in the past, with levels of 12 600 000 organisms.100 mL^{-1} (DWAF, 2001) and 6 990 000 organisms.100 mL^{-1} (Barnes, 2003) being reached. It was also argued that this site would give a good representation of the indicator and index organisms present in the river.

Sampling Frequency

Samples were taken from all three of the sites along the Berg River (Berg 1, Berg 2 and Berg 3) and from Plank 2 (Plankenburg River) on a monthly basis running from September 2007 to February 2008, with the exception of January when no samples were taken. Samples were taken from Plank 1 over a period of twelve months (excluding January 2008) from September 2007 to August 2008.

Sampling Method

Sampling was carried out according to the procedure prescribed by SANS 5667-6 (2006) with additional precautions taken to ensure both accuracy of the sample and safety of the sampler. An insulated cooler-box containing frozen ice-bricks was used to keep the samples at refrigerator temperature (4°C) until they were analysed in the laboratory. Two

autoclaved, 1 L sampling bottles (Schott) were prepared for each site and placed into the cooler box.

Firstly, the strength of flow of the river was noted (i.e. slow, medium, strong, torrential) and recorded along with the appearance of the water (i.e. cloudy, translucent, opaque, brown, milky) and any accompanying odours. The samples were taken from as close to the middle of the river as possible, in the area of the strongest flow. The water temperature was then measured and recorded. The Schott bottles were submerged 30 cm under the water surface with the neck of the bottle facing the direction of flow. In cases where the river water level was less than 30 cm, the sample was taken from the midpoint between the surface and the bottom. Once the bottle was submerged, the cap was removed and the bottle filled with water. The cap was replaced while the bottle was still submerged and the closed bottle was then placed upright into the pre-chilled cooler-box for transportation back to the laboratory.

Chemical and environmental tests

Temperature

The temperature of the water at each site was measured using the temperature probe of a digital thermometer (Hanna Instruments).

Conductivity

Conductivity is an indication of the amount of total dissolved salts in the water and is measured by the ability of the water to conduct an electric current. The conductivity of the water sample was taken with a HI 8711 conductivity meter (Hanna Instruments). The sample was calibrated according to the instructions in the manual (Hanna Instruments) and the probe was then placed into the sample until the reading on the display had stabilised. The units of measurement ($mS.m^{-1}$) were adjusted accordingly following the instructions in the manual.

pH, alkalinity and chemical oxygen demand

The pH, alkalinity and Chemical Oxygen Demand (COD) were determined according to Standard Methods (APHA, 2005).

Microbiological tests

The methods used for each microbiological test performed were chosen in accordance with the methods prescribed in the relevant standard method (APHA, 2005), as well as by taking the capacity of the laboratory facilities into consideration. Colonies that resembled those described in literature for a specific media (Merck, 2007) were isolated from the Petri dish and identified further. After it was verified that these colonies were pure, they were analysed with the relevant APIWEB-system (BioMerieux, France) for confirmation of the identification.

Aerobic colony count

The aerobic colony count technique was performed according to the method prescribed in SABS ISO 4833 (2007). In anticipation that the water samples could carry a high microbiological load, a dilution series of the water sample was made to 10^{-8}. For each dilution, 1 mL was placed into corresponding Petri dishes with a sterile pipette and Plate Count Agar (PCA) (Merck) was added aseptically. The Petri dishes were moved in a figure of eight motion to ensure an even distribution of the samples in the agar. Once the PCA had set, a layer of Bacteriological Agar (Merck) was poured over the PCA layer. The plates were then incubated at 30°C for 72 h. After incubation, the plates were inspected for typical growth.

Aerobic and anaerobic endospore formers

This test was performed according to the method prescribed in the Canadian Standard for Aerobic and Anaerobic endospore formers (MFLP-44) (Health Canada, 1998). A sterile pipette was used to add 2 mL of the water sample to 18 mL sterile physiological saline solution (PSS) in a sterile test tube. The test tube was then placed in a water-bath and heated to 75°C. The tube was kept at this temperature for 20 min before being removed and allowed to cool. A dilution series of the heat-treated water sample was made up to 10^{-8} and 1 mL of each dilution was placed into its respective sterile Petri dish. This was repeated with a second set of Petri dishes which were to be incubated anaerobically. Pour-plates were made by pouring 25 mL of sterile Tryptic Soy Agar (TSA) (Merck) into each plate aseptically. Once the TSA had set, the two sets of Petri dishes were incubated in their respective aerobic and anaerobic environments at 35°C for 48 h in order to allow both aerobic and anaerobic endospore formers to grow. The plates were then inspected for typical growth.

Staphylococcus

The test for *Staphylococcus* was performed in accordance with the method prescribed in SABS ISO 6888-1 (1999). Baird-Parker Agar (Merck) was autoclaved and Egg-yolk Tellurite solution (Merck) was added to the agar aseptically as soon as it had cooled down to 60°C. The Baird-Parker solution was then mixed thoroughly and poured into sterile petri dishes and allowed to set. A dilution range of the sample was prepared from 10^{-1} to 10^{-8}. Using a sterile pipette, 0.1 mL of each dilution was then transferred to correspondingly marked petri dishes containing Baird-Parker Agar. Each sample was spread evenly over its respective plate with a sterile glass rod or 'hockey stick' and allowed to stand for 15 min to allow the surface of the agar to dry. The plates were then incubated for a total of 48 h at 35°C and inspected after 24 h, and again after 48 h. Growth of typical colonies was recorded.

When typical colonies were observed, they were transferred onto Nutrient Agar (Merck) and incubated at 35°C for 24 h. Thereafter, further identification was performed on the colony using the API Staph system (BioMerieux, France).

Salmonella

The method for the enrichment of *Salmonella* was prescribed in SABS ISO 6579 (2003). For the primary enrichment, a Schott bottle containing 225 mL buffered peptone solution (Merck) was prepared according to the instructions and autoclaved, after which it was left to cool. A volume of 25 mL of sample water was added to the buffered peptone solution and this was then incubated at 35°C for 24 h.

For the secondary enrichment, 10 mL of the solution from the primary enrichment was transferred aseptically into 100 mL Selenite-Cysteine Broth (Merck) and 0.1 mL was transferred into 10 mL Rappaport-Vassiliadis Broth (Merck). The Selenite-Cysteine solution was incubated at 35°C while the Rappaport-Vassiliadis solution was incubated at 42°C. Both were inspected after 24 h and again after a further 24 h. At the first inspection, each of the secondary enrichment solutions were streaked out onto pre-dried Phenol Red-Brilliant Green Agar (Merck) and Xylose Lysine Deoxycholate (XLD) Agar (Merck) with an inoculation needle. These plates were incubated at 35°C for 24 h. The same procedure was followed with the Phenol-Red and XLD plates after their second incubation period. Phenol-Red and XLD plates were inspected for typical growth after 24 h. When typical growth was observed, the colonies were transferred onto Nutrient Agar (Merck) and

incubated at 35°C for 24 h. Thereafter, further identification was performed on the colony using the API system (BioMerieux, France).

Enumeration of coliforms, faecal coliforms and *Escherichia coli*

Prescribed by both the Canadian Standard (MFHPB-19) (Health Canada, 2002) as well as by Standard Methods (APHA, 2005), this method used the results obtained from an enumeration process to calculate the most probable number (MPN) of gas forming organisms, coliforms and faecal coliforms per 100 mL of water sample (reported as organisms.100 mL). The method was also used to identify the presence or absence of *E. coli* in the water sample.

Firstly, five test tubes containing 10 mL of double strength Lauryl Sulfate Tryptose Broth (LST) (Merck) and 45 test tubes containing 10 mL of single strength LST were prepared. Inverted Durham tubes were placed into all of the test tubes so that any gas production could be observed. The test tubes were then arranged in a rack with the five double strength tubes in the first row and five single strength tubes in each subsequent row, until there were ten rows in total. The following rows were then labelled according to the dilution of the sample used in each row. The first row was labelled 'double strength', the second row 'single strength' and the remaining eight rows labelled from '10^{-1}' through to '10^{-8}'. Into each of the 'double strength' tubes, 10 mL of the water sample was added aseptically. In the same way, 1 mL of the water sample was added to each of the 'single strength' tubes. A dilution series of the water sample was then made in McCartney bottles from 10^{-1} through to 10^{-8}. From each dilution, 1 mL was transferred into each of the test tubes in the row with the corresponding label (i.e. 1 mL from the 10^{-1} dilution was placed into each of the test tubes in the row labelled '10^{-1}'). The total set of test tubes was then incubated at 35°C and inspected for gas formation after 24 h, and again after 48 h. Test tubes positive for gas formation were recorded and set aside while tubes failing to form gas after a total of 48 h were assumed to be negative for gas formation.

Brilliant Green Lactose Bile Broth (BGLB) (Merck) was prepared for each positive LST tube by adding 10 mL BGLB and an inverted Durham tube to an empty test tube. These BGLB tubes were sterilised in the autoclave. An inoculation needle was used to transfer a loopful of LST from each positive tube to a tube containing BGLB. The labels from the LST tubes were transferred onto the corresponding BGLB tubes and these tubes were then incubated at 35°C for 24 h. After 24 h the tubes were inspected for gas formation and then re-incubated for a further 24 h after which they were inspected again. Gas-

positive tubes were recorded and an inoculation needle was used to transfer a loopful of the positive BGLB to test tubes containing 10 mL *Escherichia coli* (EC) Broth (Merck). The labels from the BGLB tubes were transferred to the corresponding EC Broth tubes and these tubes were then incubated in a water bath at 44.5°C for 24 h. Gas-positive tubes were recorded and a sterile inoculation needle was used to streak out the gas-positive EC Broth onto prepared plates of Levine-Eosin Methylene Blue Agar (L-EMB) (Merck) in Petri dishes. These plates were incubated at 35°C for 24 h. Plates were inspected for growth of typical colonies. In order for the purity of the colonies to be checked, typical colonies were streaked out on Nutrient Agar (Merck) and incubated at 35°C for 24 h before being examined under the microscope at ×1 000 magnification. Thereafter, further identification was performed on the colony using the API 20E system (BioMerieux, France).

The positive tubes from each dilution for EC Broth were totalled and converted into a code which was looked up on the standard MPN table (MFHPB-19). The values obtained from the table were then used to calculate the most probable number of faecal coliforms per 100 mL of the original sample.

Intestinal *Enterococci*

The prescribed method (SANS ISO 7899-2) (2004) utilises a membrane filter technique with a filter size of 0.45 µm (Whatman) to extract micro-organisms larger than 0.45 µm in diameter from the water sample.

Using a measuring cylinder, 100 mL of the water sample was measured and filtered through a sterile filter apparatus (containing the membrane filter) with the aid of a vacuum pump. After the 100 mL sample had been filtered, the membrane filter was aseptically transferred onto Slanetz and Bartley Agar (Merck) and incubated at 35°C for 44 h. These incubation conditions would promote growth of any intestinal *Enterococci* that remained behind on the filter. After incubation the colonies were inspected and those with colour ranging from pink to maroon were counted. The filter was again transferred aseptically to another Petri dish containing Bile Esculin Agar (Merck) for a further 2 h at 44°C. The colonies were then inspected again and those ranging from tan to black in colour were counted and recorded.

Listeria

This presence/absence test was performed according to SABS ISO 11290-1 (1996). A half-strength solution (1 L) as well as a full-strength solution (500 mL) of Fraser *Listeria*

Selective Enrichment Broth (Oxoid) was prepared in Schott bottles. In both cases the selective supplement was added according to the accompanying instructions. In a sterile test tube, 9 mL half-Fraser Broth was measured out and 1 mL of the water sample was added to it. This tube was incubated at 30°C for 24 h, after which an inoculation needle was used to streak out the media onto both Oxford (Oxoid) and Palcam (Oxoid) Agar. These plates were incubated micro-anaerobically at 35°C for 48 h. From the half-Fraser Broth, 0.1 mL was aseptically transferred to a test tube containing 10 mL full-strength Fraser Broth. This tube was then incubated at 35°C for 48 h, after which the broth was streaked out onto Oxford and Palcam Agars and incubated as with the half-Fraser Broth. Typical colonies were then selected and streaked out onto pre-dried Tryptic Soy Agar (TSA) (Oxoid) plates and incubated at 35°C for 24 h. A catalase test and Gram stain was performed on each typical colony. These purified colonies were identified and confirmed using the API *Listeria* system (BioMerieux, France).

Confirmation and identity of colonies present on culture-specific media

Colonies found on the culture-specific media used in the different methods were further studied so that, where possible, the bacteria could be identified to species level. Colonies were identified as areas on the growth medium that differed visibly from the rest of the medium. The colonies can be classified by whether they grow on top of or inside the growth medium, the margin of the colony, the shape of the colony, the amount of slime production, precipitation formation, colony colour, colony size, time taken for colony growth to occur, incubation temperature and oxygen availability to the colony during incubation. Firstly, colonies were identified and observed and these observations were recorded. One of each different colony observed was removed and streaked onto plates containing NA which were incubated for 48 h at 35°C and then inspected. This process was repeated until separate, pure colonies were obtained. The colonies were Gram-stained and inspected for purity under a microscope (Leitz) and once pure, further Gram stain, oxidase and catalase tests were performed. The morphology of the culture was also recorded, along with the presence or absence of endospores and the aggregation patterns of the cells. The oxidase test was performed transferring part of a colony onto laboratory filter paper with a sterile inoculation needle and placing a drop of isoamyl alcohol (BioMerieux, France) on top of the colony. After 2 min it was observed whether or not a colour change of the colony to purple took place.

For the catalase test, a portion of the colony was placed on a glass microscope slide and three drops of 3% hydrogen peroxide (H_2O_2) were placed on top of the colony. It was then observed and recorded whether or not an immediate formation of bubbles occurred (APHA, 2005). After these three tests had been conducted, the colony underwent a final identification step through the use of the API identification system (BioMerieux, France). Colonies were exposed to the specific API test corresponding to the cell morphology and culture-specific media that was used. The various API tests and the respective cultures that can be identified by them are shown below:

Staphylococci – API Staph;

Streptococci – API 20 Strep;

Escherichia coli – API 20 E;

Bacillus – API 50 CH;

Listeria – API Listeria.

Each test was performed according to the instruction manual pertaining to that specific API test (BioMerieux, F-69280 Marcy l'Etoile, France).

Results and Discussion

Berg 1

Site description - Berg 1

This site along the Berg River was 50 m upstream of the bridge (R45) crossing the Berg River when approaching Franschhoek from Stellenbosch (Fig. 1). The site was downstream of the confluence of the Berg and Franschhoek Rivers.

The recent completion of the Berg River Dam is thought to have affected the results obtained in the laboratory. The new dam constantly released a regulated amount of water into the Berg River during the course of 2008 (Berg River Consultants, 2008) which resulted in a more consistent flow rate of the river and made the seasonal flow and depth fluctuations less extreme. The Berg River dam was completed during the five months that Berg 1 was being sampled and the river depth increased from an average depth of 15 cm to 1 m.

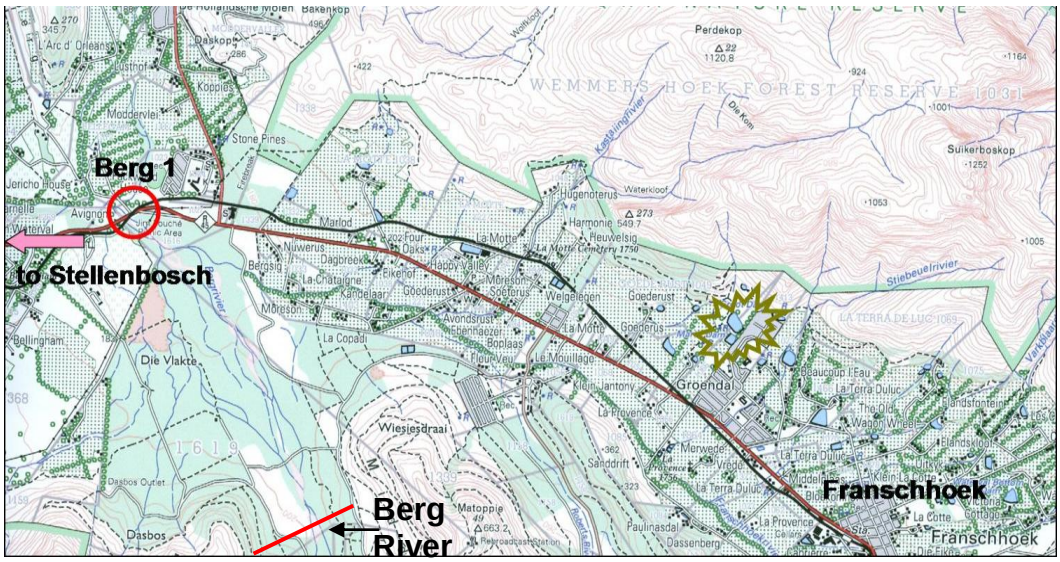

Figure 1 Map of Franschhoek and surrounds with the position of site Berg 1 marked.

Environmental and chemical results – Berg 1

Table 1 contains the environmental and chemical data obtained from the analysis of the samples taken at Berg 1 between October 2007 and February 2008. The temperature of the river water varied between 15.4°C and 20.0°C. This temperature range during the sampling period was about only 5°C but at least 15°C lower than the optimum growth of *E. coli*. While this lower temperature range is more conducive for growth of mesophilic and psychrophilic bacteria, the temperature would not have been high enough to cause the enteric bacteria to proliferate unpredictably (Leclerc *et al*., 2001). Enteric bacteria grow optimally at 35-37°C and then only in the presence of suitable and optimum concentrations of carbon, nitrogen and phosphorous sources (Leclerc *et al*., 2001).

The pH (Table1) of the water ranged from 5.74 to 7.08. A pH below 6 could have been due to environmental factors such as the acidity of the soil. This is below the WHO recommendations which state that the pH of river water should range between 6 and 9 (DWAF, 1999; WHO, 2006). However, it is known that the soil alongside the Berg River is acidic and this could explain the lower pH values (Conradie, 2002).

The alkalinity of the water varied from 12.5 to 18.8 mg $CaCO_3.L^{-1}$. This indicated that the water had a low buffering capacity, meaning that it offered little resistance against the effect of environmental changes on the pH. The alkalinity of a solution should be above 80 mg $CaCO_3.L^{-1}$ if it is to offer effective buffering protection against the environment (Spellman, 2008).

The COD was much lower than expected, with three out of the four values complying with the regulations for irrigation water (DWAF, 1996a+b). In three out of the four samples, the COD was below 30 $mg.L^{-1}$, which meant that the river water was safe for irrigation purposes (DWAF, 1999). The COD of the fourth sample was 84.6 $mg.L^{-1}$ which is above the South African limit for irrigation water (DWAF, 1999). The conductivity results did not indicate any abnormal salt levels.

Table 1 Environmental and chemical data for samples taken from Berg 1

Sampling date	Temperature	pH	Alkalinity	Conductivity	COD	Rainfall*
	°C		mg $CaCO_3.L^{-1}$	$mS.m^{-1}$	$mg.L^{-1}$	mm
October 2007	15.9	6.49	12.5	9	16.2	30
November 207	15.4	6.00	12.5	7	25.2	14
December 207	16.9	5.74	18.8	18	84.6	17
February 2008	20.0	7.08	12.5	10	18.9	17

*Rainfall figures for the Drakenstein region obtained from the South African Weather Service (2008).

Microbiological results – Berg 1

The data obtained for coliform, faecal coliform and *E. coli* levels at Berg 1 are given in Table 2. The number of coliforms detected varied between 440 and 77 000 organisms.100 mL^{-1}. The number of faecal coliforms was found to be the same as the number of coliforms in two out of the four samples. Typical *E. coli* growth on L-EMB Agar (Merck) (Fig. 2) was observed for all of the samples and according to Health Canada (2002) the faecal coliforms detected are thus *E. coli*. Therefore, the load of faecal coliforms is also the load of *E.coli* in the samples. This assumption was verified with an API 20 E test using a typical colony on the L-EMB Agar which gave a 85%, 89%, 92% and 83% I.D for *Escherichia coli* for each of the four respective samples. Taking the above into consideration, the sample taken in November 2007 was the only sample with *E. coli* levels (440 organisms.100 mL^{-1}) that fell within the South African guidelines for irrigation water (1 000 organisms.100 mL^{-1}) (DWAF, 1996b). According to the DWAF (1996b) guidelines for effects of *E. coli* on crop quality a concentration of 1-1 000 *E. coli* counts per 100 mL, there will be a likelihood of

contamination of vegetables and other crops eaten raw resulting in transmission of human pathogens.

Table 2 Coliform, faecal coliform and *E. coli* data of the Berg 1 samples

Sampling date	Coliforms (organisms.100mL^{-1})	Faecal coliform (organisms.100mL^{-1})	E. coli
October 2007	70 000	70 000	TG
Novmeber 2007	440	440	TG
December 2007	17 000	3 500	TG
February 208	54 000	540 000	TG

TG = typical growth

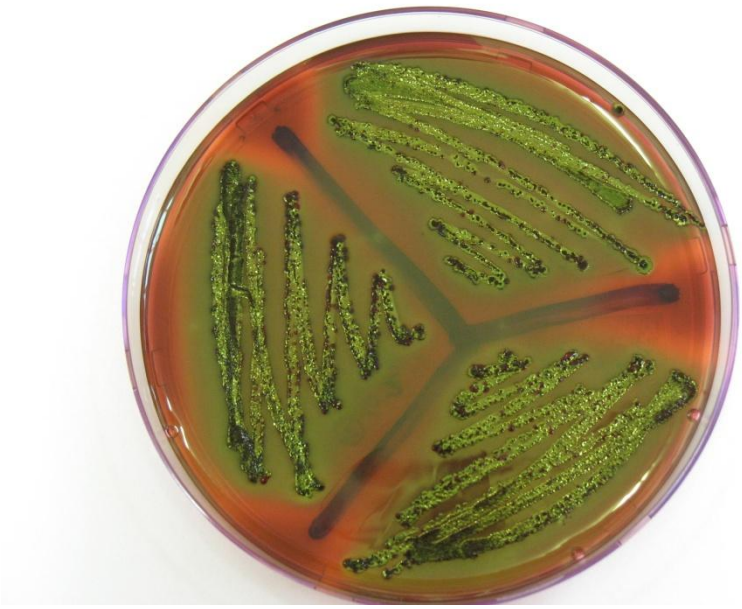

Figure 2 Typical *E. coli* growth on L-EMB Agar.

The data presented in Table 3 are the results that were obtained for all of the remaining microbiological tests. The results for the ACC, aerobic and anaerobic sporeformers, staphylococci and intestinal *Enterococci* were quantified and the levels detected in the different samples could thus be compared with each other. However, the tests for *Salmonella* and *Listeria* only measured the presence or absence of typical growth and these results could, therefore, not be compared quantitatively.

The sample taken in February 2008 was the only sample in which very high levels (32 000 cfu.mL^{-1}) of microbes were found for the aerobic colony count (ACC) (Table 3).

This result should be considered high as DWAF (1996a) warns of an increased risk of infective disease transmission when the ACC is greater than 1 000 cfu.mL^{-1}. The remaining three samples all had ACC levels between 100 and 1 000 cfu.mL^{-1} (Table 3), which according to DWAF carry a slight risk of microbial infection as it is an indication of inadequate water treatment (DWAF, 1996a). This was also the only sample on which aerobic sporeformer growth was detected while anaerobic sporeformers were only detected in November 2007. Typical *Salmonella* growth (Fig. 3) was observed in October and November 2007 and typical *Listeria* growth (Fig. 4) was observed in October 2007 (Table 3). Intestinal *Enterococci* were detected in December 2007 and February 2008. The presence of *Salmonella* and intestinal *Enterococci* in the samples is another indication that the river was polluted with faecal matter.

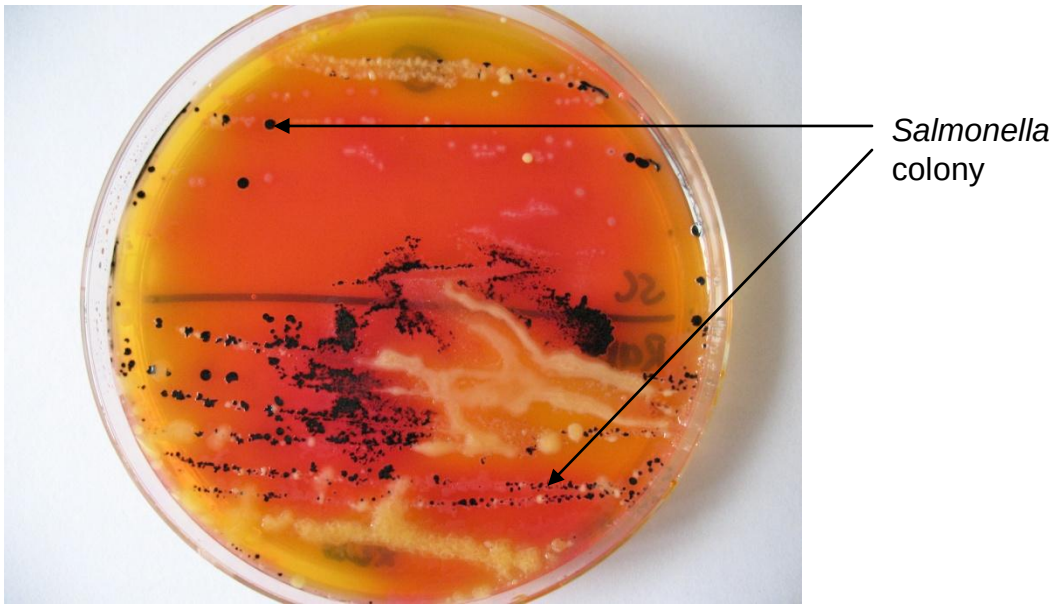

Figure 3 Typical growth of *Salmonella* colonies *on XLD Agar.*

Table 3 Microbiological data from the analysis of Berg 1 samples

Sampling date	ACC (cfu.mL^{-1})	Aerobic endspore formers (cfu.mL^{-1})	Anaerobic endospore formers (cfu.mL^{-1})	*Salmonella* TG/ND	*Listeria* TG/ND	Staphylococcus (cfu.mL^{-1})	*Enterococci* (cfu.100mL^{-1})
October 2007	250	ND	ND	TG	TG	ND	ND
November 2007	750	ND	16	TG	ND	ND	ND
December 2007	850	ND	ND	ND	ND	ND	15
February 2008	32 000	100	ND	ND	ND	ND	58

TG = typical growth; ND = none detected

Discussion – Berg 1

The results obtained from Berg 1 were found to vary, with some tests revealing no counts while high levels were detected in others. The counts for coliforms and faecal coliforms were high with three out of the four samples carrying loads in excess of the South African and international guidelines for irrigation water (DWAF, 1996b; WHO, 2006). The counts for both aerobic and anaerobic endospore formers were low in the two instances where growth was detected, while growth was absent on the other two occasions.

Listeria was detected on one of the four sampling occasions while *Salmonella* was detected on two of these occasions. The presence of these two potential pathogens is a further indication that faecal contamination is present in the river. This reality in itself provides great cause for concern as it indicates the possibility of the presence of other pathogens associated with faecal contamination.

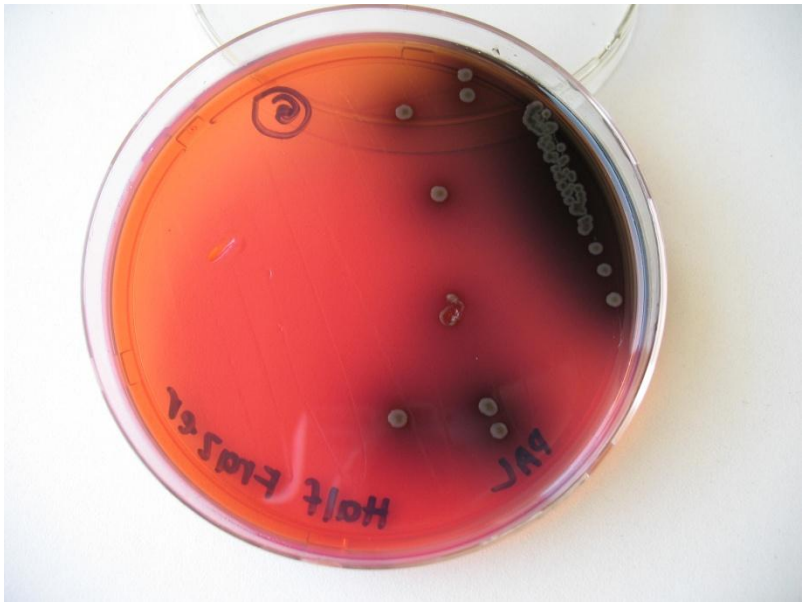

Figure 4 Typical growth of *Listeria* on Palcam Agar.

Berg 2

Site description – Berg 2

Berg 2 was also along the Berg River but downstream of Berg 1, between Franschhoek and Paarl. It was accessed by turning off the R301 onto the Fraaigelegen Road. A map of the area with the position of the site (circled in red) is shown in Fig. 5. Berg 2 was downstream of the wastewater treatment plant at Drakenstein Correctional Services, which has been reported to malfunction at times (Barnes, J.M. 2007. University of Stellenbosch,

personal communication) with untreated sewage overflowing into the Berg River. The river water at Berg 2 is used by local farmers who pump water from the river for both drip and overhead irrigation purposes. The water at Berg 2 was typically dark brown and opaque with an average depth of 1 m.

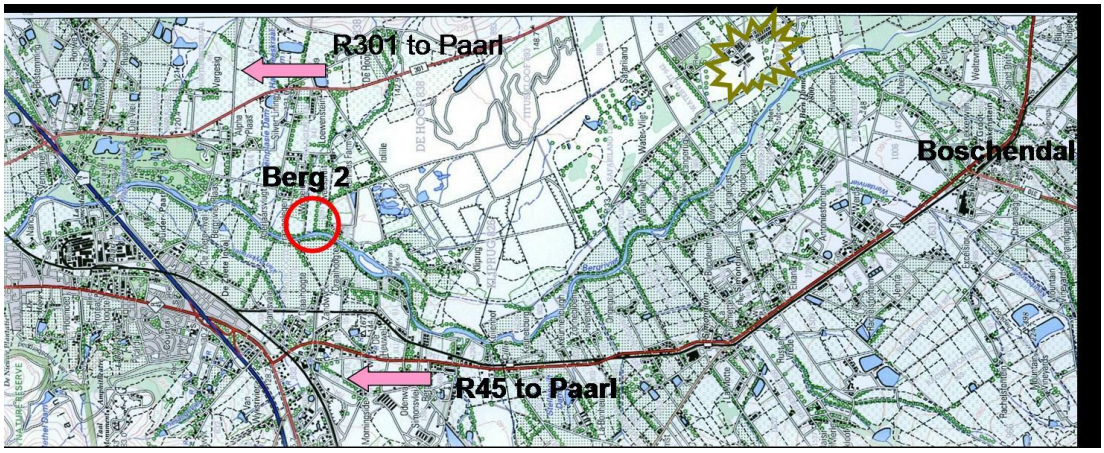

Figure 5 Map of Berg 2 site along the Berg River between Franschhoek and Paarl.

Environmental and chemical results – Berg 2

On the four sampling occasions, the temperature of the water varied from 15.3°C to 21.6°C (Table 4).

The pH ranged from 4.97 to 6.98 with the 4.97 result being very low (DWAF, 1999; WHO, 2006). The soil surrounding the Berg River is acidic (Conradie, 2002), and this could be one explanation for the lower pH vaules. However, the low pH could also be an indication of acidification due to pollution of the river and this should be kept in mind, particularly during microbiological analysis. The alkalinity of all four of the samples that were taken was below 80 mg $CaCO_3.L^{-1}$, indicating a generally poor buffering capacity of the river water during the sampling period (Spellman, 2008). The conductivity of the water was low (Table 4), indicating low levels of inorganic pollutants (LRCA, 2007). The COD values obtained from three of the four samples (Table 4) were less than 30 $mg.L^{-1}$, complying with DWAF's (1999) requirements for irrigation water.

Table 4 Environmental and chemical data for samples from Berg 2

Sampling date	Temp °C	pH	Alkalinity mg $CaCO_3.L^{-1}$	Conductivity $mS.m^{-1}$	COD $mg.L^{-1}$	Rainfall mm
October 2007	15.3	4.97	5	8	40	30
November 2007	17.4	6.04	25	19	21	14
December 2007	21.0	5.70	25	18	23	17
February 2008	21.6	6.98	13	7	23	17

Rainfall figures for the Drakenstein region obtained from the South African Weather Service (2008).

Microbiological results – Berg 2

The faecal coliform levels were less than those for coliforms, indicating the presence of non-faecal coliforms in the river water (Table 5). Typical E. coli growth was observed on L-EMB plates (Fig. 2). However, the E. coli load detected in the river water for February 2008 was the only value to exceed the South African guideline for E. coli levels in irrigation water (DWAF, 1996b). It should be noted that this particular load was exceptionally high and indicated an abnormal level of faecal contamination in the water at Berg 2.

Table 5 Coliform, faecal coliform and *E. coli* data from the Berg 2 samples

Sampling date	Coliforms (organisms.$100mL^{-1}$)	Faecal coliforms (organisms.$100mL^{-1}$)	*E. coli*
October 2007	7 000	950	TG
November 2007	440	69	TG
December 2007	7 000	920	TG
February 2008	200 000	200 000	TG

TG = typical growth

The ACC loads as shown in Table 6 were found to vary, and ranged from 320 to as high as 85 000 cfu.mL^{-1}. The loads of 2 260, 29 000 and 85 000 cfu.mL^{-1} are high for colony counts and this could indicate an increased risk of the transmission of infectious diseases (DWAF, 1996a).

Low levels of aerobic and anaerobic endospore formers were detected with the highest detected loads being 40 and 15 cfu.mL^{-1}, respectively (Table 6). While these loads might appear low, it should be kept in mind that single endospores can cause infection.

Typical *Salmonella* growth was obtained on XLD Agar (Merck) from the river sample taken in November 2007. The *Salmonella* colonies were clear with a black centre and the surrounding XLD Agar did not change colour to yellow (Merck, 2007) (Fig. 3).

Intestinal Enterococci were detected on three of the four samples with the 56 cfu.100 mL^{-1} being the highest (Table 6). This is another indication of the presence of faecal contamination in the river.

Table 6 Microbiological data from Berg 2

Sampling date	ACC (cfu.mL^{-1})	Aerobic endospore-formers (cfu.mL^{-1})	Anaerobic endospore-formers (cfu.mL^{-1})	*Salmonella* TG/ND	*Listeria* TG/ND	Staphylococcus (cfu.mL^{-1})	Enterococci (cfu/100mL^{-1})
October 2007	85 000	ND	ND	ND	ND	ND	43
November 2007	320	21	15	TG	ND	ND	ND
December 2007	2 260	40	ND	ND	ND	ND	23
February 2008	29 000	ND	ND	ND	ND	ND	56

TG = typical growth; ND = none detected

Discussion – Berg 2

The results from the four sample sets of data varied which indicates a variation in the water quality. The counts for the aerobic and anaerobic endospore formers were again low and were not high enough to cause alarm. Neither *Listeria* nor *Staphylococcus* was detected in any of the samples and only in one of the four samples was *Salmonella* detected. While this finding is positive, it should not be ignored as it is evident that the pollution entering the river is not constant, which makes the quality of the river water difficult to predict. The absence of *Listeria* and *Staphylococcus* at Berg 2 indicates that the levels of bacteria in river water are not constant and it is possible that different sources of contamination are responsible for the introduction of the different pathogens into river systems. By preventing or reducing different pathways of contamination, bacteria associated with those pathways (i.e. *E. coli* and faecal contamination) can potentially be reduced, or even eliminated. However, Berg 2 would need to be monitored for a longer period of time before conclusions regarding the absence of *Listeria* and *Staphylococcus* in the water can be made. Considering that *Listeria* and *Staphylococcus* were detected at Berg 1, it is possible that these bacteria were present at Berg 2, but that the concentrations were too low to be detected.

The COD was low with three of the samples having values less than 30 $mg.L^{-1}$ and the fourth was slightly elevated at 40 $mg.L^{-1}$, but still well within Category 4 which means that the water is suitable for irrigation of products that will be processed before consumption. Category 4 specifies that the COD of the sample must be less than 75 $mg.L^{-1}$ (DWAF, 1999).

All but one of the samples resulted in faecal coliform levels that fell within the international limit for faecal coliforms. This was surprising, especially when the results obtained at Berg 1 are taken into consideration, as it is upstream of Berg 2. The February 2008 sample, however, contained 200 000 organisms.100 mL^{-1} for faecal coliforms (Table 5) which did provide cause for concern. This was much higher than that found at Berg 1 and might be an indication of a source of contamination after Berg 1. The highest intestinal *Enterococci* load was also detected in February 2008. Both faecal coliforms and intestinal *Enterococci* can be traced to faecal origins and high levels of these bacteria indicates the presence of faecal pollution at Berg 2 in February 2008. The faecal coliform values for October and December 2007 correspond with the intestinal *Enterococci* loads for these two months which are lower than that detected in February 2008, but still indicate a presence of faecal pollution.

Berg 3

Site description – Berg 3

The third site along the Berg River was downstream of both of the aforementioned Berg River sites. Berg 3 was near the town Wellington and 100 m downstream of the Lady Loch Bridge (Fig. 6). In addition to all the possible upstream contamination mentioned sources above (Berg 1 and Berg 2), Berg 3 was downstream of the Wellington wastewater treatment plant which has also been reported to malfunction at times (Barnes, J.M. 2007. University of Stellenbosch, personal communication). At Berg 3, the river was wider than at Berg 1 and Berg 2 and the flow of the water was ‘moderate’. The water was dark brown and visibility was always less than 30 cm. The banks contained a lot of pollution varying from plastic bags to rusted shopping trolleys. The depth of the water in the middle of the river was unknown as the banks were very steep and made up of an extremely soft and crumbly earth which made the sampling process very difficult. As a result of this, samples were taken closer to the river bank.

Environmental and chemical results – Berg 3

The temperature of the river at the times of sampling, along with the chemical data obtained from the samples, is given in Table 7. The temperature of the water ranged from 14.0°C to 23.5°C.

The pH value for each of the four samples fell within the safe range for rivers (WHO, 2006). The alkalinity of the water for all of the samples was below 80 mg $CaCO_3.L^{-1}$ which indicates a poor buffering capacity which could result in rapid pH fluctuations (Spellman, 2008). The conductivity of the water was low, indicating a low level of inorganic pollutants (LRCA, 2007). The COD values from the samples taken in November and December 2007 were all below 30 $mg.L^{-1}$, indicating a low demand for oxygen from chemical pollution present in the water. This in turn indicates that the levels of chemical pollution in the water are low enough for the water to be acceptable for irrigation (DWAF, 1999). The COD of the samples from October 2007 and February 2008 fell slightly outside this limit and would not be safe for irrigation of produce for raw consumption (DWAF, 1999).

Table 7 Environmental and chemical data from analysis of samples from Berg 3

Sampling date	Temp °C	pH	Alkalinity mg $CaCO_3.L^{-1}$	Conductivity $mS.m^{-1}$	COD $mg.L^{-1}$	Rainfall mm
October 2007	14.0	6.75	25	14	41	30
November 2007	19.2	6.05	25	14	14	14
December 2007	23.2	6.06	38	18	14	17
February 2008	23.5	7.01	13	14	54	17

Rainfall figures for Wellington obtained from the South African Weather Service (2008).

Microbiological results – Berg 3

The coliform levels in the samples taken from Berg 3 were found to be very high and this translated to the faecal coliform load for three out of the four samples exceeding the South African guideline for *E. coli* in irrigation water (Table 8) (DWAF, 1996b). Typical *E. coli* growth on L-EMB Agar (Fig. 2) was observed for all four samples. The *E. coli* loads in these results revealed that the river water at Berg 3 can be contained faecal contamination at high levels (1 700 000 faecal coliforms per 100 mL).

Table 8 Coliform, faecal coliform and *E. coli* data from the analysis of Berg 3 samples

Sampling date	Coliforms (organisms.100mL^{-1})	Faecal coli (organisms.100mL^{-1})	*E. coli*
October (2007	20 000	20 000	TG
November (2007)	9 200	540	TG
December (2007)	20 000	5 400	TG
February (2008)	1 700 000	1 700 000	TG

TG = typical growth

High ACC loads (Table 9) were detected with the highest load reaching 440 000 cfu.mL^{-1} in February 2008. However, all of the ACC levels indicate inadequate water quality due to excessive microbial content and this will increase the chance of pathogens being present in the irrigation water that comes into contact with produce. The increase in amount of microbes that the produce is exposed to increases the chances of pathogen survival on produce that will reach the consumer (DWAF, 1999). Two of the samples contained aerobic endospore formers while no anaerobic endospore formers were detected in any of the samples (Table 9). Typical *Salmonella* growth was observed on XLD Agar (Fig. 3) in November 2007. *Staphlyococcus* was detected on Baird-Parker Agar (Merck) (Fig. 7) in two of the samples, with the February 2008 sample carrying a *Staphylococcus* load of 600 cfu.mL^{-1}. Intestinal *Enterococci* were detected in all four of the samples with the loads ranging from 1 to >1 000 organisms.100 mL^{-1} (Table 9). These levels corresponded with the faecal coliform loads detected in the samples and indicated that high levels of faecal contamination were present.

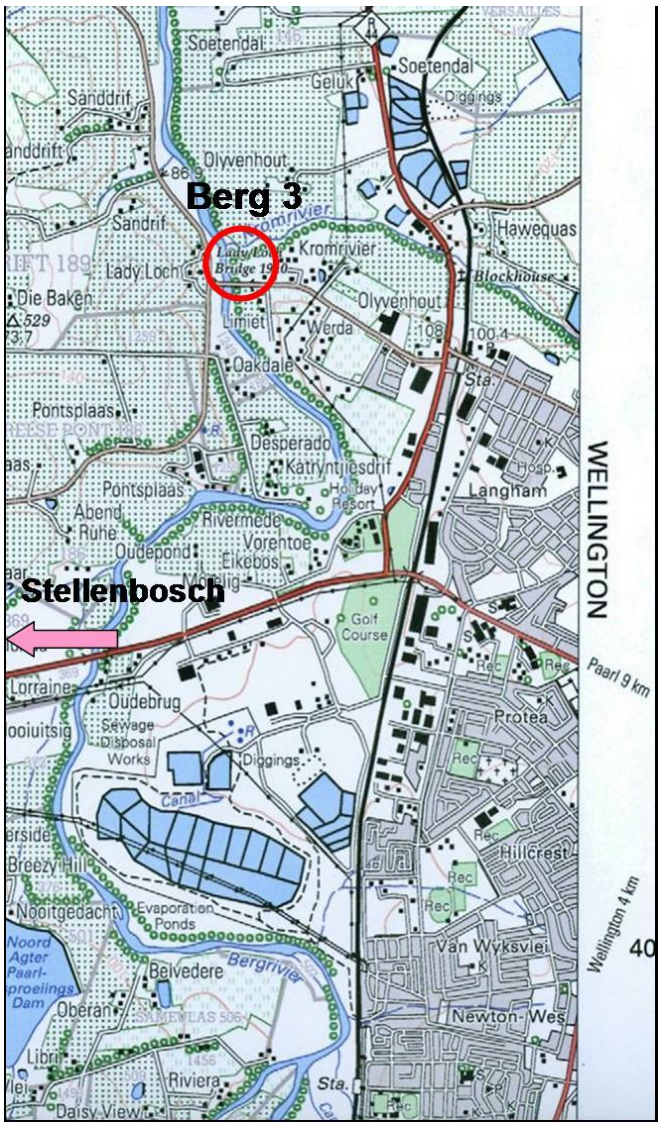

Figure 6 Map of Wellington and the surrounding area with Berg 3 marked with the red circle.

Table 9 Microbiological data from the analysis of samples from Berg 3

	ACC (cfu.mL^{-1})	A+spores (cfu.mL^{-1})	A-spores (cfu.mL^{-1})	*Salmonella* TG/ND	*Listeria* TG/ND	Staphylococcus (cfu.mL^{-1})	Enterococci (cfu.100mL^{-1})
October 2007	12 900	ND	ND	ND	ND	10	1
November 2007	1 830	53	ND	TG	ND	ND	>1 000
December 2007	8 300	ND	ND	ND	ND	ND	66
February 2008	440 000	80	ND	ND	ND	600	52

TG = typical growth; ND = none detected

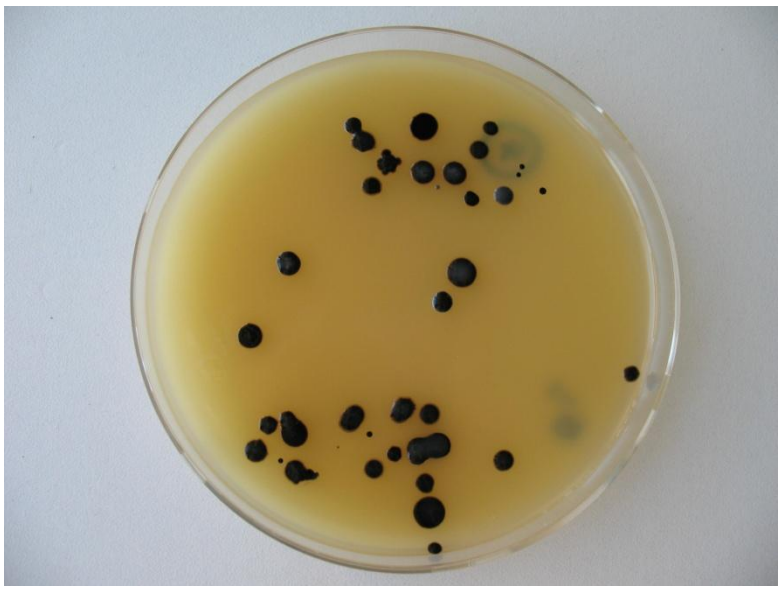

Figure 7 Typical growth of *Staphylococcus* colonies on Baird-Parker Agar.

Discussion – Berg 3

ACC values above the recommended guideline of 1 000 cfu.mL^{-1} for all four samples gives an indication that the river water at this site was polluted as all of the samples exceeded safe levels for irrigation water (DWAF, 1999). The ACC for February 2008 was particularly high at 440 000 cfu.mL^{-1}. This value was higher than the other three detected loads and indicates particularly bad water quality at the time that this sample was taken. In all four samples only aerobic endospore formers were detected and of these, only two of the four samples were positive for them with the respective values being 53 and 80 cfu.mL^{-1}. The pH range was fairly constant with the values between 6.05 and 7.01 for the four samples. The COD values were low for all of the samples but only two of the COD's were less than 30 mg.L^{-1} while the other two samples just exceeded this but were still less than 75 mg.L^{-1} which classified them as acceptable for irrigation of products to be processed (Category 4) (DWAF, 1999). *Salmonella* growth was detected in one of the four samples but *Listeria* was not detected in any of the samples. Two of the four samples were negative for *Stapylococcus* while low levels were detected in the third sample. However, the fourth sample (November 2007) tested positive and was found to carry a load of 600 cfu.mL^{-1}. This value is a cause for concern as it indicates that a contaminant was present at a high level. All of the four samples were also positive for intestinal *Enterococci* with one particular sample containing more than 250 cfu.100 mL^{-1}.

The counts for coliforms and faecal coliforms were consistently high with only one of the samples falling inside the legal limit for faecal coliforms in irrigation water (DWAF,

1996b; WHO, 2006). The lowest of the other three samples exceeding the limit was 5 400 organisms.100 mL^{-1} and the highest was 1 700 000 organisms.100 mL^{-1}. Such values indicate serious contamination of the Berg River and these levels make the use of this water for irrigation purposes questionable.

Considering that Berg 3 is the furthest downstream of the three sites on the Berg River, contamination is likely to enter the system after Berg 2, as the faecal coliform levels in the water were higher than the levels at Berg 2. While these results only reflect the state of the river at the time that the samples at the three sites were taken, the different indicator and index organisms that were present do provide insight into the nature of contamination that might be entering the river.

Plank 1

Site description – Plank 1

Plank 1 is at a low-level crossing on the Plankenburg River which flows past the Plankenbrug industrial area in Stellenbosch (Fig. 8). This specific site was sampled from in 203 (Barnes & Taylor, 2004) and reported to have “dangerously high” *E. coli* counts. Since this Barnes & Taylor report no *E. coli* values have been published. The objective of this specific study was to get an indication of the level of microbial pollution this site (Plank 1) could, if sampled over a longer period, be useful for comparison purposes. Additionally, this site was visibly polluted and the state of pollution was largely attributed to pollution entering the river from the Kayamandi settlement and Plankenbrug industrial area. It was reported (Barnes & Taylor, 2004) that the sanitation infrastructure in Kayamandi was minimal and that many of the existing facilities were not in working order. As a result of this, public sanitation facilities overflowed and effluent was washed either directly into the river or into storm water systems that, in turn, drained into the Plankenburg River.

In other cases, the lack of sanitation facilities resulted in households disposing of human and household waste directly into the Plankenburg River (DWAF, 2001). Furthermore, both industrial and sewage wastes were thought to be released illegally into the Plankenburg River by some of the local industries. The dumping of human waste could increase the levels of faecal contamination while industrial waste could alter the chemical and nutritional properties of river water, causing the inherent environmental conditions of the river water to change. This environmental change could, in turn, result in a different spectrum of microbes to be present and could also lead to organisms adapting their survival criteria in order to survive in the new environmental conditions.

The water at Plank 1 varied in depth from 15 to 45 cm over the 12 month sampling period and the flow of the river was, on every sampling occasion, moderate to fast.

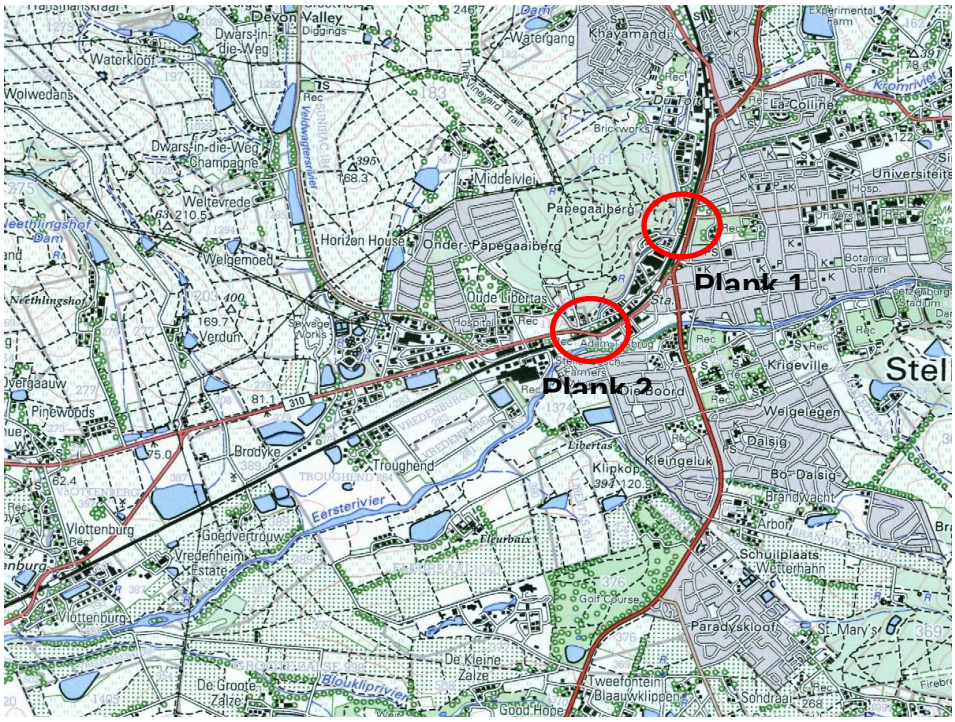

Figure 8 Map of Plankenbrug industrial area with sites Plank 1 and Plank 2 marked with the red circles.

<u>Environmental and chemical results – Plank 1</u>

The temperature of the water at Plank 1 varied between 11.8°C and 20.8°C with the range of temperatures measured given in Table 10. The pH of the samples varied from 5.64 to 6.80 (Table 10). The former value fell outside of the recommended pH range (6 to 9) for river water (DWAF, 1999; WHO, 2006). Bacteria produce acids through metabolism, if suitable carbon sources are available, and it is because of this that a low pH environment is commonly linked to the presence of bacterial contaminants. Apart from August and September 2008, the alkalinity of the water for all the samples was above 20 mg $CaCO_3.L^{-1}$ (Table 10), indicating a strong buffering capacity (Pai *et al*., 2001). Buffering capacity describes the ability of water to counteract a change in pH in order to protect itself against the environment.

The conductivity values for Plank 1 were higher than the values at any of the other sites (Table 10). All but two of the values exceeded 0.2 $mS.m^{-1}$, which is the recommended limit for water use in irrigation as levels higher than this indicate high loads of inorganic pollution. Therefore, the results in Table 10 indicate that the river contained higher levels of

inorganic pollutants than any of the other sites. The visible pollution along the banks of Plank 1 which was also observed while taking river water samples, corresponds with the findings in these results.

The COD value of three of the river water samples (Plank 1) was below 30 $mg.L^{-1}$ (Table 10) while the remaining samples exceeded this limit. In other words, if one were to use the COD levels as a guideline, there were only three occasions on which the river water could be said to have been suitable for irrigation (DWAF, 1999).

Table 10 Environmental and chemical data for the 11 month sampling period.

Sampling date	Temp °C	pH	Alkalinity mg $CaCO_3.L^{-1}$	Conductivity $mS.m^{-1}$	COD $mg.L^{-1}$	Monthly rainfall mm
September 2007	12.5	5.97	100.0	47	40.5	30
October 2007	17.9	6.41	125.0	55	66.6	14
December 2007	19.6	6.47	50.0	39	54.0	17
February 2008	20.8	6.80	No reading	No reading	No reading	17
March 2008	15.1	6.38	1125.0	70	32.4	20
April 2008	16.3	6.54	87.5	47	8.0	41
May 2008	17.7	6.61	100.0	47	26.1	69
June 2008	14.0	6.84	117.5	62	75.6	93
July 2008	flooding					182
August 2008	11.8	5.64	14.4	11	20.2	77
September 2008	12.2	6.23	14.4	12	42.1	40

Rainfall figures obtained from the South African Weather Service (2008).

Microbiological results – Plank 1

High levels of both coliforms and faecal coliforms were detected in the samples from Plank 1 (Table 11). Typical growth for *Escherichia coli* was observed on L-EMB Agar for all of the samples that produced positive results in EC broth (Fig. 2). The method (MFHPB-19, 2002) is designed so that only thermotolerant gas-forming faecal coliforms grow in EC broth. Therefore, *E. coli* should be found on the L-EMB agar and therefore it was concluded that the values of the faecal coliforms could be taken as that of *E. coli* (Health Canada, 2002).

However, contrary to the recommendation of the method (Health Canada, 2002), bacterial growth other than typical *E. coli* colonies was also observed on L-EMB Agar (Fig. 8). The API system was used to identify these atypical colonies and they were found to be *Enterobacter aerogenes* (I.D 96% API 20E). Figure 9 shows the atypical colonies on L-EMB Agar that were identified as members of *Enterobacter aerogenes*. The assumption that the method (MFHPB-19, 2002) is based on, namely that only gas-forming thermotolerant faecal coliforms will grow in EC broth and on L-EMB agar, can be

questioned. The implications of this is that, in some cases, *E. coli* loads are being over-estimated, particularly if the step of plating out the gas-positive EC broth tubes onto L-EMB agar is omitted. In addition, the presence of other potentially pathogenic faecal coliforms is being overlooked. By using L-EMB agar, it can be seen whether any colonies other than *E. coli* are present but no quantitative assessment of these other colonies is currently possible using this method.

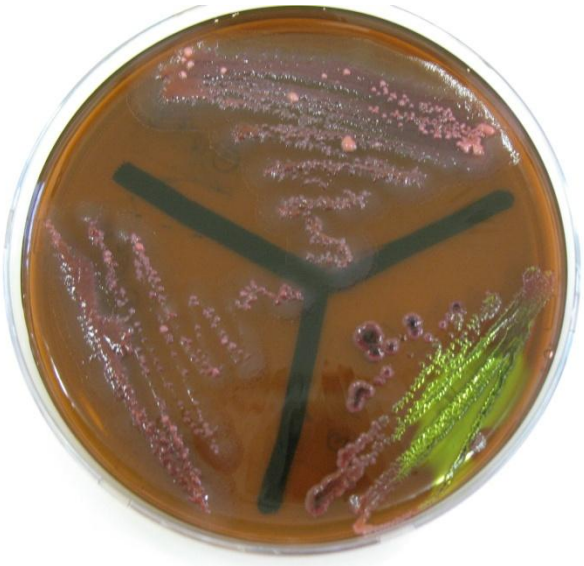

Figure 9 Growth of *Escherichia coli* colonies (green) and *Enterobacter aerogenes* colonies (pink) on L-EMB Agar.

The faecal coliform loads detected were lower than the coliform count, but the levels of the latter were still in excess of the South African guideline of <1 000 per 100 mL for *E. coli* in irrigation water (DWAF, 1996b). The *E. coli* loads detected meant that on all but one of the sampling dates, the river water was unsafe to be used for irrigation.

There is also the likelihood of contamination from vegetables and other crops eaten raw will result in the transmission of human pathogens. When the faecal coliform loads are compared to those reported by Barnes & Taylor (2004), it is clear that the counts from this study are much lower. No explanation can be given for this.

Table 11 Coliform, faecal coliform and *E. coli* data from the analysis of 11 samples from Plank 1 over a twelve month period

Sampling date	Coliforms (organisms.100mL^{-1})	Faecal coliforms (organisms.100mL^{-1})	*E. coli*
September 2007	70 000	14 000	TG
October 2007	17 000	17 000	TG
December 2007	460 000	160 000	TG
February 2008	350 000	28 000	TG
March 2008	35 000	35 000	TG
April 2008	490	490	TG
May 2008	3 300	1 300	TG
June 2008	33 000	13 000	TG
July 2008	Flooding	Flooding	Flooding
August 2008	160 000	160 000	TG
September 2008	92 000	92 000	TG

TG = typical growth

The ACC levels detected were in all cases in excess of 1 000 cfu.mL^{-1} for each of the ten samples (Table 12). This was an indication that the river carried high microbial loads that, in turn, increased the risk of causing infection if consumed (DWAF, 1996a). Six of the ten samples were positive for growth of aerobic endospore formers while four out of the ten were positive for growth of anaerobic endospore formers (Table 12).

Typical *Salmonella* growth was observed on XLD Agar for three of the four samples while typical *Listeria* growth was observed on Oxford and Palcam Agar (Fig. 4) for eight out of the ten samples (Table 12). High levels of *Staphylococcus* were detected on Baird-Parker Agar for two of the four positive samples and intestinal *Enterococci* were present in all of the samples. On one occasion (Table 12), both the *Staphylococcus* and intestinal *Enterococci* plates were overgrown, indicating high levels of these bacteria. Alternatively, it should also be considered that some of these colonies were spreaders and that the overgrown plates may simply have been due to the nature of these colonies.

All of the samples from Plank 1 contained abnormally high loads of bacteria of possible faecal origin or bacteria that indicated the presence of faecal contamination. The results also indicated an immediate need to improve the state of the river and to reduce the pollution entering it.

Table 12 Microbiological data from Plank 1

Sampling date	ACC (cfu.mL^{-1})	A+spores (cfu.mL^{-1})	A-spores (cfu.mL^{-1})	*Salmonella* TG/ND	*Listeria* TG/ND	Staphylococcus (cfu.mL^{-1})	Enterococci (cfu.100mL^{-1})
September 2007	53 000	40	10	TG	TG	80	20
October 2007	8 800	10	30	TG	TG	30 000	5
December 2007	150 000	ND	ND	TG	TG	ND	320
February 2008	25 900	ND	70	ND	TG	390	65
March 2008	15 500	ND	ND	TG	TG	ND	8
April 2008	3 600	160	ND	TG	TG	ND	43
May 2008	16 100	3600	ND	TG	TG	ND	11
June 2008	378 000	370	560	TG	ND	ND	152
July 2008	flooding	flooding	flooding	flooding	flooding	flooding	flooding
August 2008	17 000	1200	ND	TG	TG	10 000	131
September 2008	51 000	ND	ND	ND	ND	ND	94

TG = typical growth; ND = none detected

Discussion – Plank 1

The close proximity of this site to the Kayamandi settlement was used to explain most of the high pathogen levels detected in the water samples. The COD values were slightly higher than those detected at the other sites but still fell within Category 4 (less than 75 mg.L^{-1}) and according to DWAF(1999) the water should be safe for irrigation. The pH ranged between 5.64 and 6.84, showing only a slight fluctuation in pH and indicating that there is a relatively stable degree of consistency in the water. The temperature of the river water changed with the seasons with the lowest temperature being recorded as 10.2°C in August 2008 and the highest as 20.8°C in February 2008. While this temperature range might appear wide, it covers the lower growth range for mesophilic and the upper range for psychrophilic bacteria and thus could provide favourable conditions for their growth. However, the active growth of *E. coli* at this temperature range can be questioned (LeClerc *et al*., 2001).

The ACC levels were high, reflecting the presence of a large number of microorganisms in the river water which, in turn, suggests a likelihood that disease-causing organisms could be present (DWAF, 1996). The sporeformer counts for most of the months were less than 100 cfu.mL^{-1}. However, several high values were obtained between May and August 2008 with the highest count for aerobic endospore formers being 3 600 cfu.mL^{-1} and the highest for anaerobic endospore formers being 560 cfu.mL^{-1}. These values are

high and raise a cause for concern because a large number of spores could potentially be produced. Endospore formers such as *Bacillus cereus* are often found in soil and are, therefore, likely to be detected in river water if they are present in the soil. The presence of these bacilli would not be ideal as they are human pathogens and could cause disease if consumed (Bergey & Holt, 1994). These spores are capable of surviving under unfavourable conditions and could easily survive on produce until consumption.

Typical growth of *Listeria* was observed in eight out of the ten water samples. The species detected on Oxford and Palcam Agar were identified with API and found to be *Listeria grayii* I.D 94%, *Listeria ivanovii* I.D 92%, *Listeria innocua* I.D 96% and *Listeria monocytogenes* I.D 89%. *Salmonella* was also detected in eight out of the samples that were taken and is again an indication of faecal contamination. Human contamination was further confirmed through the detection of both *Staphylococcus* (four out of the ten samples were positive) and intestinal *Enterococci* (all positive). The highest load found for *Staphylococcus* was 10 000 cfu.mL^{-1} while up to 300 intestinal *Enterococci*.100 mL^{-1} were detected. Coliforms and faecal coliforms were found to be present in consistently high loads with only one of results from the ten samples being lower than the international limit of 1 000 organisms.100mL^{-1} (WHO, 2006). These results are of great concern as this water from lower down the river is regularly drawn for irrigation and the levels detected exceed all limits for irrigation as well as product consumption.

Plank 2

Site description – Plank 2

Plank 2 was accessed from the suburb of Die Boord in Stellenbosch. The site was directly upstream of a large weir, at the confluence of the Eerste and Plankenburg Rivers (Fig. 8). The rivers flow parallel to each other, separated only by a wall of sand-bags, before converging. The samples were taken on the side of the Plankenburg River where water is channelled to a canal for irrigation purposes.

From this site, a service canal runs alongside the river and carries the water to local farmers who pump river water into storage dams on their farms. This water is then used for irrigation purposes. The visual appearance of the Eerste and Plankenburg Rivers usually differed greatly, with the Plankenburg River being opaque, milky and brown in colour while the Eerste River was clear and brown, typical of river water in the Western Cape. At Plank 2, both rivers averaged a depth of more than 30 cm.

Environmental and chemical results – Plank 2

Over the four sampling periods (Table 13) the recorded water temperature ranged from 12.5°C to 21.6°C. The pH of all the samples was similar and ranged between 6.20 and 6.85 (Table 13). The alkalinity values of the September and October 2007 and also the February 2008 water samples exceeded 80 mg $CaCO_3.L^{-1}$ indicating that the water had a good buffering capacity (Spellman, 2008). The value of 350 mg $CaCO_3.L^{-1}$ obtained from the September 2007 sample was high, indicating a possible abnormality in the water composition at the time that the sample was taken. The conductivity values of the samples ranged between 38-350 $mS.cm^{-1}$ while guidelines require the value to be below 20 S $mS.cm^{-1}$. These values are comparable with the values from Plank 1, which indicated similarly high levels of inorganic pollutants at Plank 2.

The COD values from September and December 2007 and February 2008 exceeded 30 $mg.L^{-1}$ (DWAF, 1999) but were still lower than the 75 $mg.L^{-1}$ level that is recommended by DWAF for crops containing produce that are going to undergo processing.

Table 13 Environmental and chemical data for samples from Plank 2

Sampling date	Temp °C	pH	Alkalinity mg $CaCO_3.L^{-1}$	Conductivity $mS.m^{-1}$	COD $mg.L^{-1}$	Monthly rainfall mm
September 2007	12.5	6.38	350	45	51	30
October 2007	18.2	6.20	125	75	7	14
December 2007	19.9	6.78	38	22	74	17
February 2008	21.6	6.85	122	36	42	17

Rainfall figures obtained from the South African Weather Service (2008).

Microbiological results – Plank 2

The coliform levels detected in the samples varied widely, from 4 900 to 540 000 organisms.100 mL^{-1} (Table 14). While the faecal coliform levels detected were lower than the coliform levels (Table 14), the lowest faecal coliform value was still more than the South African guidelines of 1 000 cfu.100mL^{-1} (DWAF, 1996b). For all of the samples from Plank 2, typical *E. coli* growth was the only growth observed on L-EMB plates (Fig. 2) for all four samples and it was assumed that the faecal coliform represented the *E. coli* load. Barnes & Taylor (2004) also sampled this specific site over a period of seven years (1998-2004) and

reported *E. coli* values from 130 to 264 000 per 100 mL. The highest value found in this study was 54 000 *E. coli* per 100 mL.

Table 14 Coliform, faecal coliform and *E. coli* data from Plank 2 samples

	Coliforms (organisms.100mL^{-1})	**Faecal coliforms (organisms.100mL^{-1})**	**E. coli**
September (2007)	54 000	9 500	TG
October (2007)	54 000	54 000	TG
December (2007)	540 000	8 100	TG
February (2008)	4 900	22 000	TG

TG = typical growth

The ACC numbers varied between 9 400 and 41 000 cfu.mL^{-1} for the four sample sets that were analysed (Table 15). These ACC values were all in excess of 1 000 cfu.mL^{-1} which indicated high levels of heterotrophic microbes in the water and, therefore, an increased risk of infectious disease transmission (DWAF, 1996a).

Aerobic endospore formers were only detected in the September 2007 sample, while anaerobic endospore formers were detected in all of the samples with the exception of December 2008. In all of the samples containing endospore formers, the loads were less than 20 cfu.mL^{-1} (Table 15). Loads such as these would ordinarily be considered low but it should be noted that spores can resume their vegetative state under favourable conditions. Therefore, with fresh product consumed raw, even low levels of endospore formers should be noted and caution should be exercised before declaring the product safe to eat.

Typical growth of both *Salmonella* (Fig. 3) and *Listeria* (Fig. 4) was observed in three of the four samples. High *Staphylococcus* loads were detected on Baird-Parker plates for three of the four samples, with two of the positive samples carrying loads in excess of 3 100 cfu.mL^{-1} (Table 15). Intestinal *Enterococci* were also detected in all four water samples which confirmed the presence of human – particularly faecal – contamination (Table 15).

Table 15 Microbiological data from Plank 2

Sampling date	ACC (cfu.mL^{-1})	Aerobic endospore formers (cfu.mL^{-1})	Anaerobic endospore formers (cfu.mL^{-1})	Salmonella	Listeria	Staphylococcus (cfu.mL^{-1})	Enterococci (cfu.100mL^{-1})
September 2007	41 000	20	10	TG	ND	40	254
October 2007	12 000	ND	10	ND	TG	30 000	350
December 2007	22 800	ND	ND	TG	TG	ND	67
February 2008	9 400	ND	10	TG	TG	3100	7

TG = typical growth; ND = none detected

Discussion – Plank 2

Plank 2 is about 2 km downstream of Plank 1. The temperature measurements were found to follow a very similar pattern (between 12 and 21°C) to those observed over the same time period at Plank 1. The results obtained from the samples do not appear to be directly related to the temperatures of the water as the highest loads were, in most cases, not detected in the February 2008 sample. While the effect of temperature on microbial survival and proliferation was not discounted, it is thought that in the samples taken from Plank 2, the higher loads are more likely a result of an increased amount of pollution in the river. However, these conclusions are only based on four sample and in order to better assess the impact of lower temperatures on the microbial load in the river, monitoring of the river water should continue in this way throughout the winter months when the water temperature is consistently cold and more unfavourable for microbial growth. The variation in temperature could have been sufficient to alter the growth conditions for the bacteria in the river. However, the water temperatures fell within the lower range for growth of mesophilic high limits for and psychrophilic bacteria and therefore the temperature difference is not likely to have had a large influence on the growth rates of the bacteria in the river (Geldreich, 1996).

The pH values measured were between 6.20 and 6.85 and fell within the WHO guideline range for river water. These pH values were normal for river water and fell between of 6 and 9, as recommended by WHO (DWAF, 1999; WHO, 2006). These values were safely within the required pH range for river water (WHO, 2006). The COD values were also in line with those measured at the other sites, with all readings falling below 75 mg.L^{-1}, which is the maximum COD for water used to irrigate produce that is going to be processed (DWAF, 1999).

Very few endospore formers were detected, with the only sample positive for aerobic endospore formers containing 20 cfu.mL^{-1}. Three of the four samples analysed were found to contain anaerobic endospore formers at a level of 10 cfu.mL^{-1} while no growth was found in December 2007. Typical growth of both *Listeria* and *Salmonella* was observed for three of the four water samples, while dangerous levels of *Staphylococcus* and intestinal *Enterococci* were observed in two of the four samples. These levels would be dangerous for human ingestion and water containing high *Staphylococcus* levels should not be used for irrigation as it indicates a strong presence of human contamination. As was discussed for Plank 1, these results are a very clear indication of the presence of faecal pollution in the river and the proximity of Plank 1 and Plank 2 to the Kayamandi settlement makes the likelihood of the pollution entering the river from this point is very strong. However, the level of the different bacteria that were detected varied between the sampling dates, indicating that the pollution entering the river systems was varying and unpredictable. All of the faecal coliform and *E. coli* loads exceeded the international (WHO, 2006) and South African (DWAF, 1996b) guidelines for irrigation water.

The data sets gathered from the analysis of the water samples (Table 15) reflected a polluted river system containing indicator organisms at levels that exceeded the maximum permitted load for those particular organisms in irrigation water (DWAF, 1996b).

API Identification

After the final step of each of the tests that were conducted on the water samples, each of the culture-specific media was examined for typical colony growth of the particular culture in question. Both typical and atypical colonies were found on the different growth media and these were isolated for species identification with the relevant API system. The microorganisms that were isolated from culture-specific detection media and identified using the API test (BioMerieux, France) are listed in Table 16.

While it was not within the scope of this study to detect and identify all of the microorganisms present in the samples of river water, it can be seen from the results in Table 16 that microorganisms – and potential pathogens – other than those that were tested for, were present. This highlights the importance and funtion of indicator organisms as the levels that they are present in reflects the state of contamination in river water, without it being necessary to test the water separately for each microorganism.

Table 16 Specific microorganisms isolated from the Berg River (Berg 1, 2 and 3) and Plankenburg River (Plank 1 and 2) (The API identification percentage is given in brackets)

Group and media	Berg 1	Berg 2	Berg 3	Plank 1	Plank2
Coliform (L-EMB)	E. coli (85%)	E. coli (60%)	E. coli (89%)	E. coli (82%)	E. coli (91%)
	Entero. aerogenes (94%)	Entero. aerogenes (90%)	Entero. aerogenes (87%)	Entero. aerogenes (96%)	Entero. aerogenes (91%)
		Shig. sonnei (72%)		Shig. sonnei (85%)	
					Kleb. pneumoniae (92%)
	Esch. spp.(98%)	Esch. spp.(95%)	Esch. spp.(89%)	Esch. spp.(87%)	Esch. spp.(82%)
Endospore (TSA)	Bacillus spp. (96%)	Bacillus spp. (92%)	Bacillus spp. (91%)	Bacillus spp. (89%)	Bacillus spp. (93%)
Salmonella (XLD)	S. enteritidis (possible I.D)	S. enteritidis (possible I.D)	S. enteritidis (74%)	S. enteritidis (66%)	S. enteritidis (possible I.D)
	Staph. aureus (90%)	Staph. aureus (93%)	Staph. aureus (82%)		Staph. aureus (97%)
	K. pneumoniae (89%)	K. pneumoniae (possible I.D)	K. pneumoniae (92%)	K. pneumoniae (90%)	K. pneumoniae (possible I.D)
				Ser. marcescens (72%)	
			Prot. mirabilis (97%)		
Listeria (Oxford)	L. grayi (92%)	L. grayi (88%)	L. grayi (83%)	L. grayi (94%)	L. grayi (95%)
	L. monocytogenes (possible I.D)			L. monocytogenes (89%)	
		L. ivanovii (96%)	L. ivanovii (98%)	L. ivanovii (92%)	
	L. seeligeri (79%)	L. seeligeri (80%)	L. seeligeri (96%)		
	L. innocua (94%)	L. innocua (95%)	L. innocua (92%)	L. innocua (96%)	L. Innocua (85%)

Enterto. = Enterobacter; E. = Escherichia; S. = Shigella; L. = Listeria; Staph. = Staphylococcus; K. = Klebsiella

S. = Salmonella; Ser. = Serratia; Prot. = Proteus.

Conclusion and Recommendations

The state of the two rivers that were monitored in this study was in most cases found to be unacceptable with regards to the safety of the water for irrigation purposes and certainly for human contact and consumption. The regular presence of bacteria of a faecal origin is clear evidence of faecal contamination due to either direct pollution or failing wastewater treatment plants. Considering that the levels of faecal coliforms and *E. coli* exceed the South African guidelines for *E. coli* in irrigation water, it can be concluded that the water should not be used for the irrigation of any produce and, more particularly, MPFs. These results from this study highlight the danger of faecal contamination on water quality and identify some of the main pathogens that can be expected to be detected in water when faecal contamination is present.

Farmers currently drawing water from these rivers for irrigation should be alerted that the bacteria being detected in the rivers as well as the loads at which they are being detected are resulting in the water being unsafe for use and, possibly, the irrigated produce for human consumption. If the state of the rivers and the quality of the water is not improved soon, very intensive and expensive treatment methods are going to have to be investigated in order to purify the water and reduce the loads sufficiently to make it safe for irrigation.

From this exploratory study, no direct relation was found between the environmental, chemical and microbiological results. This means that one set of chemical or microbiological descriptors cannot be used to make predictions about the microbiological state of the water. Therefore, until either a link between chemical and microbiological results is found or different testing methods become standard, each parameter should continue to be tested in accordance with the prescribed tested methods for the respective microorganisms.

With the data obtained from monitoring these rivers in this exploratory study, the aim was to gain an understanding of the microbial levels in two local rivers and through this, obtain an indication with regards to the safety of the water for irrigation purposes.

Having determined that the water in the Berg and Plankenburg Rivers is, at many times, not compliant with the South African guidelines for irrigation water, it should be investigated whether or not these levels detected would pose a threat to consumer health if this water was used in irrigation. What also needs to be determined is whether potential

pathogens are carried over to produce at this specific concentration and, in particular, to what extent this occurs on products that are consumed raw.

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CHAPTER 4

SURVIVAL OF *ESCHERICHIA COLI* LOADS ON GREEN BEANS *(PHASEOLUS VULGARIS)*, SUGAR-SNAP PEAS (*PISUM SATIVUM*) AND COCKTAIL TOMATOES (*LYCOPERSICON ESCULENTUM*)

Summary

Irrigation with faecally polluted river water is one way that fruit and vegetables can become contaminated with foodborne pathogens. The risk of disease transmission from potential pathogens present in the irrigation water is influenced by the microbial load present and the numbers carried over to the produce. In this study the carry-over and survival of *Escherichia coli* on green beans, sugar-snap peas and cocktail tomatoes was assessed under controlled laboratory conditions. The beans, peas and tomatoes were exposed to *E.coli* under different combinations of exposure times (5, 15 or 30 min), drying times (30 or 120 min) and different inoculum concentration ranges (10^7, 10^5, 10^4, 10^3 and 10^2). No clear profile was observed between the different exposure and drying time combinations and in many cases large variations in counts of surviving *E.coli* for the same treatment were found. The large variations were ascribed to the formation of clumps. The varying numbers present in such clumps made it nearly impossible to obtain a well homogenised sample. In all cases a reduction of at least one log value in original inoculum number was found with the 10^2 inoculums showing no survivors.

In a follow-up study the exposure time to the inoculum was increased to 60 min and drying times were increased (6 or 12 h). These extended times were used as they are more similar to actual times occurring during the commercial cultivation of produce. Neither these parameter changes affected the variation in numbers for the same inoculum or the *E.coli* survivors. However, a reduction was found for the lower inoculum concentrations after the longer drying time. Similar *E.coli* loads to those detected on the green beans were detected on the sugar-snap peas. The number of survivors on the cocktail tomatoes was much lower than found for the beans and peas. This was attributed the 'smooth' surface of the tomato skin probably making attachment of the *E.coli* bacteria difficult. With the exception of the 10^2 inoculum range, all *E.coli* survivors detected on the three types of produce studied exceeded the guideline numbers set for fresh produce. If similar survival

patterns are to be found in the environment then results from this study should serve as a warning that the Plankenburg river water is unsafe for use in the irrigation of fresh produce.

Introduction

Escherichia coli evaluation is one of the major tools used world-wide by health managers for the curtailment of waterborne diseases as the presence of large numbers of *E.coli* is clear evidence of faecal contamination. It was shown in Chapter 3 of this thesis and reported by other researchers (Barnes & Taylor, 2004; Dalvie *et al*., 2004; Mikize, 2006) as well as in the press (Kahn, 2008) that specific rivers in the Western Cape region of South Africa are heavily polluted with *E.coli* and thus pose a potential threat to human health.

Exposure to the water from these rivers can occur in numerous ways. For instance, rivers are often used for irrigation and recreational purposes, but also for daily household purposes including drinking, cooking, washing or sanitation (Matthews, 2006). This places those who are exposed to the water at risk of contracting disease from any organisms present in the river water (Hamilton *et al*., 2006).

From an agricultural perspective in South Africa, rivers are an essential source of irrigation water (Backeberg & Odendaal, 1998; Dalvie *et al.*, 2004). The question that is now being asked by consumers, farmers, researchers and the South African Water Research Commission is ‘what impact does this contaminated irrigation water have on the safety of the produce being irrigated with it?’ (Backeberg, G. 2007. Water Research Commission, personal communication; Kirsten, F. 2007. Goedvertrou Farm, Stellenbosch, personal communication; Kahn, 2008).

In some cases, irrigation systems use overhead sprayers (Kirsten, F. 2007. Goedvertrou Farm, Stellenbosch, personal communication) which results in daily direct contact between irrigation water and the produce, increasing the chance of carry-over of bacteria (Islam *et al*., 2005; Matthews, 2006). Although a carry-over of bacteria from irrigation water onto produce has been reported (Islam *et al*., 2005; Matthews, 2006), many farmers and retailers have responded apathetically towards the situation in the past in the hope that the claims were unfounded (Backeberg, G. 2007. Water Research Commission, personal communication).

One question that has continuously been asked is if irrigation water is faecally polluted what is the *E.coli* load necessary to be sure of carry-over to produce. There are

many environmental factors that would affect microbial survival on plants (Islam *et al*., 2005; Matthews, 2006) but relatively few studies have investigated carry-over loads from irrigation water to plants and the impact of environmental factors on survival (Lang *et al*., 2004; Steele & Odumeru, 2004; Aruscavage *et al*., 2006). Both the World Health Organisation (WHO) and the South African Department of Water Affairs and Forestry (DWAF) have guidelines for the quality of irrigation water. This applies to all water being used for the irrigation of crops, irrespective of its source. The current guidelines for *E.coli* in irrigation water are not more than 1 000 organisms.100 mL^{-1} (DWAF, 1996; WHO, 1989; WHO, 2006). From the literature there is no clear indication as to how the value of <1 000 *E.coli* per 100 mL was reached.

As for irrigation water, there are also guidelines for the number of *E.coli* organisms that are permitted on produce. These guidelines are in place in order to protect the consumer and indemnify producers who adhere to safety requirements. In South Africa, the guidelines for coliforms on produce that will be consumed raw, is 200 $cfu.g^{-1}$, and 0 $cfu.g^{-1}$ for *E.coli* (DoH, 2000), meaning that no *E.coli* may be present on the produce. The *E.coli* levels in the water being used for irrigation currently exceed 1 000 organisms.100 mL^{-1} (Chapter 3) and, as stated before, it is not clear whether or not any of the *E.coli* from such an inoculum load would survive on produce. Therefore, the safety of produce that is irrigated with heavily polluted river water is in question. In order to assess the severity of this threat, it was decided to conduct a set of experiments to determine, using different inoculum concentrations, whether or not a carry-over from contaminated water to produce does take place, and how much of the contamination load is actually transferred onto the produce.

The aim of this study was to evaluate the survival of a surrogate *Escherichia coli strain* on selected produce after exposure to a range of *E.coli* inoculums at known concentrations under controlled laboratory conditions. The produce will include green beans (*Phaseolus vulgaris)*, peas (*Pisum savitum*) and cocktail tomatoes (*Lycopersicon esculentum*). Environmental parameters will include: varying inoculum concentrations; exposure time of the product to the inoculum; and the period of time after exposure to the inoculum before testing for survivors.

Materials and Methods

Escherichia coli culture

For this study, an *E.coli* culture was used in order to allow a wider range of physical and environmental aspects to be explored. The chosen strain was a non-pathogenic *E.coli* strain (ATCC 11775). A freeze-dried *E.coli* culture was obtained from the Department of Food Science, Stellenbosch University. The culture was inoculated into 10 mL Nutrient Broth (NB) (Merck) and incubated at 35°C for 24 h. A 1 mL aliquot was then used to inoculate a further 10 mL of NB, which was then incubated at 35°C for 24 h. From this 5 mL was used to inoculate 250 mL NB which was again incubated at 35°C for 24 h and 5 mL of this culture was then used to inoculate 500 mL NB. This was used as basis to construct the standard curve. An API 20E test strip (Biomerieux) was performed on this culture to verify that it was an *E.coli* (ID = 99.2%). The purity of the cultures' Gram stain was microscopically (Leitz) examined to verify cell morphology and purity.

Standard curve to facilitate culture concentration determination

A standard curve was constructed for the *E.coli* ATCC 11775 culture (M. Cameron, 2007, Department of Food Science, Stellenbosch University, personal communication). The curve was constructed by measuring the absorbance of triplicate samples (500 nm) (Spectronic 20 Genesys^{TM}, Spectronic Instruments) drawn at specific time intervals and these results correlated with the counts from the corresponding plate-counts on Nutrient Agar (NA) (Merck). A baseline blank was set using 2 mL sterile NB in a sterile cuvette. The curve (and calculated regression line)(SigmaPlot version 7.0, SPSS) is shown in Fig. 1. The culture was maintained at 35°C throughout the preparation of the standard curve.

At each specific sampling time the bottle was carefully mixed well to ensure even distribution of the bacterial cells throughout the culture. Two mL of the culture was transferred to a sterile cuvette and the OD of the culture measured (Spectronic 20 Genesys^{TM}, Spectronic Instruments) at 500 nm. The cell concentrations in subsequent studies were determined using the regression line of the standard curve in Fig. 1.

Since *E.coli* cells are known to form aggregates which would impact the determination of the cell concentration, samples were vortexed (Scientific Manufacturing, Cape Town) for 2 min followed by ultrasonication (UMC2, Ultrasonic Manufacturing Company, Johannesburg) at 50 Hz for 10 min and then vortexing again for 2 min.

Use of absorbance to determine the desired E.coli concentration range

To facilitate cell concentration load determinations, use is made of standard curves from which a culture with a specific absorbance level can be used to make dilutions to the desired concentration range. As stated before it is known that *E.coli* cultures often form aggregates (Jarvis & Kaper, 1996; Weenk, 2003) so in order to ensure that the culture was correctly diluted to the desired concentration range, two different evaluations were performed. After the initial concentration of a sample had been determined from the standard curve, the sample was diluted using PSS to give three different cell concentrations ranges (10^2, 10^5 and 10^7 cfu.mL^{-1}). A dilution series to 10^{-8} in PSS of each of these samples was prepared. Each of the respective dilution series were plated out with NA in triplicate using the pour-plate technique and incubated for 48 h at 35°C. After incubation, the colonies were counted and an average of the three replications recorded. The results obtained were compared to the desired concentration ranges in order to assess the accuracy of the actual concentrations.

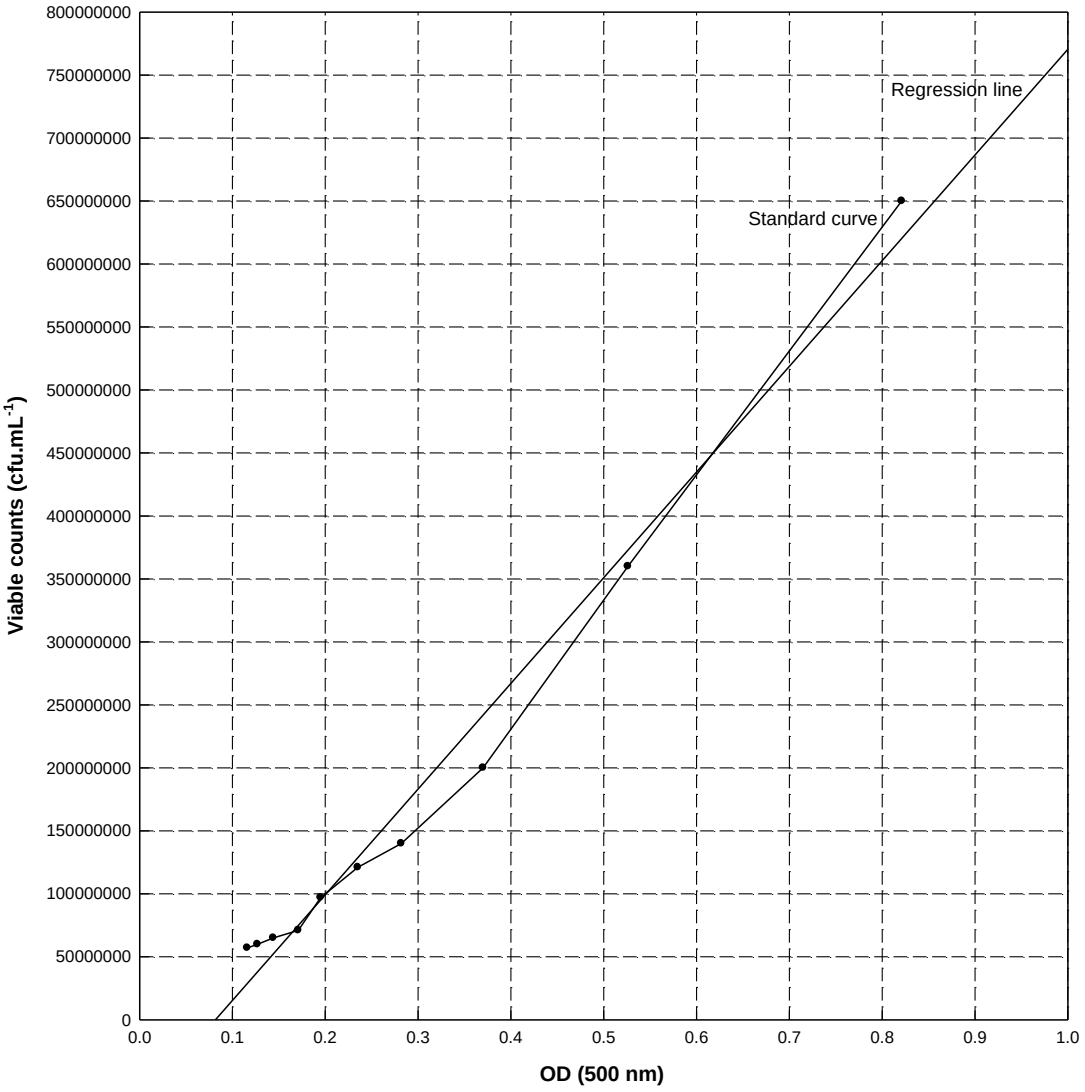

Figure 1 Standard curve of *E.coli* ATCC 11775 culture.

The second evaluation comprised two parts (as illustrated in Fig. 2) using absorbance as base for the dilutions. Both of these were performed as described above to give a range of either 10^2, 10^4 or 10^6 cfu.mL^{-1}. For the first part (Fig. 2 Part 1), a different dilution series was made from each of the samples and each of these plated out with NA using the pour-plate technique and incubated at 35°C for 48 h. For the second part (Fig. 2 Part 2), the absorbance of the same original culture was used for the dilution series to give a final concentration in the range of either 10^2, 10^4 or 10^6 cfu.mL^{-1}. For this part of the study only one dilution series was made but this was plated out on NA using the pour-plate technique a total of five times (i.e. five replications of one dilution series on NA). These plates were then also incubated at 35°C for 48 h. After incubation, the colonies on the

plates (30 - 300 cfu.mL^{-1}) were counted and the results were recorded and the average number for each concentration was calculated. The results were then compared with the desired cell concentration ranges.

Effect of exposing Green beans (Phaseolus vulgaris) to Escherichia coli

Green beans were the first produce type to be tested due to the practicality of their shape for laboratory evaluation. The surface characteristics of the bean were of interest as green beans are covered with a fine layer of hairs. It was suspected that these hairs might serve as a trap for bacteria and therefore increase the microbial load adhering to the bean.

The green beans were obtained from a local supermarket with an average length of 120 mm, a diameter of 8 mm and a mass of 6 g. Each bean was attached to a 25 cm long piece of nylon line by means of a loop around one end of the bean, secured firmly with a knot (Fig. 3). The beans were then placed inside a Bio-flow II cabinet (Labotec) (Fig. 4), where the sterilisation, exposure to the inoculum and drying process took place. The air-flow inside the cabinet was set at a constant 100 Pa. This facilitated the drying process both after sterilisation and after exposure.

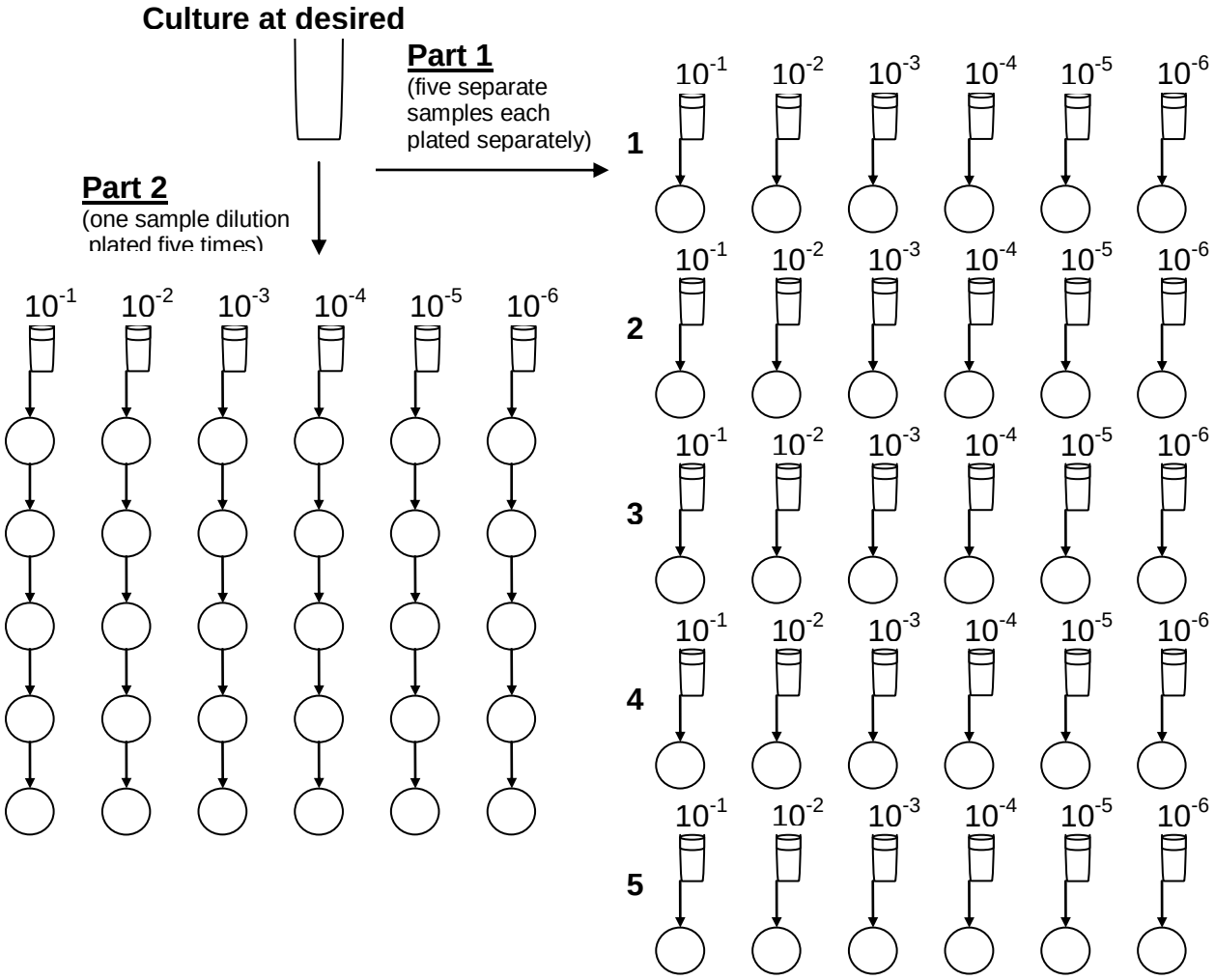

Figure 2 Diagram of the experimental set-ups using the absorbance readings from the standard curve as dilution base to determine the range of the final *E.coli* concentration.

Figure 3 Bean being exposed to inoculum (left) and sterile control bean secured by a nylon loop (right).

Figure 4 The Bio-flow II cabinet.

The beans were sterilized in 1.75% (m/v) sodium hypochlorite (NaClO) (Adams *et al*., 1989; Zhang *et al*., 2007). This step served to eliminate the chance that the organisms that have inherently colonised the beans would compete with the *E.coli* in the inoculum. The sterilisation process as recommended by Adams *et al*., (1989) and Zhang *et al*., (2007) consisted of beans being dipped in 3 consecutive 50 mL measuring cylinders containing NaClO, followed by washing in 3 measuring cylinders containing sterile distilled water.

Dipping times of 1 min and 3 min were tested in order to determine the most effective dipping time. A decision based on the effective sterilisation time was made to dip the beans into each test tube for 3 min. The beans were transferred aseptically between the measuring cylinders with a set of sterile tweezers and, once sterile, the beans were suspended from a glass rod so that they could air-dry at room temperature. After sterilisation, the beans were microbiologically analysed to determine the efficacy of the process. This was done by washing the beans in 10 mL sterile PSS inside a sterile stomacher bag and 1 mL PSS from each bag was then plated out onto NA and the plates were incubated at 35°C for 24 h before they were inspected for growth.

Exposure and drying time combinations

The *E.coli* culture in NB was incubated at 35°C for 48 h, after which the absorbance was measured. The cell concentration was then calculated using the standard curve (Fig. 1). From this culture, the volume that was needed for 600 mL culture with an end concentration in the range of 10^7 cfu.mL^{-1} was then transferred aseptically to a sterile Schott bottle and diluted with PSS up until a 600 mL volume was obtained. The actual cell concentration was also determined by serial dilutions in NA. Large test tubes were sterilised and a 50 mL volume of the 10^7 cfu.mL^{-1} inoculum was placed in each large test tube. Each bean was assigned a test tube and the rod – from which the beans were suspended for drying after being sterilised – was lowered so that each bean became submerged to the same level in the test tubes containing the specific inoculum.

Three exposure times were evaluated (5, 15 and 30 min) in combination with drying times of 30 and 120 min. The exposure times were chosen to evaluate the effect of exposure time on the carry-over/adherence of *E.coli* to the green beans. The initial combinations of exposure and drying times are summarised in Table 1.

After each relevant exposure time, beans were raised out of the culture and air dried (while still inside the Bio-flow cabinet) for the time relevant to that particular experiment (Table 1).

After drying, the beans were aseptically transferred to sterile stomacher bags containing 10 mL PSS. Each bean was analysed separately. Each bag was then carefully massaged by hand for 30 s to ensure that the bean was washed thoroughly but that no visual physical damage was done to the outside of the bean. A dilution series to 10^{-8} was made of each sample in NA and plates incubated at 35°C for 48 h. The sample from this bag underwent the same treatment as the sample from the bag that was rubbed by hand

and the end results were compared. A dilution series to 10^{-8} was made of each sample in NA and incubated at 35°C for 48 h.

The carry-over levels of *E.coli* onto beans was evaluated using inoculum concentrations in the range of 10^7, 10^5, 10^4, 10^3 and 10^2 cfu.mL^{-1} (Table 1: 'Exposure and drying time combinations').

Effect of longer exposure time on carry-over of E.coli on green beans

In order to evaluate whether the length of the exposure time had any effect on the carry-over from the inoculum onto the green beans, an experiment was conducted in which the exposure time was lengthened. This increased exposure time aimed to be closer to actual exposure times (30 to 60 min) that took place in the field (Buhr, D. 2007. Joostenberg Vlakte Farm, personal communication). The exposure time was increased to 60 min with both 30 and 120 min drying times (Table 1). After the appropriate drying time the beans were analysed as previously described.

Effect of longer drying times on the carry-over of E.coli on green beans

The effect of a longer drying time on the carry-over of bacteria onto the green beans was also examined. The longer drying times (360 and 720 min) were used to mimic the drying periods found in the field between irrigation and final harvest. For this study exposure times of the *E.coli* culture to the beans was 5 and 30 min as these periods are more representative of what takes place during irrigation and harvest (Buhr, D. 2007. Joostenberg Vlakte Farm, personal communication) (Table 1). After the appropriate drying time was complete the levels of *E.coli* present on the beans were determined as previously described.

Effect of exposing sugar-snap peas (Pisum savitum) to E.coli

In this study sugar-snap peas were exposed to different concentrations of *E.coli.* Sugar-snap peas were chosen as they are consumed raw. The peas were purchased from local grocery stores and were, on average, 8 cm long and 1.5 cm at the broadest point. The following inoculum concentration ranges of 10^5, 10^3 and 10^2 cfu.mL^{-1} were used to determine the carry-over and survival of *E.coli* on peas.

Table 1 Experimental setup showing the parameter variations (exposure and drying times) applied during the studies on the green beans.

Variation	Inoculum concentration range (cfu.mL^{-1})	Exposure time (min)	Drying time (min)	Exposures	Number of repetitions (duplicate)
Exposure and drying time combinations					
1	10^7, 10^5, 10^4, 10^3, 10^2	5	30	1	5
2	10^7, 10^5, 10^4, 10^3, 10^2	5	120	1	3
3	10^7, 10^5, 10^4, 10^3, 10^2	15	30	1	3
4	10^7, 10^5, 10^4, 10^3, 10^2	15	120	1	3
5	10^7, 10^5, 10^4, 10^3, 10^2	30	30	1	3
6	10^7, 10^5, 10^4, 10^3, 10^2	30	120	1	3
Longer exposure time					
7	10^5, 10^3, 10^2	60	30	1	3
8	10^5, 10^3, 10^2	60	120	1	3
Longer drying time					
9	10^5, 10^3, 10^2	5	360	1	3
10	10^5, 10^3, 10^2	5	720	1	3
11	10^5, 10^3, 10^2	30	360	1	3
12	10^5, 10^3, 10^2	30	720	1	3

The peas were tied to a nylon line and secured with a firm knot and then sterilised as was done with the green beans. The exposure to the *E.coli inoculums* was performed as previously described in the method for the green beans. Each variation within the

experiment was performed in duplicate and each experiment was performed in triplicate. A summary of the experimental design performed on the sugar-snap peas is given in Table 2.

Table 2 Experimental setup to determine the carry-over levels of *E.coli* on peas.

Variation	Inoculum concentration range (cfu.mL^{-1})	Exposure time (min)	Drying time (min)	Exposures	Number of repetitions (duplicate)
1	10^5, 10^3, 10^2	5	30	1	3
2	10^5, 10^3, 10^2	5	120	1	3
3	10^5, 10^3, 10^2	15	30	1	3
4	10^5, 10^3, 10^2	15	120	1	3
5	10^5, 10^3, 10^2	30	30	1	3
6	10^5, 10^3, 10^2	30	120	1	3

Effect of exposing cocktail tomatoes (Lycopersicon esculentum) to E.coli

In this study cocktail tomatoes, a produce that has frequently been implicated in food-related outbreaks (Matthews, 2006), were exposed to different concentrations of *E.coli*. The cocktail tomatoes were purchased from a local retailer and had an average mass of 10 g and height of 10 mm. These were firmly secured with a nylon line which was fixed around the widest section of the tomato. Great care was taken to not cause any physical harm to the tomatoes as the delicate skin can easily be punctured. The tomatoes underwent the same sterilisation process as the green beans and each tomato was exposed to 50 mL of the *E.coli* inoculum in sterile 50 mL test tubes. The experimental design used in this study is presented in Table 3.

Table 3 Summary of experiments and variations performed on cocktail tomatoes.

Variation	Inoculum concentration range ($cfu.mL^{-1}$)	Exposure time (min)	Drying time (min)	Exposures	repetitions (duplicate)
1	10^5, 10^3, 10^2	5	30	1	3
2	10^5, 10^3, 10^2	5	120	1	3
3	10^5, 10^3, 10^2	15	30	1	3
4	10^5, 10^3, 10^2	15	120	1	3
5	10^5, 10^3, 10^2	30	30	1	3
6	10^5, 10^3, 10^2	30	120	1	3

Results and Discussion

As far as can be attained no research has been reported in the literature where the microbial carry-over levels from contaminated water to produce under controlled conditions have been studied. This study was designed to expose produce to specific inoculum concentration ranges of the non-pathogenic *E.coli* strain, thereby mimicking irrigation techniques that bring produce into direct contact with polluted irrigation water. The exploratory nature of this research implies that results under controlled laboratory conditions are necessary before studies can be applied in the environment. Instead of administering the inoculum by overhead irrigation – as is done in the field – the produce was dipped into the inoculum and then allowed to dry before being analysed. It was anticipated that *E.coli* carry-over levels found would give an indication of *E.coli* survival on produce irrigated with polluted irrigation water in which *E.coli* was present.

Use of standard curve absorbance to determine desired E.coli concentration range

The results obtained from the concentration range dilution studies are shown in Tables 4 and 5. The first set (Table 4) was based on a standard curve absorbance of 0.621 (= 4.4 x 10^8 cfu.mL^{-1}) and this was used to dilute to the desired *E.coli* concentration ranges of 10^7, 10^5 and 10^2 cfu.mL^{-1}. After the required dilutions were prepared they were plated out on NA and incubated. The results in Table 4 are the actual numbers detected after plating for each of the three repetitions for the three desired concentration ranges. The counts were in all cases within the desired concentration range but with a wide count variation in each case.

The second data set (Table 5) was based on a standard curve absorbance of 0.582 (= 4.1 x 10^8 cfu.mL^{-1}) and used to dilute to the desired *E.coli* concentration ranges of 10^6, 10^4 and 10^2 cfu.mL^{-1}. The data in Table 5, under the headings: 'Part 1' and 'Part 2', corresponds to the experimental set-up portrayed in Fig. 2. The results (Table 5) are again the actual numbers detected after plating for each of the five three repetitions of Parts 1 and 2 for the three desired concentration ranges. The counts were in all cases within the desired concentration range but again with a wide count variation in each case.

The final actual counts as given in Tables 4 and 5 showed more variation than what had been anticipated but were in all cases within the desired concentration ranges of 10^7, 10^6, 10^5, 10^4 and 10^2 cfu.mL^{-1}. This sort of variation during standardisation of inoculum has previously been described. One of the main sources of variation was described as a "contagious distribution error" mainly from the aggregation or clumping of cells (Elliot, 1977; Weenk, 2003). Thus it was concluded that the standard curve absorbance can be used as basis to dilute the original culture to reach a desired *E.coli* concentration range. It was also clear that for each dipping study, the specific cell concentration of the inoculum to be used must be determined so as to know the concentration used.

Exposure of green beans to different E.coli inoculum ranges (10^7, 10^5, 10^4, 10^3 and 10^2 cfu.mL^{-1})

At the start of each dipping study, the specific cell concentration of the inoculum to be used was determined. Green beans were then exposed to a range of *E.coli* inoculums (10^7, 10^5, 10^4, 10^3 and 10^2 cfu.mL^{-1}) for three different time periods (5, 15 and 30 min). After exposure to the inoculum, the beans were left to dry for either 30 or 120 min (Table 1). After drying, the beans were washed in 10 mL PSS and the *E.coli* load (cfu.mL^{-1}) on the beans was determined. The inoculum concentration ranges used in this study were based

on the levels as given in Chapter 3 for the Plankenburg River. The results from the study on green beans are shown in Figs. 5, 6, 7 and 8.

In all cases (Figs. 5, 6, 7 and 8) results obtained showed *E.coli* levels on the beans to be lower than those in the initial inoculum. Thus a carry-over reduction did take place. However, the results from the different exposure (5, 15 and 30 min) and drying time (30 and 120 min) combinations showed a wide overall variation as well as count variations for each combination. No survivors were found when a 10^2 inoculum range was used.

Table 4 Use of standard curve absorbance to determine the *E.coli* concentration range.

	Actual concentration cfu.mL^{-1} (desired range = **10^7**)	Actual concentration cfu.mL^{-1} (desired range= 10^5)	Actual concentration cfu.mL^{-1} (desired range = 10^2)
Repeat 1	12.2 x 10^7	1.27 x 10^5	169
Repeat 2	14.9 x 10^7	1.71 x 10^5	195
Repeat 3	14.3 x 10^7	1.76 x 10^5	176
Mean	13.8 x 10^7 SD = 1.15 x 10^6	1.58 x 10^5 SD = 2.20 x 10^4	180 SD = 10.9

Table 5 Use of standard curve absorbance to determine the *E.coli* concentration range.

Part 1 - (five separate samples each plated separately)	Actual concentration cfu.mL^{-1} (desired range = 10^6)	Actual concentration cfu.mL^{-1} (desired range = 10^4)	Actual concentration cfu.mL^{-1} (desired range = 10^2)
Set 1	2.7 x 10^6	2.3 x 10^4	160
Set 2	1.9 x 10^6	2.7 x 10^4	180
Set 3	1.9 x 10^6	2.0 x 10^4	150
Set 4	1.8 x 10^6	1.4 x 10^4	210
Set 5	1.7 x 10^6	2.03 x 10^4	250
Mean	2.0 x 10^6	2.08 x 10^4	190
	SD = 3.57 x 10^5	SD = 4.25 x 10^3	SD = 36.3
Part 2 - (one sample plated five times)			
Repeat 1	1.8 x 10^6	4.0 x 10^4	130
Repeat 2	2.1 x 10^6	1.3 x 10^4	90
Repeat 3	2.5 x 10^6	4.0 x 10^4	120
Repeat 4	2.1 x 10^6	7.7 x 10^3	80
Repeat 5	1.7 x 10^6	2.2 x 10^4	140
Mean	2.04 x 10^6	2.45 x 10^4	112
	SD = 2.80 x 10^5	SD = 1.34 x 10^4	SD = 23.1

From the data it was clear that, especially for the 10^7 inoculum range, the longer exposure and drying times did not impact the *E.coli* numbers differently from the shorter combinations. With the 10^5, 10^4 and 10^3 inoculum ranges the extended combinations did

appear to impact the survivor numbers. Extended exposure and drying times might result in larger differences in number between the numbers of *E.coli* survivors from the different experimental combinations.

In many cases large variations in counts of surviving *E.coli* for the same treatment were found. In the case of the 10^7 range variations were found from 200 and 1 580 000 cfu.mL^{-1}. *Escherichia coli* are known to aggregate and form clumps and biofilms mainly as a result of the formation of extracellular polysaccharides. This also aids in its adherence to surfaces (Jarvis & Kaper, 1996; Weenk, 2003). *E.coli* also has a tendency to form clumps in solution (Albert *et al*., 1993; Zogaj *et al*., 2008). This characteristic may have contributed to the variation in results that were observed. Another aspect that might have contributed to the cell number variations is the adherence/attachment to the beans. This could be affected by the clumps but could also be a result of interactions between the bacteria and the product surface. In this study it was found that clumps are very difficult to break apart in a mixing process that comprised both mixing on a vortex and ultrasonication of the *E.coli* solution. A micrograph of a stained *E.coli* clump taken at an enlargement of X1000 is presented in Fig. 9. Depending on the size of the clump, it can be made up of millions of *E.coli* cells. The vast number of cells in such a clump would make it very difficult to separate all of the cells in order to break up the clump. The number of cells in a clump also means that even the presence of one such clump could dramatically increase the number of cells carried over to produce.

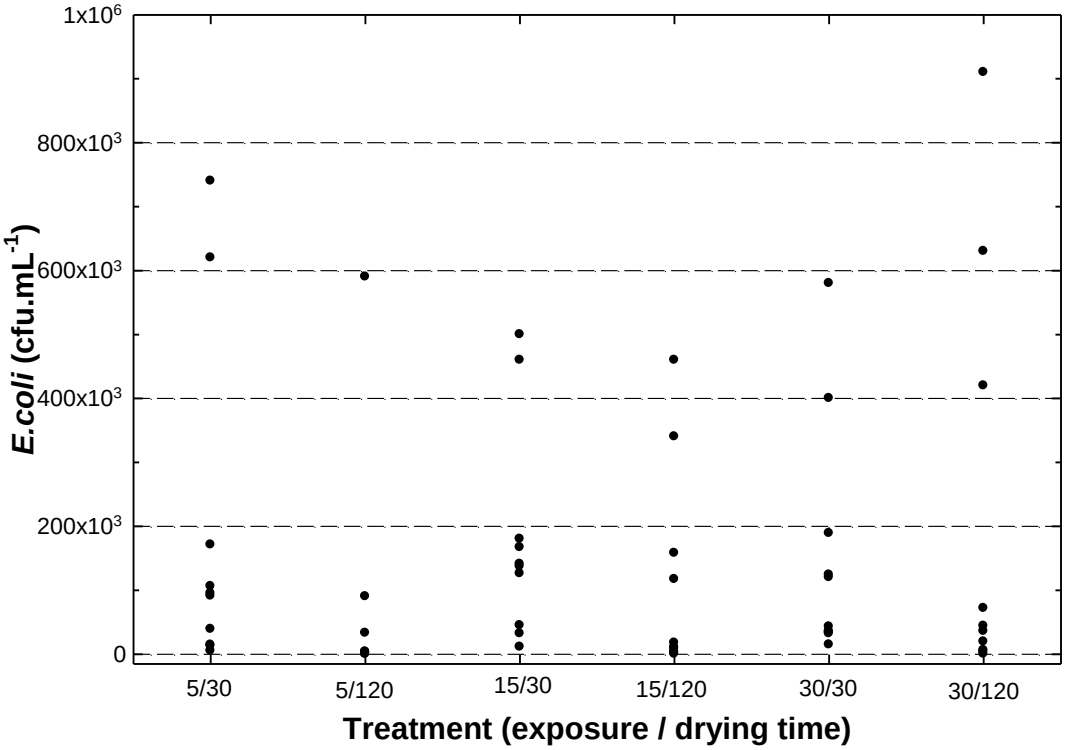

Figure 5 *E.coli* counts on green beans that had been exposed to the 10^7 inoculum range (13 600 000 cfu.mL^{-1}). The study was made up of 6 treatments, each with 10 replications.

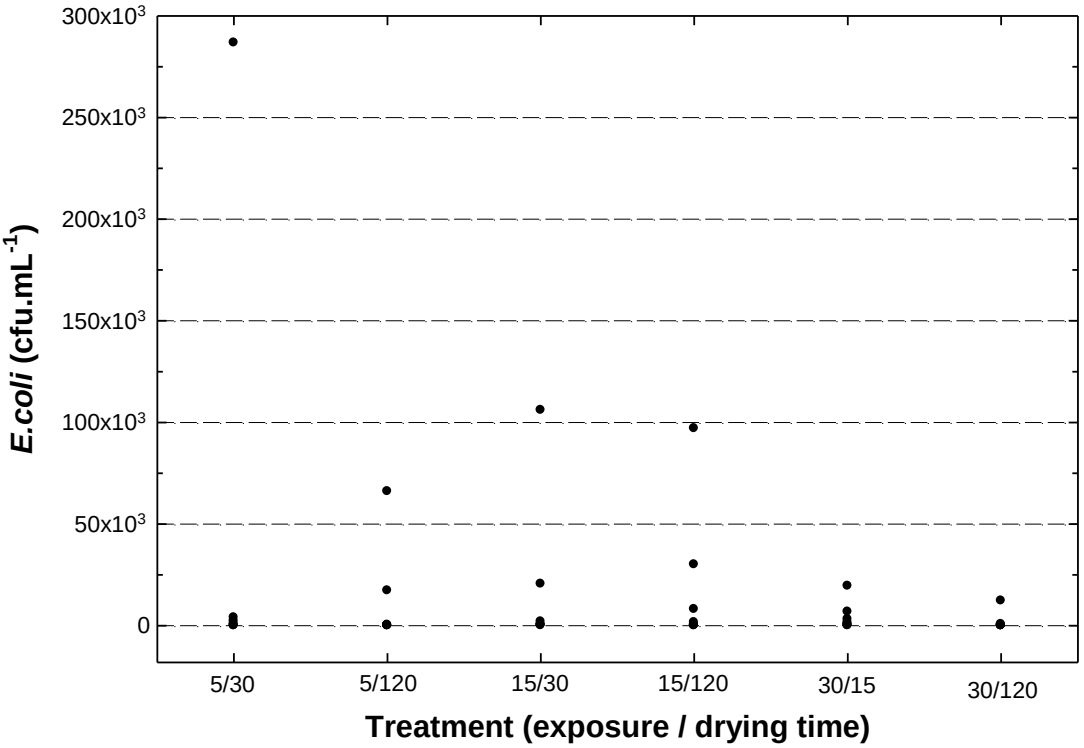

Figure 6 *E.coli counts* on greenbeans that had been exposed to a 10^5 inoculum range (= 163 000 cfu.mL^{-1}). The study was made up of 6 treatments each with 8 replications.

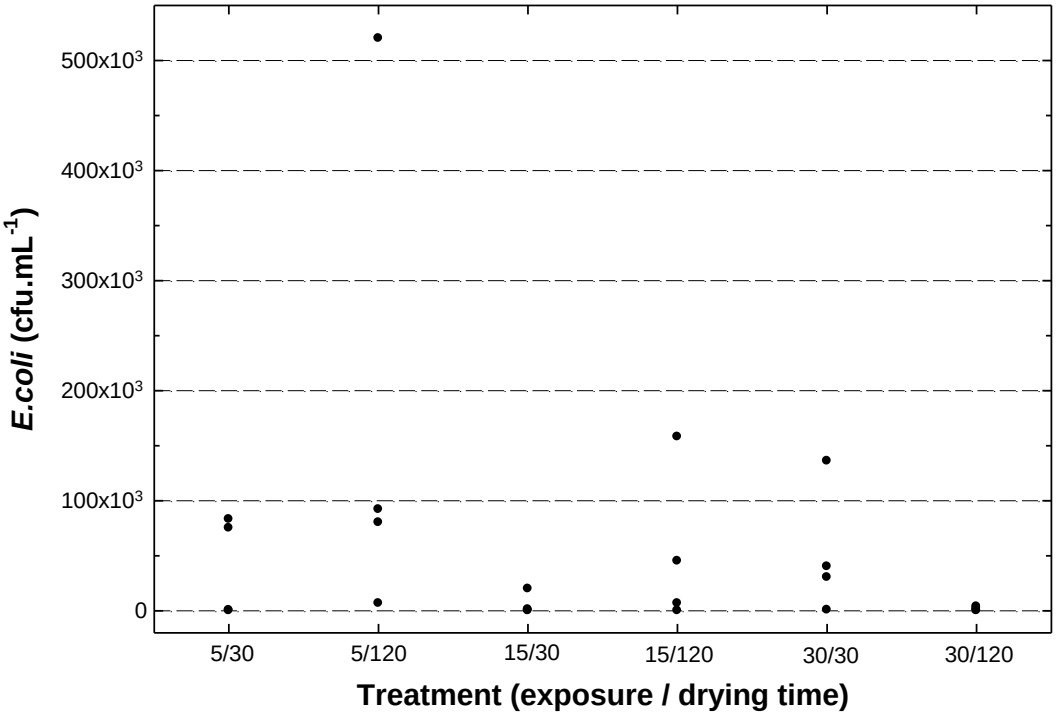

Figure 7 *E.coli counts* on green beans that had been exposed to a 10^4 inoculum range (= 81 000 cfu.mL^{-1}). The study was made up of 6 treatments each with 4 replications.

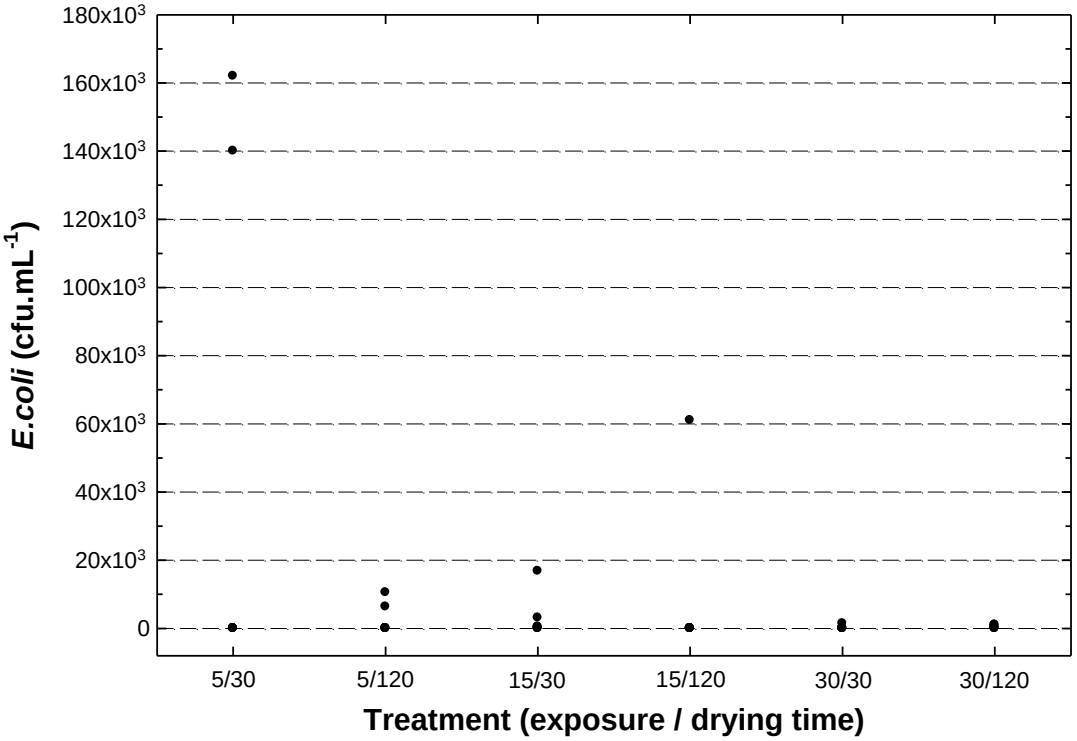

Figure 8 *E.coli counts* on green beans that had been exposed to a 10^3 inoculum range (= 16 000 cfu.mL^{-1}). The study was made up of 6 treatments each with 8 replications.

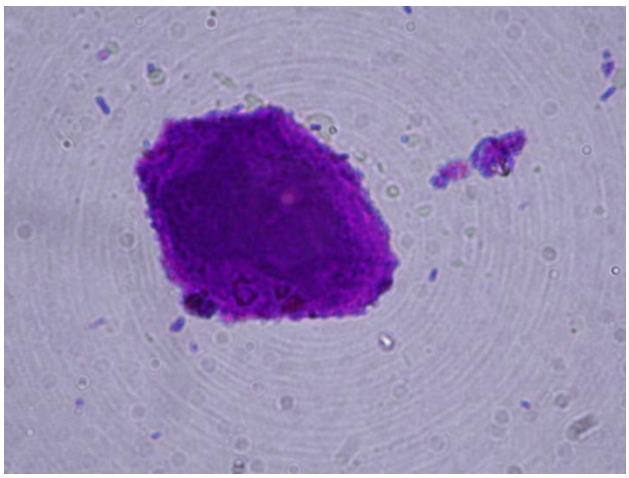

Figure 9 Micrograph of an *E.coli* clump (1 000 x).

Clumping may have been one of the major causes of the variation observed in the results obtained when using absorbance values as bases for the dilution to the desired concentration range (Tables 4 and 5). From a health side the presence of clumps heightens the risk of a disease outbreak if produce containing clumps of a potential pathogen is consumed. Considering that clumps in the inoculum were detected under laboratory conditions and after the inoculum had been well mixed on the vortex and exposed to ultrasonic treatment, it is possible that clumps will be present in irrigation water.

All of the beans on which *E.coli* were detected exceeded the South African food guideline which is zero *E.coli* per gram product (DoH, 2000). Considering that most of the beans were positive for *E.coli* it can be concluded that even irrigation water with a potential pathogen load of 1 000 cfu.mL^{-1} would not be safe as irrigation water for raw produce. It should also be noted that the *E.coli* load of 1 000 cfu.mL^{-1} (or, as given in the WHO and DWAF guidelines, 100 000 organisms.100 mL^{-1}) exceeded the South African guideline for *E.coli* in irrigation water which is <1 000 organisms.100 mL^{-1} (DWAF, 1996). It was found during the studies reported in Chapter 3 that the water in the Plankenburg River in Stellenbosch was carrying, at times, *E.coli* loads in excess of 4 000 000 organisms.100 mL^{-1}. If similar survival patterns of *E.coli* on produce are to be found in the environment, then the results from this study should serve as a warning that the river water is not safe to be used for the irrigation of raw produce.

Effect of longer exposure of beans to the E.coli inoculum (10^5, 10^3, 10^2 cfu.mL^{-1})

In the first study, especially at the lower cell concentration ranges, there was an indication that the exposure time had an effect on the carry-over from the inoculum onto the green beans. Thus for this study the exposure time was lengthened to be closer to actual exposure times (60 min) that took place in the field (Buhr, D. 2007. Joostenberg Vlakte Farm, personal communication) (Table 1).

In the first variation change, the exposure time (10^5 and 10^3 concentration range) was lengthened to 60 min combined with 30 and 120 min drying times (Table 1). The data again shows the variation in surviving *E.coli* numbers on the beans for each treatment. When the profile given in Fig. 10 is compared as a whole to the data given in Figs. 6 and 8, there was no clear difference between the data sets.

No growth was detected from beans exposed to the 1x10^2 cfu.mL^{-1} inoculum. Since no *E.coli* survivors were detected on any of the beans exposed to this range it can be assumed that this concentration is safe for irrigation. However, the surviving *E.coli* loads on green beans should be monitored under true environmental conditions before a decision is made regarding the safety of this water for irrigation.

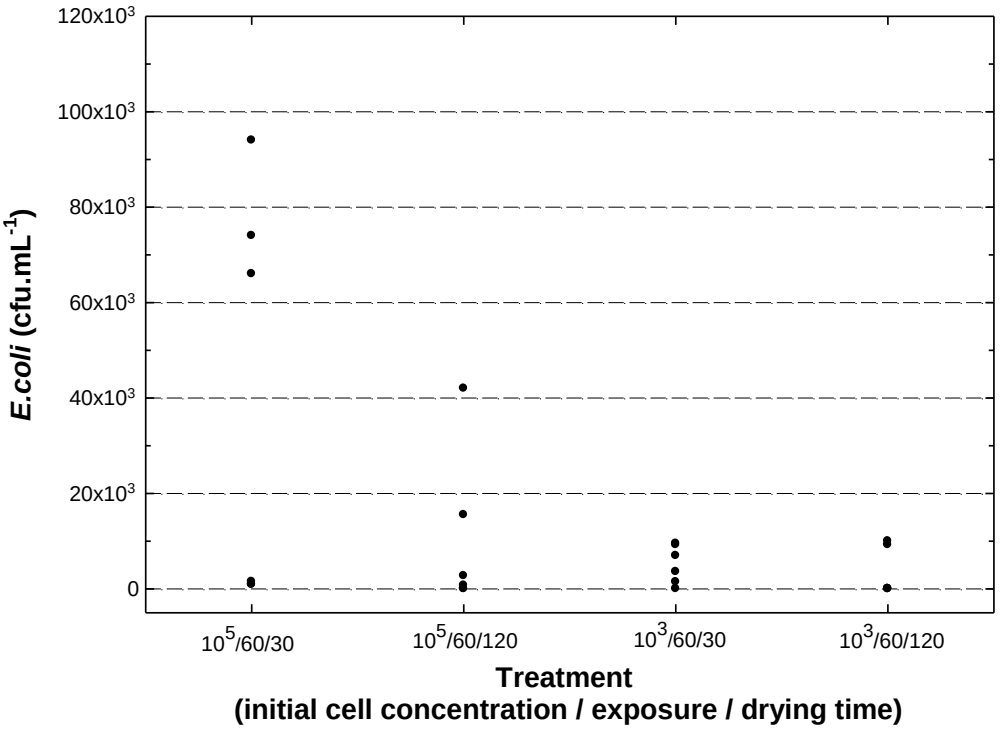

Figure 10 *E.coli counts* on green beans that had been exposed to a longer exposure (60 min) to the 10^5 and 10^3 inoculum ranges (= 173 000 and 12 300 cfu.mL^{-1}). The study was made up of 4 treatments each with 6 replications.

Effect of extended drying times (6 and 12 h) on E.coli survival

The aim of this study was to assess the survival of *E.coli* at initial different concentrations (10^5, 10^3 and 10^2 cfu.mL-1) on green beans after applying longer drying times (6 and 12 h) (Table 1). The extended drying times were used as they are more similar to the actual drying times that could occur during the commercial cultivation of produce (Minhas et al., 2005; Buhr, D. 2007. Joostenberg Vlakte Farm, personal communication). Exposure times of 5 and 30 min were again used in this study.

The data (Fig. 11) from the exposure of *E.coli* to the 10^5 concentration range followed by the 6 and 12 h drying times shows in all cases a count reduction of at least 1 log unit to below 40 000 cfu per bean. However, the large variation in counts was again found.

The results from the samples that were exposed to the 10^3 concentration range inoculum showed, with one exception, no survivors on the beans after drying process. In the case of the exposure to the 10^2 inoculum no *E.coli* counts were found on any of the beans after both the 6 and 12 h drying times.

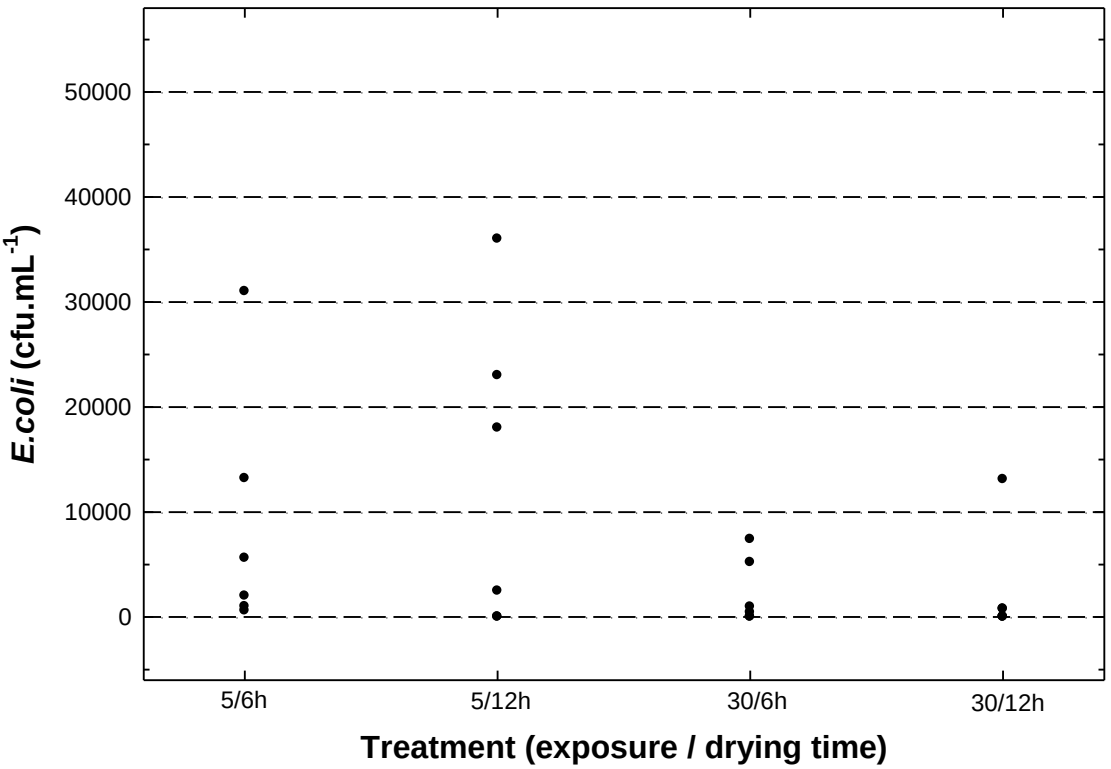

Figure 11 *E.coli counts* on green beans that had been exposed to longer drying times (6 and 12 h) to the 10^5 inoculum range (= 163 000 cfu.mL^{-1}). The study was made up of 4 treatments each with 6 replications.

Effect of exposing sugar-snap peas (Pisum sativum) to E.coli

In contrast to the range of inoculum concentrations (Table 1) used for the experiments on the green beans, only 10^5, 10^3 and 10^2 cfu.mL^{-1} inoculums were used in this study (Table 2). The peas were exposed to the relevant *E.coli* inoculum for different exposure (5, 15 and 30 min) and drying time (30 or 120 min) combinations. Each combination repetition was performed in 6 times. The experimental procedure was found to be easier than with the beans as the peas were smaller and there was less morphological variation.

The results obtained for the 10^5 cfu.mL^{-1} inoculum are presented in Fig. 17. Again a wide variation in *E.coli* numbers were found but in total a log reduction of at least 1 was found. One of the peas was found to have a load of 70 000 cfu.mL^{-1} which was particularly high while the next highest loads were between 20 000 and 40 000 cfu.mL^{-1} (Fig. 12). The other peas monitored carried much lower loads that ranged from 0 to below 20 000 cfu.mL^{-1} with most of the values closer to the lower end of this range (<5 000 cfu.mL^{-1}). The results from this study where peas were exposed to a 10^5 concentration load were similar to that obtained for the beans (Fig. 6). Even though many of the counts were zero some levels must still be regarded as high and, more importantly, these all exceed the South African guideline of 0 *E.coli* per 1 g fresh produce (DoH, 2000) and could be considered as not suitable for human consumption in a raw form.

From the peas exposed to the 10^3 concentration load, only two were found to be carrying *E.coli* (loads of 810 and 340 cfu.mL^{-1}, respectively). No *E.coli* was detected on the rest of the peas. Although these results indicate that this inoculum concentration could be safe, it should be remembered that clumps might be carried over making the peas unsafe for human consumption (DoH, 2000).

No *E.coli* was detected on the peas exposed to the 10^2 concentration range and it could possibly be considered a safe concentration for *E .coli* to be present in irrigation water.

Effect of exposing cocktail tomatoes (Lycopersicon esculentum) to E.coli (10^5, 10^3 and 10^2 concentration ranges)

The experimental plan (Table 3) for determining the survival of *E.coli* on cocktail tomatoes was identical to that of the sugar-snap peas. The tomatoes were exposed to *E.coli* at inoculum concentration ranges of 10^5, 10^3 and 10^3 for different combinations of exposure (5, 15 and 30 min) and drying times (30 or 120 min) and then tested for the presence of *E.coli*.

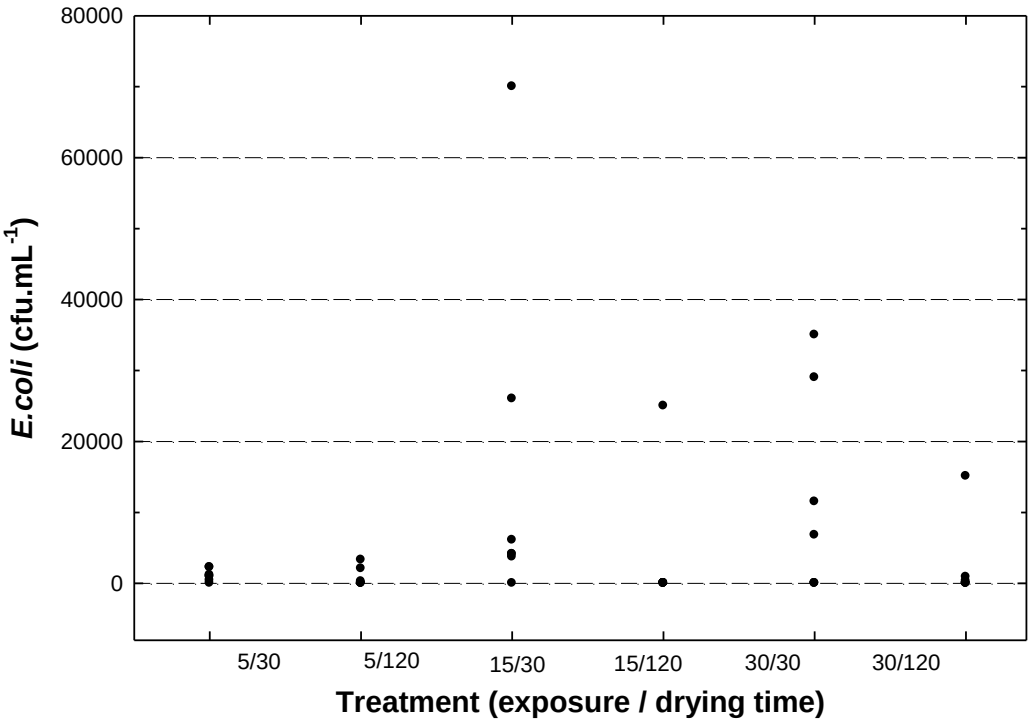

Figure 12 *E.coli counts* on peas that had been exposed to the 10^5 inoculum range (= 228 000 cfu.mL^{-1}) for either 5, 15 or 30 min and then dried (30 or 120 min). The study was made up of 6 treatment combinations each with 6 replications.

The studies on the tomatoes were more challenging to execute than those performed on the beans and peas as it was very difficult to successfully secure the nylon line firmly enough around the tomato without puncturing its skin. The results of the levels of *E.coli* survivors detected at the inoculum concentration ranges of 10^5 and 10^3 are presented in Figs. 13 and 14. The survivors found on the tomatoes for all three inoculum concentrations used in this study were considerably lower than those found on both the peas and the beans. Eight of the 36 repetitions were positive for the presence of *E.coli*, with the highest load detected being 1 050 cfu.mL^{-1}. The other *E.coli* loads at the 10^5 concentration range varied between 240 and 980 cfu.mL^{-1}. The remaining 28 tomatoes were negative for *E.coli*. These lower loads could probably be attributed to the 'smooth' surface of the tomato skin. This was interesting as tomatoes have been implicated in food-related outbreaks on numerous occasions, yet the lowest loads of *E.coli* were found on them. This indicates that if other produce had been irrigated with the same contaminated water an outbreak could have been more serious.

The results obtained for the 1x10^3 cfu.mL^{-1} inoculum (Fig. 14) were lower than those for the 10^5 inoculum range (Fig. 13) but followed a very similar profile. These results (Fig.

14) were marginally higher than expected; considering the nature of the surface of the tomato skin. However, all of the loads were still less than the original inoculum concentration. It is uncertain whether this was due to presence of clumps in the inoculum or to *E.coli* not surviving on the tomato skin. The results from this study showed the unpredictability of the carry-over of *E.coli* from water onto produce and the subsequent detection. This experiment revealed that products can still carry survivors and be unsafe for consumption, even after exposure to a low *E.coli* inoculum concentration (DoH, 2000).

Unlike the results for the two above inoculum range, no survivors were detected on the tomatoes inoculated with the 10^2 inoculum. These zero values are in line with those obtained for the beans and peas under the same experimental conditions. Considering the above statement and the aforementioned smooth skin of the tomato, it can be suggested that tomatoes exposed to this inoculum concentration would be safe for consumption. However, the uncertainty regarding the formation of bacterial clumps might requires a more conservative safety limit for irrigation water and more replications would have to be done to be certain that there is no contamination carry-over.

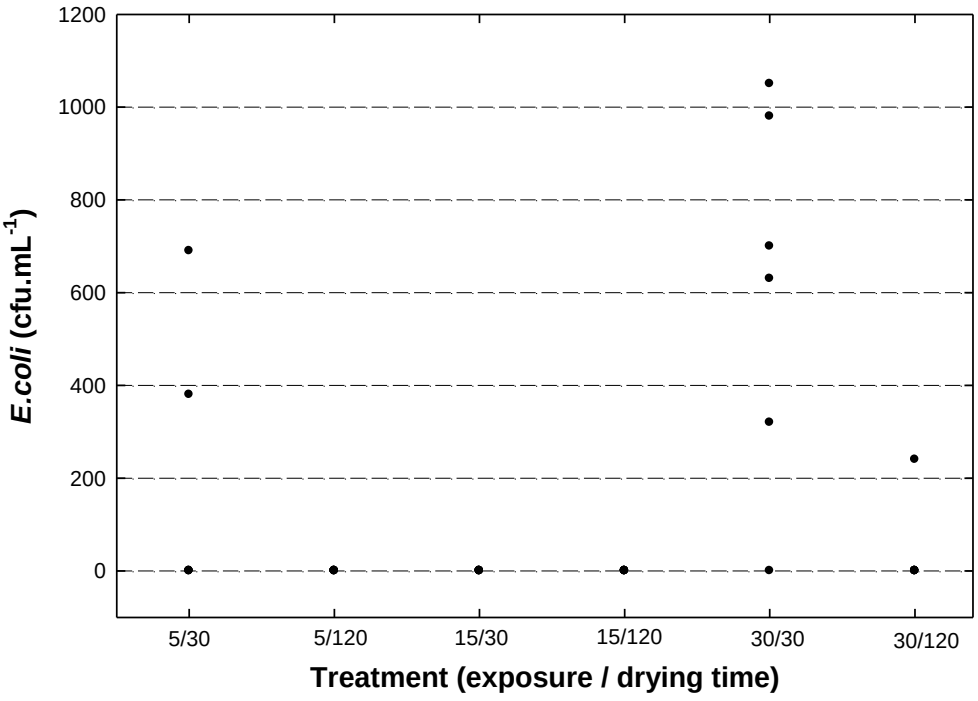

Figure 13 *E.coli* survivors on cocktail tomatoes after exposure to the 10^5 concentration inoculum range (= 197 000 cfu.mL^{-1}). The study was made up of 6 treatment combinations each with 6 replications.

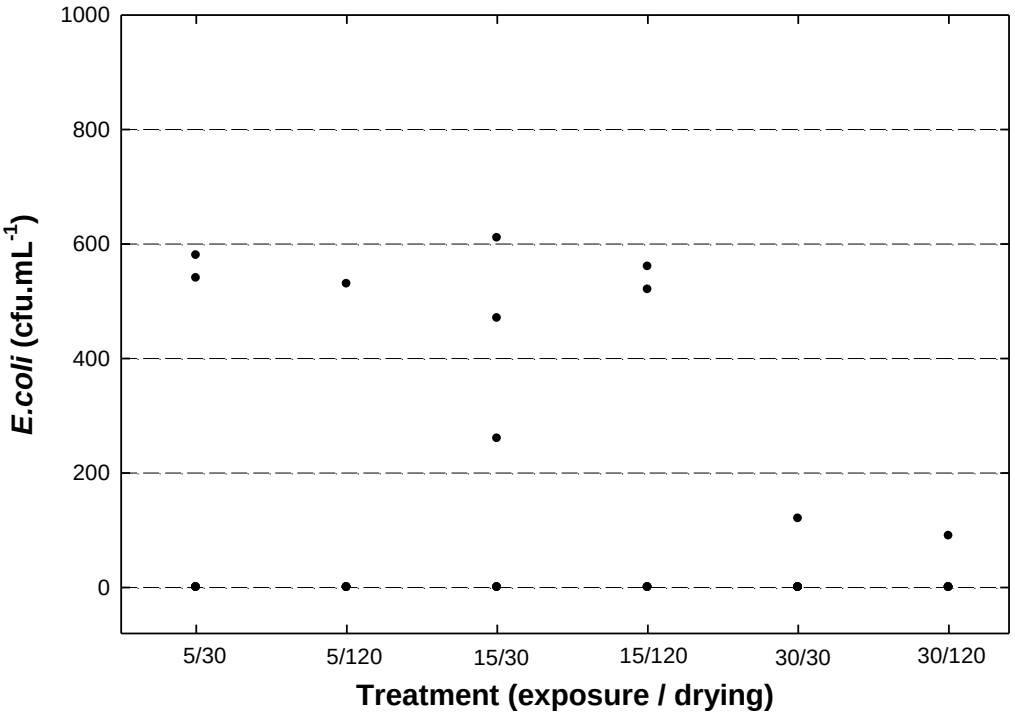

Figure 14 *E.coli* survivors on cocktail tomatoes after exposure to the 10^3 concentration inoculum range (= 2 450 cfu.mL^{-1}). The study was made up of 6 treatment combinations each with 6 replications.

Conclusions and Recommendations

The results reported from the studies in this chapter serve to give an indication of how the survival of *E.coli* on produce is affected under environmental conditions experienced in isolation. The assessment of the accuracy of the inoculum dilutions revealed a discrepancy between the actual and desired *E.coli* inoculum concentrations. However, in all cases the method of using absorbance as bases for the dilution to a desired inoculum range worked well but as a result of the varying values within the desired range it was essential to determine the final count of the inoculum used in the dipping studies. This meant that the original dipping count was only known 24 h after the dipping was completed. If one considers the procedures that were used to ensure that the culture is mixed thoroughly, it can be concluded that at least one additional factor was preventing an even distribution of *E.coli* cells throughout the culture. One of these is that direct exposure to a "highly diluted" inoculum by means of a dipping process is known to result in a varying rate of adherence of *E.coli* to produce (Lang *et al*., 2004). Past literature has also ascribed the variation to

several causes of which “contagious distribution” (aggregated or clumping) (Elliot, 1977) was thought to be one of the most important factors.

From the results obtained, it was shown that *E.coli* is carried over from water onto produce. In all cases reduced loads were found on the produce in comparison with the concentration in the initial inoculum. *Escherichia coli* was found to survive on green beans after exposure to the 10^7, 10^5, 10^4 and 10^3 inoculum ranges. In all cases no carry-over of cells from a 10^2 concentration was found. It was also found that neither an increased exposure time to the *E.coli* inoculum nor a longer drying time after exposure to the inoculum influenced the load of *E.coli* survivors detected on the beans.

The fine covering of hairs on the surface of green beans is possibly the primary reason for the highest number of *E.coli* survivors being detected on them, followed by carry-over to the sugar-snap peas. The lowest numbers of survivors were observed on the cocktail tomatoes. The results from the studies suggests there is a strong possibility that the nature of the surface of the product plays a role in the attachment of bacteria with a ‘round’, ‘smooth’ surface being less conducive for attachment. Therefore, if several different products are irrigated with the same contaminated water, the products with the rougher, less regular surface with will pose a greater safety threat to the consumer.

The studies described in this chapter were performed under ideal laboratory conditions which, leads to the minimisation of the impact of external environmental influences. It cannot be forgotten that the reality of agriculture involves environmental factors which will have an effect on the survival of *E.coli* and other water-borne pathogens. However, this study was necessary to ascertain the potential for pathogen carry-over. The data obtained could be used as a base against which further results can be compared with. It is anticipated that changes in environmental factors such as wind, heat, humidity and ultra-violet light would be likely to influence the survival patterns of *E.coli* that were observed in these experiments.

By using *E.coli* as an indicator organism throughout this study (Mara *et al*., 2007), the results obtained do clearly indicate that the carry-over and survival of potential pathogens from contaminated irrigation water to produce does take place. Using the results from this experiment as a model, they will hopefully provide insight into how pathogens might attach and survive on produce and through this, indicate the severity of the health risk to consumers when raw or minimally processed produce is irrigated with polluted water.

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CHAPTER 5

GENERAL DISCUSSION AND CONCLUSIONS

A safe and abundant supply of water is not only of paramount importance but it is inextricably linked to every industry in our country. Water use in South Africa is dominated by agricultural irrigation which accounts for >60% of all water used. Over the last few years it has been shown that many of the South African rivers that are drawn from for agricultural irrigation purposes are heavily polluted and are in many cases used for the irrigation of fresh produce. Unfortunately, the use of this polluted and contaminated water results in the contamination being carried through to all of the aspects that the river water is used for. In the case of river water being used for irrigation, carryover of pathogenic bacteria from water to the fresh produce takes place.

Waterborne pathogens not only degrade the environment, but enter the food chain as well. Some of the pathogens have the ability to adhere to or even penetrate vegetables and fruit and can infect humans and farm animals. The risk of disease transmission is especially high when the foodstuff is eaten raw or just lightly cooked.

In an exploratory study over a five month period, the quality of three selected sites from the upper Berg and two from the Plankenbrug Rivers were assessed. Faecal coliform counts ranging from 540 to 1 700 000 cfu.100ml^{-1} and 490 to 160 000 cfu.100ml^{-1} were found for the Berg and Plankenbrug Rivers, respectively. These high faecal counts were a clear indication of faecal contamination taking place. Other indicator and index microbes including *Salmonella*, Staphylococcus, *Listeria*, endosporeformers, *E. coli* and intestinal *Enterococci* were frequently isolated from all five sites sampled. From the exploratory study it was concluded that the water from all the sites were not suitable for use in irrigation practices as they regularly exceeded the guidelines for faecal coliforms and *E.coli* as set out by South African authorities.

A further study over 12 months was done on a site on the Plankenburg River in order to observe microbial level variations over a longer period. Over the period faecal coliforms and *E.coli* counts were found to be present in consistently high loads and again the other indicator and index species were found to be present. The close proximity of this site to an informal settlement was used to explain most of the high pathogen levels detected in the water samples. These levels are in excess of safe levels and could pose a risk to consumers if levels as high as these were to be ingested.

One question that has continuously been asked is if irrigation water is faecally polluted what is the *E. coli* load necessary to be sure of carry-over to produce. There are many environmental factors that would affect microbial survival on fresh produce but relatively few studies have investigated carry-over loads from irrigation water to produce and the impact of environmental factors on survival. The *E.coli* levels in the Plankenburg river water being used for irrigation currently exceed 1 000 organisms.100 mL^{-1} and, as stated before, it is not clear how many if any of the *E.coli* from such high inoculum loads would survive on produce. Therefore, the safety of produce that is irrigated with this water is in question. In order to assess the severity of this threat, it was decided to conduct a study to determine, using different inoculum concentrations, how many of such high contamination loads are actually transferred onto the produce under controlled laboratory conditions.

In this study the carry-over and survival of *E. coli* on beans, peas and cocktail tomatoes was assessed. The results obtained do clearly indicate that the carry-over and survival of potential pathogens from contaminated irrigation water to produce does take place. No clear profile was observed between the different exposure and drying time combinations and in many cases large variations in counts of surviving *E.coli* for the same treatment were found. In all cases a reduction of at least one log value in original inoculum number was found with the 10^2 inoculums showing no survivors. The large variations were ascribed to the formation of bacterial clumps. The varying numbers present in such clumps made it nearly impossible to obtain a well homogenised sample.

The fine covering of hairs on the surface of beans is possibly the primary reason for the highest number of *E.coli* survivors being detected on them, followed by carry-over to the peas. The lowest numbers of survivors were observed on the tomatoes. The results suggest that the nature of the surface of the product plays a role in the attachment of bacteria. Therefore, if several different products are irrigated with the same contaminated water, the products with the rougher, less regular surface with will pose a greater safety threat to the consumer. If similar survival patterns are to be found in the environment then results from this study should serve as a warning that the Plankenburg river water is unsafe for use in the irrigation of fresh produce.

Irrespective of clumping, the pathogenic loads detected in the rivers were at levels that posed a definite risk to the health of the consumer. Therefore, under environmental conditions similar to those created in the laboratory, a similar rate of survival on produce irrigated with this river water could be expected to be observed. While it is acknowledged that environmental conditions may affect the survival of pathogens on produce, these

conditions are unpredictable and vary seasonally, or even daily. The survival patterns at ideal conditions should, therefore, be used to develop the standards for acceptable pathogenic loads in river water in order to always be able to ensure safety of the produce.

While it is encouraged that the abovementioned recommendations be considered, it is strongly urged that the relevant authorities do not wait on further results before addressing the problems presented in this study. The current state of the Berg and Plankenburg Rivers is unsafe for human contact as well as for use in irrigation. The detected pathogen levels were repeatedly high and further deterioration of the systems and infrastructure that resulted in the contamination, could only lead to the situation worsening in the future. This would, in turn, translate to an increased risk of an outbreak that would put the health and lives of consumers at risk. If these rivers are to going to continue being used for the irrigation of fresh produce, immediate action needs to be taken to improve the state of the rivers by reducing the pathogenic load in them. The rivers should be monitored regularly and the results should be made public so that farmers are aware of the dangers they are exposing their produce to by irritating with this water. Finances should be made available for facilities to be repaired and upgraded to prevent unnecessary contamination of the rivers taking place. While other sources of contamination, such as an insufficient sanitation infrastructure in informal settlements, might be more difficult to contain immediately, the apathy towards such situations is going to have to change if a solution to this continuous stream of pollution is to be found. The solution is going to require the cooperation of the relevant authorities and communities but a drastic change is necessary if river water is going to continue being used for irrigation. In more affluent agricultural sectors the treatment of water prior to irrigation is a possibility, but in poorer areas the producers are simply reliant on whatever is available and will continue to use river water for irrigation, irrespective of its quality. For this reason, the long term solution lies in improving the overall state of the rivers and not just in treating the irrigation water.

In the meantime, as much awareness as possible should be created to encourage consumers to take precautionary steps themselves. However, the greatest responsibility currently lies with retailers who need to become stricter in terms of the quality standards that they set. The enforcement of these standards through regular testing of produce will increase the safety of food for the consumer in the short-term, until the quality of river water is restored to safe levels.