PROFILING OF POTENTIAL PATHOGENS FROM PLANKENBURG RIVER WATER USED FOR THE IRRIGATION OF FRESH PRODUCE

by

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ABSTRACT

The increased consumption of fresh produce has been shown to be related to increases in foodborne disease outbreaks and these have in many cases been ascribed directly to carry-over of pathogens from contaminated irrigation water. In South Africa, rivers are the main source of irrigation water but many have been found to be unsuitable for irrigation of fresh produce because of the unacceptably high levels of faecal contamination.

The main aim of this study was to do a baseline evaluation of the microbiological quality of the Plankenburg and Eerste Rivers and to determine which bacterial contaminants are present. Two sampling sites were selected for the Plankenburg (Plank-1 and -3) and one for the Eerste River (Eerste-1). The microbiological analysis included aerobic colony count (ACC), aerobic and anaerobic sporeformers, *Staphylococcus*, *Salmonella*, *Listeria*, enterococci, coliforms, faecal coliforms and *E. coli* using standard methods. The faecal contamination levels for both rivers exceeded the DWAF and WHO guidelines of <1 000 *E. coli* per 100 mL water for irrigation of fresh produce intended to be consumed raw. The Plankenburg River sites always had higher coliform contamination levels (1 200 - 13 000 000 MPN per 100 mL water) than the Eerste River site (230 - 79 000 MPN per 100 mL water). There was also a high incidence of index organisms including *Salmonella*, *Staphylococcus, Listeria* and endosporeformers. The isolation of intestinal enterococci suggested the presence of potential pathogens that can cause disease outbreaks. The baseline data also showed large variations in microbial loads over the 15 month study with the faecal coliform counts ranging for Plank-1 from 1 200 to 7 000 000 MPN.100mL^{-1}, Plank-3 from 10 to 460 000 MPN.100mL^{-1} and Eerste-1 from 28 to 79 000 MPN.100mL^{-1}. The water temperatures at all three sites ranged from 12.1° to 21.7°C with COD values in most cases below 100 mg.L^{-1}.

As the baseline study showed large variations in microbial loads over the 15 month study period an assessment using the Colilert-18 system of the weekly, daily and hourly variations, for 6 weeks over a period of 4 months was conducted at site Plank-2. This site was specifically used as it is an irrigation source point for nearby fresh produce farmers and is about 2 km further downstream from an informal settlement. The weekly variation trend for total coliforms (TC) showed a decrease over the entire sampling period with the highest count of 3 200 000 MPN.100 mL^{-1} during the warmer period. The *E.coli* (Ec) counts showed a similar trend with the highest count of 440 000 MPN.100 mL^{-1} also in March. The daily variation trends were the same for both the TC and Ec and counts found to increase from Monday to Thursday followed by a decrease to Sunday. The highest counts were on Thursday with average TC and Ec counts of 1 900 000 and 160 000 MPN.100 mL^{-1}, respectively. The hourly variation trends were similar for both TC and Ec with counts increasing from 06h00 to 12h00 followed by a decrease to 18h00. The increases in TC and Ec counts found during the weekly, daily and hourly variation trend studies clearly suggests that the 15 month sampling that was done once a month on Mondays at 08h00 could be considered an underestimation of the contamination levels of the Plankenburg and Eerste Rivers.

The overall weekly variation trend for the water temperature showed a decrease over the sampling period while the daily and hourly variation trends showed an increase from 06h00 to 18h00. The overall weekly trend for pH differed from that of the temperature with an increase over the sampling period. The analysis of covariance showed no correlation ($p < 0.05$) between the physico-chemical (temperature and pH) and the microbial variables (TC and Ec). Therefore it was concluded that temperature and pH had no direct impact on either the total coliform or *E. coli* counts.

Both the Plankenburg and Eerste Rivers were found to be unsuitable for the irrigation of fresh produce intended to be consumed raw due to the high levels of faecal contamination that exceeded DWAF and WHO guidelines. Irrigation with such water could pose a health risk because of presence of potential pathogens that could be carried-over to fresh produce.

UITTREKSEL

Die toenemende gebruik van vars produkte hou direk verband met die toename in voedseloordraagbare siektes. Alte dikwels kan dit toegeskryf word aan die teenwoordigheid van patogene in besproeiingswater. In Suid Afrika is riviere die hoofbron van besproeiingswater maar dit is al gevind dat meeste ongeskik is vir gebruik as besproeïngsbron as gevolg van die onaanvaarbare hoe vlakke van fekale besmetting.

Die hoofdoel van hierdie studie was om 'n basislyn evaluasie van die mikrobiologiese kwaliteit van die Plankenburg en Eerste Riviere te doen en ook vas te stel watter bakteriese kontaminante teenwoordig is. Twee bemonsteringpunte is geselekteer vir die Plankenburg (Plank-1 en -3) en een vir die Eerste Rivier (Eerste-1). Mikrobiologiese analises met standaard metodes het die volgende ingesluit: aërobe kolonie telings (AKT), aërobe en anaërobe spoorevormers, *Staphylococcus*, *Salmonella*, *Listeria*, enterococci, koliforms, fekale koliforms en *E. coli* met gebruik van standaard metode. Die fekale besmettingsvlakke vir beide riviere het die DWAF en WHO leistreep van <1 000 *E. coli* per 100 mL water vir besproeiing van vars produkte wat rou geëet kan word oorskry. Die Plankenburg Rivier bemonsteringspunte het in alle gevalle 'n hoër kolivorm besmettingsvlak (1 200 - 13 000 000 MPN per 100 mL water) as die Eerste Rivier punt (230 - 79 000 MPN per 100 mL water) gehad. Daar was ook 'n hoër voorkoms van indeksorganismes insluitend *Salmonella*, *Staphylococcus, Listeria* en endosporevormers. Die voorkoms van ingewand enterococci was 'n addisionele aanduiding van die voorkoms van patogene wat ernstige gesondheidsrisikos vir die verbruiker kan inhou. Die basislyn data het groot variasies in die mikrobe vlakke oor die 15 maand van studie getoon. Die faecal koliforms vir Plank-1 het gewissel van 1 200 tot 7 000 000 MPN.100mL^{-1}, vir Plank-3 van 10 tot 460 000 MPN.100mL^{-1} en vir Eerste-1 van 28 tot 79 000 MPN.100mL^{-1}. Die water temperature het gewissel van 12.1° tot 21.7°C met die CSB waardes in meeste gevalle minder as 100 mg.L^{-1}.

Aangesien daar sulke groot variasies in mikrobe ladings oor die 15 maande tydperk voorgekom het, is die Colilert-18 sisteem gebruik om die weeklikse, daaglikse en uurlikse variasies vas te stel vir 6 weke oor 'n periode van 4 maande by die Plank-2 bemonsteringspunt. Daar is spesifiek op die bemonsteringspunt gefokus omdat dit as 'n besproeiingsbron gebruik word deur groente produsente. Dit is ook gelee ongeveer 2 km stroomaf van 'n informele nedersetting.

Die weeklikse variasies in totaal koliforms (TC) het 'n afname oor die hele bemonsteringsperiode getoon, met die hoogstes telling van 3 200 000 MPN.100 mL^{-1} gedurende die warmer tydperk. Die *E.coli* (Ec) tellings het 'n soortgelyke neiging getoon, met die hoogste telling van 440 000 MPN.100 mL^{-1} ook in Maart. Die daaglikse neigings was dieselfde vir beide die TC en Ec en die tellings het vermeerder van Maandag tot Donderdag, met 'n afname tot Sondag. Die hoogste telling was op Donderdag met gemiddelde TC and Ec tellings van 1 900 000 and 160 000 MPN.100 mL^{-1}, respektiewelik. Die uurlikse variasie profiel was soortgelyk vir beide TC and Ec met tellings wat vermeerder het van 06h00 tot 12h00 gevolg deur 'n afname tot 18h00. Die

toename in TC en Ec getalle soos vasgestel gedurende die weeklikse, daaglikse en uurlikse variasie het duidelik getoon dat die bemonsterings wat een maal per maand op Maandae om 08h00 gedurende die 15 maande tydperk uitgevoer is, tot ‘n erg onderskatting van die besmettings vlakke in die Plankenburg en Eerste Riviere gelei het.

Die algehele weeklikse variasies vir die water temperatuur het ‘n verlaging oor die bemonsteringstydperk getoon terwyl die daaglikse en uurlikse variasie neigings ‘n verhoging van 06h00 tot 18h00 getoon het. Die weeklikse neigings vir pH het van die van die temperatuur verskil. Die analises van kovariante het geen korrelasie ($p < 0.05$) tussen die fisiese-chemiese (temperature en pH) parameters en die mikrobe veranderlikes (TC en Ec) getoon nie. Dus is daar afgelei dat temperatuur en pH geen direkte impak op die totale kolivorm of *E. coli* tellings gehad nie.

Die data van die studie het duidelik getoon dat water van beide die Plankenburg en Eerste Riviere nie geskik is vir gebruik vir besproeiing van vars produkte wat rou geëet gaan word nie. In beide gevalle het die fekale besmettingsvlakke die DWAF en WHO leistreep oorskry. Besproeiing met sulke water hou ‘n gesondheidsgevaar in as gevolg van die teenwoordigheid van potensiële patogene wat oorgedra kan word na vars produkte.

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Language and style used in this thesis are in accordance with the requirements of the *International Journal of 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

Fruit and vegetables are considered by the general consumer to be nutritious and healthy mainly because of the high vitamin, mineral and fibre content. Already in 1990, the World Health Organisation (WHO, 1990) recommended an increased intake of fresh produce for healthier living. Some of the health benefits associated with the increased consumption of fresh produce include a reduction in the risk of cardiovascular diseases and cancer due to the high antioxidant content (Carlsen *et al.,* 2010). As a result many countries and international organisations have considered the consumption of fresh produce as a major public health objective (Naska *et al.,* 2000). On the negative side there has been an increase in post-harvest spoilage and fresh produce associated disease outbreaks (CDC, 2010).

Fresh produce has been associated with several large disease outbreaks (Elviss *et al.,* 2009; Matthews, 2009; CDC, 2010). It has been estimated that in the United States alone, more than 7 million cases of illnesses and about 5 000 deaths per year are caused by such foodborne outbreaks (CDC, 2009). The types of raw commodities involved in such outbreaks include lettuce, spinach, tomatoes, uncut and pre-cut salads, pears, spinach and others. According to Beuchat (2006) one of the main reasons for the outbreaks is as a result of pre-harvest contamination of fresh produce with potential pathogens (Beuchat, 2006). Potential pathogens that have been isolated from fresh produce include *Salmonella, Escherichia, Staphylococcus* and *Listeria* (Roberts *et al.,* 2009; Sela & Fallik, 2009). Beuchat (2006) also reported that one method of potential pathogens being introduced to fresh produce is through irrigation with contaminated water (Mathews, 2009).

Many South African rivers have been found to be unsuitable for irrigation of fresh produce (Barnes & Taylor 2004; Germs *et al.,* 2004; Olaniran *et al.,* 2009; Paulse *et al.,* 2009) mainly because of the high levels of faecal contamination. Indicator and index organisms exceeded the DWAF and WHO guidelines of 1 000 *E. coli* per 100 mL water for irrigation of fresh produce (WHO, 1989; DWAF, 1996). The uMngeni River in Kwazulu-Natal was reported to have *E. coli* counts of 1 000 000 cfu per 100 mL water (Olaniran *et al.,* 2009). The Diep, Berg and Plankenburg Rivers in the Western Cape have also been reported to have heavy loads of faecal indicators sometimes with *E. coli* loads of >500 000 per 100 mL water (Paulse *et al.,* 2009). Such highly polluted river systems will certainly pose serious health risks (Zamxaka, 2004a) since in the above examples the rivers are used for drinking, irrigation and other recreational purposes.

Many cases of disease outbreaks, after using faecally contaminated water to irrigate fresh produce, have been reported world wide (Das *et al.,* 2009; Sela & Fallik, 2009). In South Africa a typhoid outbreak by *Salmonella typhi* that had been isolated from faecally contaminated water, was

reported by Keddy *et al.* (2010). Other waterborne disease outbreak incidences have also been reported in the Eastern Cape region (Zamxaka *et al.,* 2004b). Therefore, it is clear that faecally contaminated water can play an important role in the carry-over of potential pathogens from the irrigation water to fresh produce and if the conditions are favourable may lead to a foodborne disease outbreak.

One of the South African rivers used for irrigation of fresh produce, the Plankenburg River, has been reported to be highly contaminated with faecal matter (Barnes & Taylor, 2004; Paulse *et al.,* 2009). Potential pathogens isolated from the river water included: alpha-haemolytic *Streptococcus* of groups A and B; a haemolytic *Streptococcus; Enterobacter, Klebsiella*, *Citrobacter, Acinetobacter and Pseudomonas* spp; *Proteus mirabilis, Proteus vulgaricus, Enterococcus faecalis* and coagulase negative Staphylococci. The health implications associated with these pathogens include gastroenteritis in adults and diarrhoea in children (Anon., 2004; Pu, 2010). Thus the continuous use of faecally contaminated river water for irrigation of fresh produce may lead to a foodborne outbreak with serious health and economic implications.

For this reason in 2007, the South African Water Research Commission initiated a research project to investigate the link between the quality of irrigation water and the safety of produce (Backeberg, 2006). The main objective of the research was to investigate which bacterial and viral contaminants are found in polluted irrigation water sources and highlight their potential risks and carryover potential to crops cultivated using such water sources. This should give an indication whether faecally contaminated water used for irrigation of fresh produce can potentially lead to disease outbreaks. Studies done on the Plankenburg River as part of the WRC project found the river to be unsuitable for irrigation of fresh produce because of the high levels of indicator (coliforms, faecal coliforms and *E. coli)* and index organisms *(Salmonella, Listeria, Staphylococcus* and *Enterococci)* (Ackerman, 2010). Although no disease outbreak as a direct or indirect use of the Plankenburg River has been reported, the persistent faecal pollution of the river and its use for irrigation may possibly result in one. It is thus important to monitor the river for faecal pollution over a longer period of time and to give warning to the authorities so that corrective actions can be implemented to prevent potential disease outbreaks.

The objective of this study was firstly to continue monitoring the microbial quality of the Plankenburg and Eerste Rivers for 14 months (Jan 2009 to May 2010) as part of the ongoing Water Research Commission project (K5/1773//4) on the extent of contamination found in these specific rivers. In order to achieve this, irrigation water will be sampled and both microbiological and physico-chemical tests done to confirm the occurrence profiles, types and quantities of microbes present. The isolation and enumeration of indicator and index organisms will be done to determine the level of faecal contamination. The resulting data will be used to determine the quality of the water according to DWAF and WHO guidelines. The aim of the second phase of the study will be to determine the weekly, daily and hourly faecal load profiles, and thirdly, to determine

if the river water temperature and/or pH has an effect on the microbial load profiles.

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CHAPTER 2
LITERATURE REVIEW

A. BACKGROUND

The increase in obesity and heart diseases has forced people to live a more active and healthy lifestyle which includes eating more fresh fruits and vegetables. Most of our daily requirements for vitamins, minerals and fibre are provided by fresh fruits and vegetables because they have a higher nutrient density (Darmon *et al*., 2005). Fruits and vegetables are also high in antioxidant content which helps against stress related diseases (Carlsen *et al*., 2010). 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, 1990) recommends an intake of at least five portions of fresh fruit and vegetables per day. The WHO has also issued reports stating that correct fresh produce intake alone could save millions of lives a year and that 31% of heart disease cases are due to an insufficient intake of such foods (Johnston *et al*., 2005). As a result of the WHO recommendations (WHO, 1990) consumption of fresh produce has increased by >30% per capita (Carlsen *et al*., 2010) in the United States.

The consumption of fresh produce is not only good for the consumer's health but also for the South African economy with the import and export industry in Western Cape (WESGRO, 2010). The fruits exported include apples, pears, apricots and peaches. The other positive aspect about increased consumption of fresh produce is that of job creation especially for a developing country such as South Africa.

There is a general health and food safety concern about the increased consumption of fresh produce due to the contamination that might make them carriers of potential pathogens (Beuchat, 2002; Matthews, 2006). The fact that they are minimally processed raises more concern since pathogens are known to survive minimal processing (Islam *et al*., 2005; Mathews, 2006). This is because thermal processing which involves heat is excluded in minimal processing. This then means that the pathogen population can multiply to infectious levels resulting in foodborne outbreaks. This will have a negative effect on the economy, the import/export industry and this will ultimately increase the unemployment and poverty rate. The concern is not only about the financial integrity of the country but also about the health of the people.

The Food & Agricultural Organisation of the United Nations (FAO, 2010) has reported an increase in global population. In order to meet the increase in demand as a result of the global population growth, the world production of fruits and vegetables was reported to have increased by 4.5% and 3.7% respectively, from 1993 to 1999 (FAO, 2010). This increase in production is expected to rise at a rate of at least 3% for vegetables and about 1.6% for fruit per year.

To satisfy the demand for the global supply of safe fresh produce, additional distribution steps had to be implemented for export/import purposes (Garrett *et al*., 2003) which make the produce susceptible to additional microbial contamination (Garrett *et al*., 2003). This might result in a foodborne disease outbreak since the processing of whole or cut fresh produce has no thermal kill step to destroy possible pathogens. The safety of fresh produce mainly depends on cleaning, sanitation and disinfection regimes (Busta *et al*., 2003; Harris *et al*., 2003). The effectiveness of these regimes depends directly on the quality of the fresh produce which means that a high level of pre-harvest contamination could result due to inefficient disinfection treatments. It is therefore important to minimise pre-harvest contamination of fresh produce to ensure the effectiveness of disinfection regimes in preventing disease outbreaks (Garrett *et al*., 2003).

The risk of an outbreak is made greater by the fact that the majority of the South African population is poor, more susceptible to pathogens and cannot afford health care should there be an outbreak. The spread of infection is made easier and faster by the fact that most of the poor population live in informal settlements where there are only basic sanitation facilities and waste removal in many cases is ignored (Barnes & Taylor, 2004). The informal settlements are generally erected near natural water sources such as rivers and streams to supplement water requirements for easy disposal of solid faecal waste (Barnes & Taylor, 2004).

Studies have also shown that the carry-over of pathogens from irrigation water to crops does take place (Stine, 2004; Matthews, 2006). It has also been reported that pathogens in water can lead to infections through: ingestion of the contaminated water; consumption of fish from contaminated rivers or streams; fresh produce irrigated with contaminated water; and also through swimming and washing in contaminated rivers (Arnone & Walling, 2007). This then means that the faecal contamination of irrigation water can lead to waterborne and foodborne outbreaks.

B. POLLUTION OF SOUTH AFRICAN RIVERS

It has been reported that 80% of the world's diseases, especially those occurring in developing countries are due to contaminated surface waters (Pandey, 2006). According to a report by Haldenwang (2009), 77% of the water in South Africa is sourced from surface water such as rivers and dams. It is also important to note that the availability and use of the water depends on its quality. Pathogenic bacteria associated with incidences of gastroenteritis were isolated from untreated drinking water that had been sourced from contaminated rivers (Pavlov *et al*., 2004). Therefore the availability of safe water sources will decrease with increased pollution because of the increased risk of potential disease outbreaks such as diarrhoea, cholera, dysentery and skin infections (Haldenwang, 2009) by potential pathogens.

There are other reports in literature stating that potential pathogens are introduced into the river systems through faecal contamination (Griesel & Jagals, 2002; Keshav *et al*., 2010). The

Keiskammahoek River in the Eastern Cape, was found to have faecal pollution from a sewage treatment plant (Fatoki *et al*., 2003). This posed a health issue as the water was being used by the community for drinking, irrigation and other recreational purposes. Thus, the treated wastewater effluent that was discharged into the Keiskammahoek River was found to have a high *Listeria* load (Odjadjare, 2010) and shown to be the major contamination source of the specific river. Similarly, the preliminary assessment of the Chunies River in Limpopo showed the water to be of poor microbiological quality mainly because of faecal pollution (Germs *et al*., 2004). The river was considered to be unsuitable for domestic use or for use as irrigation source because of the unacceptable high microbial levels.

Escherichia coli is generally used in water assessment programmes as an indicator for faecal contamination (Fremaux *et al*., 2009). The South African River Health Programme (RHP, 2008) has reported faecal contamination in several rivers in the KwaZulu-Natal Province. The monitoring of the rivers lasted for four years starting in 1999 and ending in 2002. The uMngeni River had *E. coli* counts of up to 1 million per 100 mL water. Olaniran *et al*. (2009) also reported high faecal coliform counts (255 cfu. mL^{-1}) in the uMngeni and Palmiet Rivers in Kwazulu-Natal. *E. coli* isolates with virulence factors being detected in both river waters. Therefore, irrigation with the water may result in a disease outbreak.

The Mayville and Bellaire streams both had *E. coli* counts of above 100 000 cfu per 100 mL due to inadequate sanitation from nearby informal settlements (RHP, 2008). The *E. coli* counts for the Aller and Umhlangaan Rivers were above 200 000 cfu per 100 mL due to damaged sewage works. In another case the Umlazi and Slangspruit Rivers had *E. coli* counts of above 600 000 cfu per 100 mL and also as a result of damaged sewage works. The microbiological quality of the Mhlathuze River was also found to be poor because of high levels of faecal coliforms (Bezuidenhout *et al*., 2002). The sampling period was from March 1998 to November 1999.

In the Western Cape Province, a study by Barnes & Taylor (2004) reported heavy pollution in the Plankenburg River. The study lasted for five years starting in 1998 ending in 2002. The faecal coliform pollution reached a high of 12 000 000 *E. coli* per 100 mL water. The limit of 2 000 *E. coli* per 100 mL for irrigation water was reportedly (Barnes & Taylor, 2004) exceeded in 97% cases during the four year sampling period. Another study done on the Plankenburg River from June 2004 to June 2005 reported total faecal coliform and *E. coli* counts of 210 000 000 and 3 500 000 microorganisms per 100 mL, respectively (Paulse *et al*., 2009). Also in September 2007 to February 2008, Lötter (2010) reported faecal coliform counts as high as 460 000 *E. coli* per 100 mL water. Ackerman (2010) also reported a high faecal coliform of 160 000 *E. coli* per 100 mL water for the Plankenburg River. The sampling period was from September 2007 to September 2008. In all the cases the source of contamination was ascribed to sewage contamination from the nearby informal settlement of Kayamandi. The Diep River was reported to have *E. coli* counts of 160 000 cfu per 100 mL water (Paulse *et al*., 2009) for the year 2005. The highest faecal count was 1 800

000 cfu per 100 mL water. Sewage spills were suspected to be the source of contamination since the sampling site was situated below the Potsdam Wastewater Treatment Works. The Berg River was also found by Paulse *et al*. (2007) to have high counts ranging between 36 and 17 000 000 *E. coli* per 100 mL water. The sampling period was from June 2004 to June 2005. In this case the sampling site was near to the Mbekweni informal settlement which was suspected as the contamination source.

In the Gauteng Province, the Juksei River was reported to have *E. coli* counts that were as high as 30 000 000 cfu per 100 mL water in September 1994 (De Wet *et al*., 1999). The Tshwane River was also reported to have faecal coliform counts of between 5 900 and 23 000 cfu per mL water (Nevondo & Cloete, 1999). In the Eastern Cape Province the Tyume River is used for drinking purposes and was reported to have faecal coliform counts that exceeded guidelines (Zamxaka, 2004a). Potential human pathogens (*Escherichia coli*, *Salmonella* and *Vibrio cholerae*) were isolated from the Tyume River (Zamxaka, 2004b). In the Venda region six rivers were reported to have faecal coliform counts ranging from 1 500 to 63 000 cfu per 100 mL water (Obi *et al*., 2002).

Some of the pathogens isolated in the Plankenburg River showed resistance to specific antibiotics and chlorination treatments (Barnes & Taylor, 2004). The potential pathogens detected included β-haemolytic *Streptococcus* (group A and B), α-haemolytic *Streptococcus*, *Enterobacter* spp., *Klebsiella* spp., *Citrobacter*, *Acinetobacter*, *Pseudomonas* spp., *Proteus mirabilis, Proteus vulgaricus*, *Enterococcus faecalis* and coagulase negative *Staphylococcus*. Some of these isolated potential pathogens were tested for resistance to antibiotics and chlorination. The *E. coli* strains showed high resistance to the antibiotics amoxicillin and co-trimoxazole. The β-haemolytic *Streptococcus* group A were found to be resistant to co-trimoxazole and erythromycin. *E. coli* O157:H7 was found to prevail in treated drinking water around the Amathole District in the Eastern Cape (Abong`o & Momba, 2008). Therefore, pathogens with resistance genes can survive disinfection treatments. Thus, irrigation with faecally contaminated water may contaminate the fresh crops with potential pathogens that have resistance genes which allow them to survive minimal processing treatments. Therefore, irrigation of fresh produce with contaminated water poses a definite health risk to the consumer.

Haldenwang (2009) described the state of South African rivers as being unacceptable. The report also recognised the failing of municipal sewage treatment infrastructure and informal settlements as major contamination issues. Therefore, the polluted water poses a health issue since many of the rural areas use the river as a source of drinking water (Aneck-Hahn *et al*., 2009). The high *E. coli* counts reported in previous studies show that the use of river water for irrigation of fresh produce poses a health and food security risk to both the consumer and agricultural industry.

C. DISEASE OUTBREAKS

Waterborne disease outbreaks

Waterborne disease outbreaks are closely linked to gastroenteritis (Risebro & Hunter, 2007). The clinical symptoms include diarrhoea, abdominal cramps and vomiting (Majowicz *et al*., 2007; Gómara *et al*., 2008). Contaminated drinking and recreational waters have been associated with many outbreaks (Das *et al*., 2009; Dale *et al*., 2010). In these cases the water was found to have high levels of faecal contamination making it an excellent transmission vehicle for disease-causing-enteric-pathogens such as *Campylobacter, E. coli, Cryptospiridium, Shigella* and Norovirus.

The number of reported waterborne outbreaks since 1920 has increased in the United States (Craun *et al*., 2006) reaching a high of 36 162 cases per year in 2002. Many of the outbreaks involved bacterial pathogens like *Salmonella typhimurium, Vibrio cholerae, Escherichia coli* O157:H7 and *Campylobacter jejuni*. A surveillance report by the CDC (CDC, 2009b) reported a total of 78 waterborne outbreaks in the US alone during 2006 with 20 of the outbreaks associated with untreated recreational water venues such as rivers, lakes and dams. It was further reported that eight of the outbreaks were caused by bacteria. Parasites and viruses were involved in three outbreaks. It was also reported that all eight of the bacterial outbreaks resulted in acute gastroenteritis illness. *E. coli* O157:H7 was involved in three cases and *Campylobacter jejuni* in one. The remaining four cases involved *Shigella*.

In August 2000, a waterborne outbreak in France linked to faecal contamination of groundwater was investigated (Gallay *et al*., 2006) and *Campylobacter coli*, Rotavirus and Norovirus were identified as possible causative agents. The risk of illness increased with the increased drinking of the tap water and the sources of contamination were reported as a combination of agricultural run-off and failure in the chlorination step of the wastewater treatment. In a study by Das *et al*. (2009) on an outbreak in a village in India found the source of the outbreak to be drinking water that was contaminated with sewage. The causative agent was identified as *Vibrio cholerae* O1. In another study, drinking water from a groundwater system that got contaminated through a sewage spill caused an outbreak in La Neuveville, Switzerland (Maurer & Sturchler, 2000). In this case *Campylobacter jejuni* was isolated as the causative agent. These studies clearly show how faecal contamination of water sources can lead to disease outbreak.

In a study by Harris *et al*. (2003), a water well supplying drinking water to the local town of Walkerton was found to be contaminated with *E. coli* O157:H7 by cattle manure from a nearby farm. The study showed how agricultural run-offs can contaminate surface waters resulting in an outbreak. The study by Barnes & Taylor (2004) also showed how run-offs from an informal settlement can contaminate surface waters. In this study, the Plankenburg River in the Western Cape had a high level of faecal contamination. Although there was never a reported case of disease outbreak, the level of contamination in the water was found to be a potential health and

food safety hazard. A study by Parker *et al*. (2010) also showed stormwater run-offs from the coastal North Carolina areas in the US as having high levels of faecal contamination that may result in a potential disease outbreak.

Other studies have shown evidence of poor surface water quality with the isolation of pathogens such as shiga toxin producing *E. coli* in concentrations that can lead to disease outbreaks (Ashbolt, 2004; Ram *et al*., 2009). Not only do these reports and studies show evidence of poor surface waters but also of high faecal pollution levels. According to the principles of disease ecology, society and its environment are at equilibrium which means that any change occurring in society will also have an impact on the environment (Mayer, 2000). The increase in population growth and urbanization together with poor water supply and sanitation are the factors that lead to waterborne diseases in many developing countries (Ashbolt, 2004).

Some of the major water pollution sources include waste effluents from wastewater treatment plants, industrial waste, run-offs from informal settlements and even from animal slaughter farms (Murkhejee *et al*., 2007; Ram *et al*., 2009). A study by Cho *et al*. (2010) showed that rainfall run-offs can also contribute to the contamination of nearby streams and rivers. In those cases the faecal indicator bacteria counts increased to levels greater than 24 000 MPN per 100 mL water. It was also reported that 98% of the people living in rural areas defecate in open fields and that 90% of the pollution of river systems is due to human waste (Pandey, 2006). Therefore, it is not surprising to have faecal contamination of rivers from nearby informal settlements since the standard of living is similar to that in rural areas.

Furthermore, the majority of waterborne disease related deaths occur in developing countries (Arnone & Walling, 2007) with 15% of the deaths being those of children under the age of 5 years old. Surface water in developing African countries has been known to cause diseases such as cholera (Ashbolt, 2004; Stine, 2004). The cholera epidemic that affected Mozambican refugees in Malawi was linked to contaminated drinking and recreational water (Swerdlow *et al*., 1997). *Vibrio cholerae* O1 was identified as the causative agent. This is why an adequate water supply can improve the health and quality of life in a community.

The importance of quality water supply is also important to South Africa as failure to supply safe potable water impacts waterborne disease outbreaks in the country. Water around the Gogogo region in the Eastern Cape Province, South Africa was found to cause high incidences of waterborne outbreaks related to diseases such as cholera and typhoid (Zamxaka *et al*., 2004b). In the study done by Keddy *et al*. (2010) that investigated the South African typhoid fever outbreak in 2005, faecally contaminated water was identified as the probable cause of the outbreak. This was based on the isolation of *Salmonella typhi* from the water. A study on the microbiological quality of water supply in the rural areas around the Eastern and Western Cape Province in South Africa was done by Mackintosh & Colvin (2003). He found the water to be of poor microbiological quality for both provinces.

There is not much data about the occurrence of disease outbreaks in South Africa possibly due to a lack of proper surveillance systems. Factors influencing a surveillance system include public awareness, reporting of disease, and resources for investigation of possible outbreaks (Craun *et al*., 2006; Risebro & Hunter, 2007). Therefore many incidences of possible disease outbreak occurrences go unnoticed.

Foodborne Disease Outbreaks

Food is recognised as an excellent vehicle for pathogens associated with disease (Newell *et al*., 2010) and therefore, can be a source of disease outbreaks. Especially the increase in the consumption of fresh and fresh-cut produce has been associated with the increase in foodborne outbreaks (Solomon & Sharma, 2009). The demand for fresh fruits and vegetables necessitates the use of minimal processing that result in microbial quality issues due to inadequate removal of spoilage organisms and disease causing pathogens (Sela & Fallik, 2009). Enteric pathogens isolated from many of the outbreaks include *Salmonella*, *Escherichia coli* O157:H7 and *Listeria* (Matthews, 2009; Roberts *et al*., 2009). The types of fresh produce associated with disease outbreaks include tomatoes, seed sprouts, fresh herbs, cantaloupes and leafy green vegetables such as lettuce, spinach and cabbage (Johnston *et al*., 2005; Elviss *et al*., 2009; Matthews, 2009).

In the past, fruits and vegetables were considered as minor and infrequent pathogen sources (Doyle, 2000) because animal-based products were identified as major food sources of pathogens (Greig & Ravel, 2009). However, changes in foodborne outbreak trends are showing fresh produce as being a major source (Newell *et al*., 2010). Findings in a study by DeWaal *et al*. (2007) identified fresh produce as one of the food commodities, second to seafood, with the highest foodborne outbreak cases. This is why it is important to understand the factors driving foodborne outbreaks in order to reduce the incidence rate.

The major factors that have contributed to the change and increase in foodborne diseases include the increasing global food market that exports food from countries having poor food safety procedures (Solomon & Sharma, 2009; Newell *et al*., 2010). This is because exporting of fresh produce increases storage and transportation time which additionally allows potential survival of the pathogens (Quested *et al*., 2010). The other major factor influencing foodborne disease outbreaks is the change in eating habits (Newell *et al*., 2010). The increase in consumption of fresh fruits and vegetables increases the chances of an outbreak because there is no thermal-kill step to destroy any potential pathogens (Newell *et al*., 2010; Quested *et al*., 2010). The increased demand for organic food may also potentially increase foodborne outbreaks because the change in farming practices for organic food allows the survival of pathogens (Newell *et al*., 2010). In a study done by Oliveira *et al*. (2010), *E. coli* counts were found to be higher on organic fresh lettuce than on conventionally cultivated types.

In developing countries the factors contributing to foodborne illness also include poverty, poor education and hygiene, contaminated water, food and eating utensils (King *et al*., 2000). Other factors that increase the severity of the foodborne illnesses in developing countries are the high level of malnutrition and immune-compromised patients. Therefore it is very important that foodborne outbreaks are prevented in such countries because an outbreak may lead to death due to the low immunity.

Foodborne outbreaks in the US have been estimated to cause about 76 million illnesses and 5 000 deaths per year (King *et al*., 2000; CDC, 2009a). Therefore, not only are these foodborne illnesses a health and food safety hazard but they are also an economic hazard. The cost of foodborne outbreaks in the US was estimated at 152 billion dollars per year (Scharff, 2010). This estimate was based only on health-related costs for acute and long-term foodborne illnesses. The health-related costs included medical costs for buying of drugs and other treatment products and quality-of-life losses where death occurs. The estimated cost for a non-typhoidal *Salmonella* illness case was $9 146. The estimated cost for an *E. coli* case was $1 399. It is important to note that such costs cannot be afforded in a developing country which is why prevention of disease outbreaks is important in South Africa.

Since 1996, the FDA has dealt with foodborne outbreak cases of Salmonellosis and *E. coli* O157:H7 linked to raw or minimally processed seed sprouts (Johnston *et al*., 2005). Japan has also had confirmed outbreaks of *E. coli* O157:H7 linked to radish sprouts. A Montana *E. coli* O157:H7 outbreak associated with lettuce was reported in July 1995 (Ackers *et al*., 1998). It resulted in 13 hospitalisations whereby one developed into a haemolytic-uremic syndrome case. Multistate *Salmonella* outbreaks associated with uncooked tomatoes during 1990 and 1993 were identified in Illinois, Michigan, Minnesota and Wisconsin (Herdberg *et al*., 1999). *Salmonella javiana* was responsible for the 176 cases in 1990 and *Salmonella montevideo* was responsible for the 100 cases in 1993.

In a report by the Centres of Disease Control (CDC, 2009a) detailing the surveillance of foodborne disease outbreaks in 11 States in the USA, nearly 30 000 cases were reported for the year 2006. The causative agents were viruses, bacteria, fungi and parasites (CDC, 2009a). Of those reported cases, 11 resulted in death with 10 of the deaths ascribed to bacterial food pathogens. For the year 2006, *E. coli* O157:H7 claimed six lives while *Listeria monocytogenes* claimed two followed by *Salmonella enteriditis* and *Clostridium botulinum* each claiming one life. Other reported outbreaks include three *E. coli* O157:H7 cases from leafy vegetables; two *Salmonella* cases from tomatoes; and one Shiga toxin-producing *Escherichia coli* (STEC) on fruit salad (CDC, 2009a).

Since then, an analysis of the preliminary foodborne data of 1996 to 2008 for the USA has shown a decrease in some of the foodborne outbreak incidences in terms of contamination of food with pathogens (CDC, 2010). A sustained decrease in reported foodborne illness cases due to

Campylobacter, Listeria, Salmonella, Shiga toxin-producing *Escherichia coli, Shigella* and *Yersinia* was identified. Meanwhile an increase of foodborne illness cases caused by *Vibrio* was found. It is important to note that the data analysed was for the US. Therefore, it is possible that the incidences of foodborne outbreak everywhere else in the developing world is still increasing. This is due to the risk of infection being higher as a result of the poor water supplies and sanitation (Ashbolt, 2004; Barnes & Taylor, 2004). This is why it is important to understand the contamination of fresh produce so as to prevent the infection of food by potential pathogens.

Much attention has been given to leafy green vegetables since outbreak incidences associated with them have increased by 39% between 1996 and 2005 (Solomon & Sharma, 2009). These incidences have highlighted some important emerging produce-pathogen concerns such as the survival of pathogens in the environment and their subsequent carry-over to fresh produce. Therefore, it is important to understand how contamination of fresh produce during irrigation takes place.

D. CONTAMINATION OF FRESH PRODUCE

The contamination of rivers with runoffs and other sources such as spills from municipal waste water treatment plants can introduce pathogens into the rivers. Most pathogens are known to survive in water and therefore pose a risk for the contamination of fresh produce through irrigation (Stine, 2004). Studies have shown the survival of *Listeria, Salmonella* and *E. coli* in water (Lu *et al*., 2004; Islam *et al*., 2004; Ells & Truelstrup, 2006). Therefore, a better understanding of fresh produce contamination with pathogens and their survival and possible growth on the surface of the fresh produce is needed to help curb foodborne outbreaks (Stine, 2004) from fresh produce.

It is known that continued irrigation with contaminated water results in the accumulation of pathogens on the surface of fresh produce (Islam *et al*., 2005; Ells & Truelstrup, 2006; Aiello *et al*., 2007). The accumulation may also lead to the formation of biofilms increasing microbial resistance to antibiotics and disinfectants (Chaidez *et al*., 2005; Solomon & Sharma, 2009). Biofilms are exopolymeric substances that give protection to pathogens against disinfection treatments (Sela & Fallik, 2009). The microbial population within a biofilm attach to the surface preventing them from being washed off during washing steps. Apart from biofilm formation, internalisation of the pathogen into the plant tissue also allows its survival against disinfection and washing treatments (Pu, 2010).

Modes of internalisation include uptake of the pathogen through infiltration via the internal gas spaces, root system and damaged tissues (Solomon & Sharma, 2009). Drastic temperature on the surface of the plant was found to cause expansion of the gas spaces which then allows pathogens to enter. A study by Kroupitski *et al*. (2009) also showed how the change in light can induce stomata to open allowing for the attachment and internalisation of pathogens into the plant

tissue. In another study by Solomon *et al*. (2002), *E. coli* O157:H7 migrated to the edible parts of a lettuce plant via the root system. Thus, it is important to keep the microbial load on food surfaces to a minimum so as to prevent possible internalisation by potential pathogens.

Studies have reported salad vegetables as having high levels of heterotrophic bacteria (Heaton & Jones, 2008; Eni *et al*., 2010). This can also include pathogens (Beuchat, 2006). Other fresh produce including cantaloupe and lettuce were found to have high total coliform counts (Stine, 2004). The mere presence of high heterotrophic bacteria and coliform counts on fresh produce suggests the possibility of a health risk from both spoilage and pathogenic organisms. This is because most of this type of produce is either eaten raw or subjected to minimal processing. The levels of heterotrophic bacteria after 3 - 5 days of storage were found to be higher than at harvest which suggests not only survival but growth of the bacteria (Stine, 2004). Other studies have reported the survival and growth of microbial organisms on the surface of fresh produce (Pu, 2010).

African developing countries are known to be large exporters of fresh produce and their lack of implementation of high level food safety measures makes their exports questionable in terms of potential pathogenic loads (Stine, 2004). For example mean microbial loads on fruit and vegetables sold in Nigeria were reported to be very high (Eni *et al*., 2010). In the study *E. coli* and *Salmonella* were isolated from the fruit and vegetables. In developed countries the food industry has to implement food safety measures such as Hazard Analysis Critical Control Point (HACCP) to minimise and eliminate contamination sources (Buchanan & Doyle, 1997). These food safety measures include disinfection and washing steps to remove pathogens from the surfaces especially of fresh produce. Therefore, implementation of food safety measures is another factor that influences foodborne outbreaks by preventing post-harvest contamination of the food.

However, according to Matthews (2009), the implementation of food safety measures should not be a major issue since the conditions on the plant tissue surface limit the survival of microbes . These include changes in temperature and relative humidity, exposure to UV light and presence of epiphytic microbes which compete with pathogens for nutrients. It was reported by Stine (2004) that dry conditions that have a low relative humidity had a higher inactivation rate. The characteristics of the crop surface may also influence the microbial survival rate as pathogens are known to survive longer on the surface of cantaloupes than on lettuce or bell peppers (Stine, 2004). Some of the crops surfaces were found to allow for better attachment and protection from disinfection and washing treatments.

UV light was also found to have an influence as some of the pathogens were exposed to light and others kept in the shade (Stine, 2004). It was then found that those that were shaded from the light survived longer than those exposed to light. However biofilms are known to protect pathogens from detrimental conditions on the plant surface (Sela & Fallik, 2009). There is evidence

that pathogens can co-exist with epiphytic microbes allowing their survival and growth (Solomon & Sharma, 2009).

Other factors influencing the carry-over, survival and growth of pathogens on fresh produce are: the specific type of pathogen; the type of vegetable; and the type and length of environmental and storage conditions (Harris *et al*., 2003). Pathogens such as *Salmonella* are known to survive on the skin of tomatoes, and grow to alarming concentrations inside sliced tomatoes (Herdberg *et al*., 1999). This organism was reported to grow on whole and sliced tomatoes at 20° to 30°C (Stine, 2004). Other studies have also shown the survival of *E. coli* O157: H7 and *Salmonella* after carry-over to the surface of carrots (Islam *et al*., 2005). Pathogenic parasites and *Salmonella* were also isolated from sprouts in Norway (Robertson *et al*., 2002). Therefore different types of pathogens have different survival rates and these must be taken into consideration during routine microbial testing of the crops.

Pathogens such as *E. coli* O157:H7 have the ability to survive extreme environmental conditions (Harris *et al*., 2003). *Escherichia coli* O157:H7 and other pathogens can live in both fresh and salt water due to their resistance to acid and salt conditions. This means that there is a chance of them being carried over to crops during irrigation with contaminated river water. *Escherichia coli* O157:H7 also has a resistance to chlorine (Harris *et al*., 2003) which means it can survive the washing and disinfection step during the processing of fresh produce. *Enterobacteriaceae* species can also survive freezing and high temperatures which indicates that the pathogen will be able to survive the low storage temperatures used for fresh produce.

The risk of pathogen transmission from irrigation water to crops also is dependent on the: level of water contamination; survival profile of the specific pathogen in water and on crop surfaces; and the growth ability of the organism on the specific crop surface (Steele & Odumeru, 2004). Additional factors influencing carry-over to fresh produce during irrigation include: the size of the space between the ground and crop's vegetative part; the hydrophobicity and contours on the surface; and, the type of irrigation system used.

Continuous irrigation with contaminated water is known to lead to aggregates forming and a subsequently higher pathogenic load (Sela & Fallik, 2009; Solomon & Sharma, 2009). Some of the pathogens that can form aggregates are known to be resistant to chlorination treatments (Barnes & Taylor, 2004) and can potentially survive processing until consumption by consumer. The pathogenic load in the aggregate may exceed the infectious dose required by a healthy individual to become infected. Therefore, irrigation of fresh crops with contaminated water is a health hazard and should be given attention immediately.

Enteric pathogens such as *Salmonella*, *Shigella*, *Vibrio* and *Campylobacter* have also been isolated from the rivers in the Venda region (Obi *et al*., 2005) that are used for irrigation of fresh produce. Similarly *Salmonella* was isolated in the Tshwane River and the counts were between 7 000 and 46 000 cfu per mL (Nevondo & Cloete, 1999). The presence of the pathogenic *E. coli*

0157:H7 was detected in the Ganges River, India by Hamner *et al*. (2007). In this case, the isolates tested positive for the Shiga toxins stx_1 and stx_2. Enterotoxigenic *E. coli* (ETEC), which is associated with acute watery diarrhoea cases in children, was detected in surface water of Bangladesh (Qadri *et al*., 2005). The isolates were characterised as being potentially resistant to antibiotics erythromycin, ampicillin and others. Ram *et al*. (2009) also isolated ETEC from the Saryu River that showed resistance to antimicrobial agents. A study by Mieta *et al*. (2010) also characterised entero-invasive *E. coli* and *Shigella* as both having virulent genes. These pathogens were isolated from diarrhoeal human stool samples therefore, faecally contaminated river water can introduce potential enteric pathogens to fresh produce through carry-over.

Thus it can be concluded that water plays a central role in the transmission of pathogens to fresh produce. It can also be concluded that once the plant surface is contaminated with pathogens, the chances of survival until consumption by the consumer is high. It also seems likely that minimal processing treatments are inadequate for the removal of potential pathogens from the plant surface. Therefore, it is important to identify and detect major contamination sources in order to prevent pre-harvest contamination of fresh produce.

E. CONTAMINATION SOURCES OF FRESH PRODUCE

The contamination of fresh produce has greater food safety risks compared to the contamination of other foods. This is because most fresh produce is packaged or sold as ready-to-eat-products. Normally the preparation step before consumption involves rinsing or washing with water and sometimes the fresh produce is consumed without any washing step. The preparation of other foods involves suitable processing steps such as cooking which involves heat. It is this preparation step that lowers food safety risks by eliminating disease causing microorganisms. This is why the contamination of fresh produce should be controlled in order to prevent foodborne outbreaks.

Although food pathogens have been identified as the main causative agents with the raw and minimally processed food as main carriers, there always remains the question of how the pathogens got to be on the food product. This question is valid in the sense that the natural microbial community found on fruit and vegetables are normally non-pathogenic bacteria, yeast and moulds (Sela & Fallik, 2009). Studies have shown a direct link between the fresh produce and contamination source (Beuchat, 2002; Matthews, 2006). The contamination sources are normally associated with the environment that the food is exposed to before and after harvest. The pre-harvest sources include soil, irrigation water, manure, compost, fertilizer, dust, humans, animals and insects. The post-harvest sources include handling, processing equipment, rinse water, storage environment, distribution, transportation and preparation during processing.

Pre-harvest contamination

The major contamination routes during the pre-harvest period are soil and irrigation water (Heaton & Jones, 2008). Both resources are necessary for crop production so exclusion from production is not an option. They are part of the natural environment and are therefore subject to faecal contamination. Thus the prevention of pre-harvest contamination of fresh produce with potential pathogens depends on the quality of soil and irrigation water used for crop farming.

Soil

As a pre-harvest contamination source, soil is sometimes seen as a major source of pathogens that could lead to foodborne outbreaks associated with fresh produce. This perception of soil has to be considered carefully as normal unpolluted soil is made up of mostly non-pathogenic microbes. These types of microbes can regulate and negate pathogen growth by the secretion of inhibitory substances such as antibiotics, bacteriocins, organic acids, and others (Jay, 2000). The soils acidity, pH, moisture and temperature are also limiting factors to the growth of certain pathogens and thus it is these facts that, when taken into consideration, should not allow the soil to be considered as a major pathogen source.

It is also well documented that the microbial ecosystem of a healthy soil is free of enteric pathogens such as *E. coli* O157:H7 (Islam *et al*., 2005). Therefore enteric pathogens get introduced to soil through contamination. One of the ways that soil gets contaminated is via the application of organic animal or human fertilizer (Heaton & Jones, 2008). The other way is by direct contamination from wild animal and livestock (Beuchat, 2006). Furthermore, runoffs from nearby informal settlements and agricultural land pollute river systems used for irrigation with potential pathogens changing the irrigation water into a major source of contamination with dire health risk consequences (Barnes & Taylor, 2004).

However, organic compost or fertiliser should not play a role in contaminating soil or produce with pathogens since the production process is regulated by standards and retail guidelines, but it does. Studies also attest to the fact that proper composted fertilizers should have no pathogens due to the high temperatures associated with the exothermic reaction of composting that kill pathogens (Murkhejee *et al*., 2007). In certain cases composted materials used for agriculture are made from faecal matter of animals which has been known to contain high concentration of pathogens (Stine, 2004; Matthews, 2006).

Therefore, the repeated use of such compost fertilisers may result in the build-up of pathogens in the soil. This is a definite health hazard since the properties and characteristics of the soil have been shown to allow for their survival and growth (Stine, 2004). Therefore, faecal contamination of soil is also a food safety hazard since pathogens can enter the plant tissue via the root system (Solomon & Sharma, 2009) allowing survival of pathogens against minimal processing treatments.

Irrigation water

Contaminated irrigation water should also not be a pathogen source for soil or the produce simply because of guidelines, but it happens. The World Health Organisation (WHO, 1989) recommends as a guideline, <1 000 faecal coliforms per 100 mL of irrigation water used for fresh produce. Due to the import and export of fresh produce between continents, many countries have adopted this specific WHO guideline as part of their food safety standards and regulations. Based on such international food safety standards, most import and export companies are encouraged to use water of good quality during the production of the fresh produce. Common irrigation water sources include municipal drinking water, treated waste water and river water. Most of these irrigation water sources should be free of pathogens.

Municipal drinking water is meant for human consumption and is therefore regulated by legislation (DWAF, 1996) based on a zero pathogen policy for drinking water. Treated waste water is regulated with strict guideline authorities such as the WHO to ensure that the microbiological quality of crops will not be affected (Hespanhol & Prost, 1994; Blumenthal *et al*., 2000). The DWAF guideline for irrigation of fresh produce is also <1 000 faecal coliforms per 100 mL water (DWAF, 1996b).

River water should also be free of pathogens because freshwater systems are naturally low in nutrients necessary for microbial growth and survival (Solomon *et al*., 2002). Another reason why river water can be free of pathogens is where a biological self-purification process in streams and river systems takes (Ralph, 1972). However, the level of faecal contamination to river systems has increased drastically (Solomon *et al*., 2002) due to the impact of increases in population, urbanisation and informal settlements being built near water ways. Therefore the biological self-purification process is no longer functional in today's world.

In South Africa river water is the most commonly used source of irrigation water, especially in rural areas (Wenhold *et al*., 2007) where small household farming is the major source of food for most families. If the water used undergoes no purification treatment process, there is a likelihood of it having high microbial loads and pathogen presence mainly from faecal contamination. Therefore, the microbial quality of irrigation water is a possible major pre-harvest contamination source of fresh produce.

Post-harvest contamination

With regards to post-harvest contamination sources, bad hygiene is one of the major causes during harvesting, handling and preparation (Matthews, 2006; Collignon & Korsten, 2010). An unclean production environment is another source of contamination to processing equipment, storage chambers and delivery vehicles during distribution. Microorganisms can aggregate and

form biofilms in food processing facilities (Chmielewski & Frank, 2003). This becomes a hazard as the biofilm may harbour spoilage and pathogenic microorganisms. *Escherichia coli* O157:H7 has been found to have the ability of forming biofilms on stainless steel surfaces (Ryu & Beuchat, 2005). The strains isolated were also found to have high resistance to chlorine. Therefore, post-harvest contamination of fresh produce from the processing plant is a definite hazard and should be managed by the implementation of safety measures.

Governments around the world are encouraging production companies together with retail outlets to implement Good Manufacturing Practices (GMPs) and Hazard Analysis Critical Control Point (HACCP) as food safety production systems. Another food safety production system aimed at farming companies is Good Agricultural Practices (GAPs). Studies have shown the importance of implementing GAPs and these include routine microbiological analyses of produce, equipment, handlers, rinse and irrigation water to ensure food safety (Gomes da Cruz *et al*., 2006).

It is a known fact that the efficiency of disinfectant washing and sanitising as applied during the processing of fresh produce is sometimes inefficient (Solomon & Sharma, 2009). This can lead to contamination of the equipment and storage chamber used in the processing plant during cutting, packaging and storage. Thus, the success of implementing food safety measures is as good as the microbial quality of the harvested fruit or vegetable.

F. PRESENCE OF POTENTIAL PATHOGENS IN CONTAMINATED IRRIGATION WATER

It has been established that irrigation water contaminated with faecal material will probably have a high load of potential pathogens. Therefore, consumption of fresh produce contaminated with potential pathogens from irrigation water may lead to a disease outbreak. The types of microbial pathogens responsible for disease outbreaks include bacteria, viruses and parasites (Beuchat, 2006). Some of the known bacterial pathogens include members of the genera *Escherichia*, *Salmonella, Campylobacter, Listeria, Shigella, Vibrio, Yersinia, Clostridium, Staphylococcus, Bacillus* and other emerging pathogens such as *Enterobacter sakazakii*. The known viral pathogens include *Calicivirus, Norovirus, Hepatitis A* and *B*. The parasitical pathogens include *Cyclospora* and *Giardia* (Barnes & Taylor, 2004; Sela & Fallik, 2009). Only the bacterial pathogens will be discussed for the purpose of this particular study.

Microbial pathogens involved in disease outbreaks

Escherichia coli

This organism is used as an indicator for faecal pollution and presence of other enteric pathogens (DWAF, 1996; Buchanan & Doyle, 1997; Stine, 2004; Fremaux *et al*., 2009). This is because it is known to occur in human and animal sewage and manure compost made from animal faecal

matter (Beuchat, 2006; Sela & Fallik, 2009). The six recognised *E. coli* pathotypes are enterohaemorrhagic (EHEC), enterotoxigenic (ETEC), enteroinvasive (EIEC), enteroaggregative (Eagg EC), enteropathogenic (EPEC) and diffusely adherent (DAEC). The EHEC strains have the ability to produce shiga toxin 1 (Stx1) and 2 (Stx2) which are also known as verotoxin 1 and 2, respectively (Buchanan & Doyle, 1997; Ochoa *et al*., 2007; Pu, 2010). The toxins damage cells by releasing cytokines such as tumour necrosis factor.

The EHEC strain *E.coli* O157:H7 was recognised as a foodborne pathogen since the two haemorrhagic outbreaks in 1982 associated with hamburgers. The one occurred in Oregon resulting in 19 hospitalizations out of 26 cases. The other occurred Michigan with 21 cases of which 14 were hospitalizations. The symptoms associated with *E. coli* O157:H7 are bloody diarrhoea also known as haemorrhagic colitis (HC), acute renal failure, seizures, coma, stroke, colonic perforation, pancreatitis and hypertension (Buchanan & Doyle, 1997; Ochoa *et al*., 2007; Pu, 2010). In children it is known to cause haemolytic uremic syndrome (HUS). Other foods associated with the outbreak include poultry, seafood, unpasteurised apple juice, radish and alfalfa sprouts, raw milk, lettuce, water and yoghurt.

The survival and growth of *E. coli* O157:H7 depends on intrinsic and extrinsic factors such as temperature, pH and water activity. The minimum growth temperature is approximately 8° - 10°C and the pH for optimum growth is between 5.8 and 7.5. Studies have shown its growth at low pH levels in acidic foods such as mayonnaise, sausages, apple cider and cheddar cheese (Buchanan & Doyle, 1997; Harris *et al*., 2003). Other studies have attested to signs of resistance to heating, radiation and antimicrobial agents. It can also survive in environments with low water activity at refrigeration temperatures. Some of these *E. coli* types have been isolated from river water and therefore could pose a potential carry-over problem during irrigation (Qadri *et al*., 2005; Ram *et al*., 2009). Therefore, contamination of fresh produce with *E. coli* types could lead to serious foodborne outbreaks and should therefore be prevented.

Salmonella

Salmonella causes a disease called salmonellosis. The recognised symptoms are enteric fever and foodborne illness syndrome which are characterised by vomiting, diarrhoea, headache, malaise, anorexia and abdominal cramps (Anon., 2004). The disease may be caused by the presence of a minimum of 15 cells with an incubation period of between 6 to 48 hours.

It has also been reported that most of the *Salmonella* serotypes including *Salmonella enteritidis* are host specific and occur in foods made from the host such as meat from cows or chickens (Wiedmann & Nightingale, 2009). It is also known that pathogens are shed through the faecal matter of the host animal. This is why most of the *Salmonella enteritidis* cases are generally associated with foods such as meat, poultry and seafood. The preparation of these foods involves some form of heating that normally ensures elimination or inactivation of some of the pathogens.

The presence of the same pathogens on fresh crops pose a food safety risk since the food is either consumed raw or undergoes minimal processing which is inefficient in the elimination of pathogens. An *IpaB* virulent gene was detected in a *Salmonella* isolate (Mieta *et al*., 2010). Therefore, contamination of fresh produce with *Salmonella* should be prevented to avoid disease outbreaks.

Shigella

Shigella causes shigellosis which is also known as bacillary dysentery. It is a host-dependant bacterium that does not survive heat treatments and pH values below 4.5 (Anon., 2004). The minimum infective dose for shigellosis is 10 cells per infection. The symptoms include diarrhoea, abdominal pain, fever and vomiting. *Shigella* has been reported to grow at 22° - 27℃ on sliced fruits and vegetables (Stine, 2004). The *Ial* and *IpaH* virulent genes that are typical of EIEC strains was also isolated in *Shigella* strains from diarrhoeal stool samples (Mieta *et al*., 2010). Therefore the presence of *Shigella* on fresh produce must be seen as a definite food safety hazard.

Campylobacter jejuni

Campylobacter is a fragile bacterium and can be eliminated fairly easily. This is because it cannot grow at temperatures below 30℃ and has a slow growth under favourable conditions (Anon., 2004). It is also sensitive to low oxygen, acidic conditions, disinfectants and heat. The symptoms associated with its disease are bloody diarrhoea, abdominal cramps, headaches, muscle pain and nausea. Some *Campylobacter* strains can produce an enterotoxin that is known to cause food poisoning (Stine, 2004).

Clostridium

In contrast to *Campylobacter, Clostridium perfringens* can survive most processing steps because members of this genus produce endospores that are resistant to heat and disinfectants. The endospore allows them to survive the high temperatures generated during processing, composting and the wastewater treatment steps (Stine, 2004). *Clostridium perfringens* and *Clostridium botulinum* are both toxin-producing pathogens (Anon., 2004), but only *C. botulinum* will be discussed. Its toxin causes botulism and the symptoms are nausea, vomiting, fatigue, dizziness, headache, dryness (mouth, skin, and throat), constipation, double vision, difficulty in breathing and sometimes death. The toxin can be inactivated by the sterilization heat treatment of 80℃ for 10 minutes. A study done on several salad vegetables found a high prevalence of *Clostridium* on most of the supermarket vegetables. Thus, *Clostridium* species can definitely survive treatment steps and then reach the consumer. It is therefore important to prevent the contamination of fresh produce with *Clostridium* species.

Yersinia enterocolitica

It is one of the most alarming pathogens since it can grow at refrigeration temperatures (Anon., 2004). It can be eliminated if proper preservation methods are used since it is sensitive to heat treatments, sodium chloride (5%), and acidity (pH 4.0). *Yersinia* related disease is recognized by symptoms such as intense abdominal pains, diarrhoea, fever and vomiting. Presumptive *Yersinia* was isolated on lettuce leaves (Johannessen *et al*., 2002) therefore, survival is possible. Investigation of multiple *Yersinia* infections in Finland reported that the consumptions of Iceberg lettuce to be the source of the infections (Jalava *et al*., 2004). Therefore, the survival of *Yersinia* can definitely lead to potential disease outbreaks.

Vibrio

There are several different *Vibrio* species implicated in disease outbreaks and they include *V. cholera, V. parahaemolyticus* and *V. vulnificus* (Anon., 2004). *Vibrio cholera* is one of the few pathogens that produce a toxin which causes the disease. These toxin-producing pathogens are deadly since the toxin is thermo-stable and can survive heat treatments. The cholera toxin results in symptoms including mild to severe diarrhoea, vomiting, dehydration and sometimes death. It has an infectious dose of 1 000 000 cells per infection and they can survive at temperatures below 10℃ and varying levels of sodium chloride.

Staphylococcus aureus

It is another bacterial pathogen that produces a toxin that causes severe gastroenteritis (Anon., 2004). The symptoms include nausea, vomiting, retching, abdominal cramps, sweating, weak pulse and shallow respiration. The cells can grow at temperatures between 35° to 47.5℃ and many produce toxins at populations exceeding 10^5 cells per g. *Staphylococcus* has also been isolated from strawberries and mushrooms (Johannessen *et al*., 2002).

Bacillus cereus

This spore forming member of the genus *Bacillus* also produces a toxin (Anon., 2004). The symptoms are first emetic with nausea and vomiting. These are then followed by diarrheal symptoms which include diarrhoea and abdominal cramps.

Listeria monocytogenes

It is a well known pathogen that causes listeriosis (Anon., 2004). The initial symptoms associated with listeriosis are mild fever, diarrhoea and malaise. The secondary symptoms are meningitis, encephalitis, endocarditis, osteomyelitis and death of foetus in the case of pregnant women. It also has been implicated in foodborne outbreak cases associated with lettuce, tomatoes and cabbage (Beuchat, 2006). *Listeria* can survive extended periods of unfavourable condition and high salt

concentrations (Stine, 2004). It can also grow at temperatures below -0.4℃ and at the pH range of 4.39 to 9.4. A study by Alegre *et al*. (2010) showed how *Listeria innocua* was able to survive and grow at a temperature of 5℃ on the surface of fresh-cut peaches. Therefore its survival at refrigeration temperatures may be depended on the environmental conditions. *Listeria monocytogenes* was also isolated from strawberries (Johannessen *et al*., 2002) therefore, contamination should be prevented to control disease outbreaks.

G. GENERAL CONCLUSIONS

It is clear from the above review that water and foodborne disease outbreaks are a serious concern. There is also evidence of an increase in the number of outbreaks associated with fresh produce. There also has been a concern with the quality of irrigation water used to irrigate fresh crops. Thus, both waterborne and foodborne pathogens have been linked by carry-over from contaminated water to fresh produce. This means that the prevention of foodborne outbreaks associated with fresh produce at-harvest can probably be managed by preventing the contamination of irrigation water sources.

It has been reported that many South African rivers are heavily polluted with faecal matter and it is clear from literature that faecal contamination is the major source of pathogens in river water. The gastroenteritis that is characteristic of foodborne outbreaks are ascribed to enteric pathogens from the intestinal tract of humans and animals as the causative agents. Thus, it can be assumed that the major sources of faecal pollution are obviously waste waters from malfunctioning wastewater treatment plants or from run-offs from nearby animal farms. In the case of the Plankenburg River, the nearby informal settlement may be the major source of faecal contamination. This is because of the lack of proper sanitation and hygiene whereby faecal waste is disposed of in stormwater drains or directly into the river. Therefore, prevention of disease outbreaks is dependent on the efficient removal of faecal waste so as not to contaminate the environment. Proper treatment processes should be applied to the waste in order to destroy the pathogens preventing them from being re-introduced to the environment since most of the treated waste water is flushed into the river systems.

It has been stated in literature that contaminated irrigation water is one of the major pre-harvest contamination sources of fresh produce. Therefore, it can be concluded that the microbial quality of irrigation water is the driving factor influencing the increase in disease outbreaks associated with fresh produce. Furthermore, it can be said that the microbial quality of irrigation water determines the microbial quality of fresh produce. Although legislation is in place to ensure food safety of fresh produce, the guidelines being used are unrealistic for many of the developing countries that have a high level of faecal pollution of rivers. More research needs to be done to accurately measure the food safety of fresh produce in relation to the level of contamination in irrigation water sources.

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CHAPTER 3

MICROBIAL BASELINE STUDY OF SELECTED SITES ON THE PLANKENBURG AND EERSTE RIVERS

SUMMARY

Fresh produce is recommended as part of a healthy diet to help prevent illness such as cardiovascular diseases. Studies have linked fresh produce including lettuce to foodborne outbreaks and have shown that polluted irrigation water can be a major source of contamination. Thus, the aim of the study was to assess the microbiological quality of the Plankenburg and Eerste Rivers for irrigation of fresh produce that will be consumed raw or undergo minimal processing. The microbiological quality tests included aerobic colony count (ACC), aerobic and anaerobic sporeformers, *Staphylococcus*, *Salmonella*, *Listeria*, enterococci, coliforms, faecal coliforms and *E. coli* using standard methods. Determination of pH, alkalinity, conductivity and chemical oxygen demand (COD) was also done on the water samples. The results indicated high levels of faecal contamination for both rivers with faecal coliform levels that exceeded both the DWAF and WHO guidelines of <1 000 per 100 mL water. The *E. coli* count for the Plankenburg River was higher (1 400 000 cfu per 100 mL water) than that for the Eerste River (79 000 cfu per 100 mL water). Intestinal *Enterococci* for the Plankenburg River was also higher (1 760 cfu per 100 mL water) than the Eerste River (350 cfu per 100 mL water). *Staphylococcus* was absent in the Eerste River but present at both the Plankenburg River sites. Aerobic and anaerobic sporeformers for both rivers were present in the summer months of December and January. There was also a high incidence of pathogens *Salmonella* and *Listeria* in both rivers. The ACC for both rivers were high (454 to 160 000 cfu per mL water). Therefore, it was concluded that both rivers, in terms of microbial loads, are unsuitable for fresh produce irrigation purposes. In contrast, the physico-chemical results showed the water to be safe as far as crop yield production is concerned. The pH, alkalinity, conductivity and COD were generally in range of the DWAF guidelines.

INTRODUCTION

An increase in the number of foodborne outbreaks has been reported (Lynch *et al.*, 2009). One of the driving forces leading to the increase in foodborne outbreaks is the increase in consumption of fresh produce (Ells & Hansen, 2006; Quested *et al.*, 2010). Contaminated tomatoes and leafy green vegetables including lettuce and spinach are reported to be responsible for most of the disease outbreaks related to fresh produce (Abadias *et al.*, 2007; Pu, 2010). The causative agents of these outbreaks include *Salmonella*, *Listeria* and *Escherichia*. Many of the species of these genera are considered to be serious human pathogens (Sela & Fallik, 2009). They are mostly

present as part of the microbial community of faecal matter of humans and other warm blooded animals such as farm cattle (Harris *et al*, 2003; Ateba & Bezuidenhout, 2008).

One of the major sources of contamination of fresh produce has been shown to be polluted irrigation water (Griesel & Jagals, 2002; Islam *et al.*, 2005; Johnston *et al.*, 2005; Abong'o & Momba, 2008). On a national level little was known about the level of pollution and specifically faecal contamination of South African rivers that are tapped for irrigation purposes. To get a clearer understanding of the problem, a national study was started in 2007 by the South African Water Research Commission (Backeberg, 2006) to determine the extent of the microbial contamination level of the river water used for irrigation and the carry-over of contaminants to fresh produce. The data obtained so far showed that most of the irrigation samples from South African rivers and irrigated produce evaluated had faecal coliforms and *E. coli* counts higher than the WHO and DWAF guidelines (Lötter, 2010). Many other potential pathogens including *Salmonella, Staphylococcus, Listeria, Vibrio* and intestinal enterococci were also reported to be present (Keshav *et al.*, 2010). The Department of Water Affairs and Forestry (DWAF) stated that there is a risk of a disease outbreak if faecal coliform counts in irrigation water exceeds 1 000 cfu per 100 mL water (DWAF, 1996). The World Health Organisation has a similar guideline of 1 000 faecal coliforms per 100 mL water for the irrigation of fresh produce consumed raw (WHO, 1989).

The Plankenburg River, passing through the town of Stellenbosch, is used by farmers in the surrounding areas for irrigation of fresh produce such as beans, strawberry, pears and grapes (Lötter, 2010) during the summer months. This is mainly because the water from the river is readily available and easily accessible. The Plankenburg River has in the past been reported to be highly contaminated with faecal matter. For the period May 1998 to February 2003, the river was reported to have a high faecal coliform count of 1 200 000 *E. coli* per 100 mL (Barnes & Taylor, 2004). In June 2004 to June 2005, faecal coliform counts as high as 3 600 000 *E. coli* per 100 mL was reported by Paulse *et al.* (2009). In September 2007 to February 2008, Lötter (2010) reported faecal coliform counts as high as 460 000 *E. coli* per 100 mL water. A high faecal coliform count of 160 000 *E. coli* per 100 mL water was reported by Ackermann (2010) for the period of September 2007 to September 2008. Therefore, according to the DWAF and WHO guidelines previous results of studies done on the Plankenburg River indicate that the water is microbiologically unsuitable for irrigation of fresh produce to be consumed raw.

The objective of this study was to continue monitoring the microbial quality of the Plankenburg and Eerste Rivers for 15 months (Jan 2009 to May 2010) as part of the ongoing Water Research Commission project study on the extent of contamination found in this specific river water at three selected river sites.

MATERIALS AND METHODS

Sampling sites

Two local rivers were chosen for sampling purposes. The first river is the Plankenburg River which runs along an informal settlement and it will be referred to as Plank-1 and Plank-3 sampling sites. The second river is the Eerste River (Jonkershoek river section) which runs along a residential area and it will be referred to as Eerste-1 (Fig. 1).

Plank-1 – This sampling site is located along the Plankenburg River just below the Kayamandi informal settlement (Fig. 1). Previous studies have shown this site to be heavily contaminated with faecal matter (Barnes & Taylor, 2004; Paulse *et al.*, 2009; Huisamen, 2010; Lötter, 2010).

Eerste-1 – This site is situated alongside Die Boord residential area and it forms part of the Eerste River (Fig. 1). Previous studies also reported the site to have lower faecal contamination than the site for Plank-1 (Huisamen, 2010; Lötter, 2010).

Plank-3 – This site is about 2km downstream from the site for Plank-1 (Fig. 1). It is situated at the confluence point between the Plankenburg and Eerste Rivers. It is also an irrigation source point where farmers draw their water for irrigation of crops. The fresh produce irrigated includes grapes, citrus fruit, pears and green beans (Lötter, 2010).

Sampling frequency

Both rivers were sampled once a month for 15 months. The sampling was done on Mondays between the hours of eight and nine in the morning. The sampling of water was done according to the SANS 5667-6 (2006) guideline. One litre samples were taken near the middle of the river 30 cm below the surface facing toward the river flow direction. The samples were placed in a cooler bag filled with ice and transported back to the laboratory for analysis in duplicate within two hours after sampling.

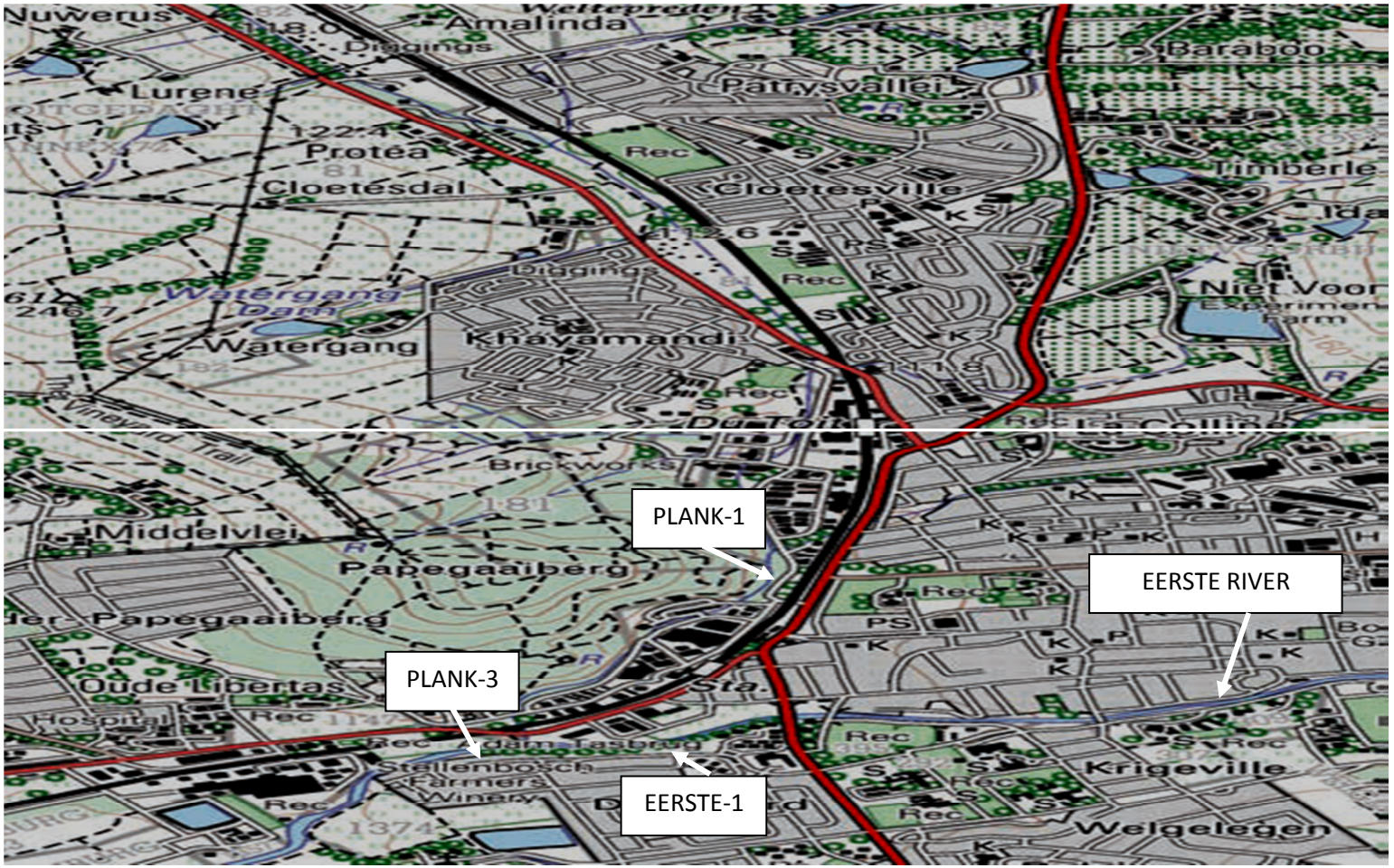

Figure 1 Location of the sampling sites for the Plankenburg (Plank-1 and Plank-3) and Eerste Rivers (Eerste-1).

Microbiological Analysis

The aerobic colony count was used to give an indication of the size of the microbial population in the water. The aerobic and anaerobic sporeformers were used to establish the presence of *Bacillus* and *Clostridium* strains. Total coliforms, faecal coliform, *E. coli* and intestinal enterococci were used as indicator organisms for faecal contamination (Busta *et al.*, 2003). The "index organisms", *Staphylococcus*, *Salmonella* and *Listeria,* were used as indicators for the possible presence of related pathogens (Busta *et al.*, 2003).

Aerobic Colony Count

A dilution series of the sampled water was prepared in sterile physiological saline solution (PSS) (APHA, 2005). Pour plates using Plate Count Agar (Merck) were prepared and after setting an additional layer of agar was poured over the set surface (SABS ISO 4833, 2007). The plates were then inverted and incubated at 30℃ for 72 h. After the incubation, plates with colonies between 30 and 300 were counted. Typical colonies were identified as cream colonies and the data reported as cfu.mL^{-1} of sample (colony forming units) (Merck, 2005).

Aerobic and anaerobic sporeformers

A dilution series of the sampled water in PSS was prepared and then placed in a 75℃ waterbath for 20 min (MFLP-44) (Health Canada, 1998). Pour plates using Trypticase Soy Agar (Merck) were prepared in duplicate and incubated at 35℃ for 48 h. A set of duplicate plates were inverted and incubated anaerobically using an AnaeroJar (Oxoid) and an AnaeroCult A strip (Merck) at 35℃ for 48 h. Typical colonies were identified as yellowish-brown coloured colonies and the data reported as cfu.mL^{-1} of sample (Merck, 2005).

Staphylococci

A dilution series of the sampled water was prepared. Set plates were also prepared (SABS ISO 6888-1, 1999) using Baird-Parker Agar (Merck) and aseptically spreading the 0.1 mL aliquot of the dilution series evenly on the surface of the plate using a sterile glass hockey stick. The plates were left to dry after which they were inverted and incubated at 35℃ for 48 h. Typical colonies were identified as being black with a clear zone and the data reported as cfu.mL^{-1} of sample (Merck, 2005).

Salmonella

The pre-enrichment and enrichment steps required for the detection of *Salmonella* species using Buffered Peptone Water (Merck), Rappaport medium (Merck) and 100 mL Selenite Cysteine medium (Merck) were conducted as described by the SABS ISO 6579 (2003) standard. Streaking

onto pre-dried Xylose Lysine Deoxycholate (Merck) plates was used after which the plates were inverted and incubated at 35℃ for 24 h. Typical colonies were identified as black colonies and the presence of *Salmonella* reported as either present or absent (Merck, 2005).

Listeria

The detection of *Listeria* (SABS ISO 11290-1, 1996) requires both a primary and secondary enrichment step using Frazer Broth (Merck). A streaking technique for the enrichment broths was used on the pre-dried Oxford Agar (Merck) and Palcam Agar (Merck) plates for the enrichment. After streaking, the plates were inverted and incubated anaerobically using an AnaeroJar (Oxoid) and AnaeroCult A strips (Merck) at 35℃ for 24-48 h. Typical colonies were identified as grey-green with a black zone and the presence of *Listeria* reported as either present or absent (Merck, 2005).

Intestinal Enterococci

A 0.45 µm membrane filter was used to filter 100 mL of sample (SANS ISO 7899-2, 2004) and the filter then aseptically placed on a pre-dried Slanetz and Bartley Agar plate (Merck). The plates were inverted and incubated at 35℃ for 44 h. After incubation, colonies were examined and the filter then placed on a Bile Esculin Azide Agar plate that had been preheated to 44℃. The plate was then incubated at 44℃ for two hours. Typical colonies were black and the data reported as cfu.100 mL^{-1} of sample (Merck, 2005).

Coliforms, Faecal coliforms and E. coli

Detection of these organisms from the sampled river water was done using the multiple tube fermentation (MTF) method (MFHPB-19) as described by Health Canada (2002) and Standard Methods (APHA, 2005). It involved the inoculation of sampled river water into double and single strength Lauryl Sulfate Tryptose (LST) Broth (Merck), Brilliant Green Lactose Bile (BGLB) Broth (Merck) and *Escherichia coli* (EC) Broth (Merck) containing 4-methylumbelliferyl-β-D-glucuronide (MUG) as per the instructions. The coliform, faecal coliform and *E. coli* counts were determined using the Most Probable Number (MPN) tables. Pre-dried Levine Eosin Methylene Blue (L-EMB) plates (Merck) were also prepared as per instruction and typical *E. coli* colonies were identified as having a metallic green sheen (Merck, 2005). The dilution factor of the positive EC-MUG tubes that resulted in typical colonies was used to determine the *E. coli* count from the MPN tables. The MTF results are expressed as MPN per 100 mL water.

Physico-Chemical Analysis

The parameters that were monitored (APHA, 2005) included temperature, pH, alkalinity, conductivity and chemical oxygen demand (COD). Conductivity was determined using a Hanna

Instruments (HI8733) conductivity meter. A DR2000 spectrophotometer (Hach Co. Loveland, CO) was used to colorimetrically determine the COD.

RESULTS AND DISCUSSION

The data collected over the 15 month sampling period for the microbiological and physico-chemical analysis of the sampled water from the Plankenburg and Eerste Rivers are discussed below. The results for each sampling site are discussed separately. The figures representing the data were generated using the Statistica9 (StatSoft) programme.

Microbiological Results

Plank-1

Results for the Plank-1 sampled water are presented in Table 1. The aerobic colony count (ACC) ranged from 454 to 116 000 cfu.mL^{-1} of water. The ACC data is representative of the total microbial content including spoilage microorganism (Merck, 2005; Sela & Fallik, 2009). Therefore a high ACC content in water could lead to spoilage of fresh produce irrigated.

Aerobic sporeformers were only detected in December 2009 at a level of 22 400 cfu.mL^{-1}. This is an indication that aerobic sporeformers such as *Bacillus* species were sometimes present in the water. Anaerobic sporeformers were only detected in July and December 2009 and the counts were 52 and 12 320 cfu.mL^{-1} respectively. Therefore, it was then concluded that anaerobic sporeformers such as *Clostridium* species were present in the water. Both *Bacillus cereus* and *Clostridium botulinum* are known foodborne pathogens (Kim *et al.*, 2010; Harris *et al.*, 2003) and irrigation with water containing them could lead to colonization and formation of biofilms on the surface of fresh produce (Niemera & Cook, 2010). This could possibly result in disease outbreaks or faster spoilage of the fresh produce.

The highest count for staphylococci was 430 cfu.mL^{-1} in April 2010. Typical colonies on the Baird-Parker plates were identified as round black colonies with a clear zone (Merck, 2005). The data could also be an indication that Enterotoxin-producing *Staphylococcus aureus* strains that can cause food poisoning may possibly be present in the water (Cha *et al.*, 2006). The intestinal enterococci count ranged from 31 to 1 760 cfu.mL^{-1} with typical colonies having a tan to black colouration (Merck, 2005). *Enterococcus faecalis* strains are generally used as indicators for faecal contamination (Agudelo *et al.*, 2010) and this additionally indicated faecal contamination of the water.

Table 1 Microbiological results (average of duplicates) of river water samples taken from the Plank-1 site for the period March 2009 to May 2010.

Date	**Aerobic Colony Count** (cfu.mL^{-1})	**Aerobic Sporeformers** (cfu.mL^{-1})	**Anaerobic Sporeformers** (cfu.mL^{-1})	**Staphylococcus** (cfu.mL^{-1})	**Salmonella**	**Listeria**	**Enterococci** (cfu.100 mL^{-1})	**Coliforms** (MPN.100 mL^{-1})	**Faecal Coliform** (MPN.100 mL^{-1})
Mar-09	46 000	ND	ND	ND	ND	ND	198	13 000	2 300
Apr-09	28 200	ND	ND	ND	TG	TG	180	1 400 000	1 400 000
May-09	3 200	ND	ND	ND	ND	ND	259	23 000	2 300
Jun-09	1 930	ND	ND	ND	ND	ND	270	1 300	350
Jul-09	116 000	ND	52	ND	TG	TG	145	230 000	45
Aug-09	454	ND	ND	ND	TG	TG	376	1 300 000	330 000
Sep-09	1 276	ND	ND	ND	TG	ND	31	79 000	49 000
Oct-09	93 000	ND	ND	ND	ND	TG	61	1 700 000	1 300 000
Nov-09	4 600	ND	ND	ND	TG	TG	318	79 000	49 000
Dec-09	1 870	22 400	12 320	135	TG	ND	ND	330 000	79 000
Jan-10	5 900	ND	ND	ND	ND	ND	188	230 000	49 000
Feb-10	8 820	ND	ND	ND	TG	ND	560	13 000 000	170 000
Mar-10	103 000	ND	ND	ND	ND	TG	ND	130 000	79 000
Apr-10	32 400	ND	ND	430	TG	TG	1 760	6 400 000	280 000
May-10	8 960	ND	ND	ND	TG	TG	ND	79 000	640

ND = Not Detected; TG = Typical Growth; MPN = Most Probable Number; cfu = colony forming units

Salmonella and *Listeria* were present in the water of 60% and 53% of the samples, respectively, over the 15 month sampling period. Typical *Salmonella* colonies were identified as colourless with a black centre and those for *Listeria* were identified as having a greyish colouration surrounded by a black zone on their respective media (Merck, 2005). Both pathogens are considered as food safety hazards (Mead *et al.*, 1999). Their presence in the water also confirms faecal contamination as source of pollution since both pathogens generally occur in faecal matter of warm blooded animals or infected humans (Griff *et al.*, 2003; Sauders *et al.*, 2005; Renter *et al.*, 2006). The presence of index organisms indicating possible presence of related pathogens was also reported in previous studies done by Lötter (2010). This may also suggest that the survival of pathogens is possible in flowing streams. Therefore continuous faecal contamination of the river water may lead to a possible build up of related disease causing foodborne pathogens in the water.

The coliform count (Table 1) ranged from 1 300 to 1 700 000 MPN.100 mL^{-1} of water and the faecal coliform count ranged from 45 to 1 400 000 MPN.100 mL^{-1} of water. The faecal coliforms were confirmed as typical *E. coli* on L-EMB plates (Merck, 2005). Therefore the maximum *E. coli* count was also considered to be 1 400 000 MPN.100 mL^{-1} of water. According to the DWAF guideline (<1 000 faecal coliform per 100 mL), the water can be considered as unsuitable for irrigation of fresh produce intended to be consumed raw (DWAF, 1996). The high *E. coli* counts also suggest high faecal contamination of the water at this particular sampling site (Plank-1) which is consistent with previous studies done (Barnes & Taylor, 2004; Paulse *et al.*, 2009; Lötter, 2010). The detection of *E. coli* is indicative of animal or human faecal pollution sources (Busta *et al.*, 2003). Thus, nearby informal settlements, local industries, animal farms and slaughterhouses may possibly have been the pollution sources.

When taking this study's maximum faecal coliform loads into consideration, there appears to be a general decrease in the faecal contamination of the Plankenburg River since Barnes & Taylor in 2004 reported a maximum *E. coli* count of 1 200 000 MPN.100 mL^{-1} Plankenburg water for the year 2000. Similarly, for the year 2005, Paulse *et al.* (2009) reported an *E. coli* count of 3 600 000 MPN.100 mL^{-1} water for the Plankenburg River at the same sampling site and in 2007, Lötter (2010) reported a maximum *E. coli* count of 1 600 000 MPN.100 mL^{-1} water for the same site.

This general decrease in *E. coli* could be as a result of dilution factors, decay rates, biofilm formation or even the settling and retention of organisms in the sediment, as has been shown to be possible in flowing streams (Dukta & Kwan, 1980). Furthermore, the decrease in *E. coli* could also be due to a decrease in contamination from the nearby settlement. Even though the counts are lower than that reported in previous studies (Barnes & Taylor, 2004; Paulse *et al.*, 2009; Ackermann, 2010; Lötter, 2010), when using the DWAF and WHO guidelines as basis it is still not suitable for irrigation of fresh produce and poses a serious food safety and health hazard.

In this study, as shown in Fig. 2, higher *E. coli* counts of up to 1 400 000 MPN.100 mL^{-1} were found during the dryer months of March/April and the lower *E. coli* count of 45 MPN.100 mL^{-1} of water

during the wetter and colder months of June/July. Similar profile patterns were reported by Ackermann (2010) whereby a high *E. coli* count of 160 000 MPN.100 mL^{-1} water was reported in the dry month of December 2007. Thus, seasonal changes may possibly be impacting the *E. coli* load. But it must also be taken into consideration that the lower *E. coli* counts during the winter months could be as a direct result of dilution through increased rainfall.

However, the opposite could be true since higher flow rates associated with heavy rainfall could also lead to higher *E. coli* counts due to the re-suspension of bacteria from the river sediments (Dukta & Kwan, 1980). The study of Ackermann (2010) reported a high *E. coli* count of 160 000 MPN.100 mL^{-1} water in August 2008 following heavy rainfall at the end of July 2008. Therefore rainfall and river flow rates may impact the *E. coli* load through dilution and re-suspension resulting in seasonal variation profiles as presented in Fig. 2.

Eerste-1

The ACC from the Eerste-1 site ranged from 39 to 98 700 cfu.mL^{-1} of water (Table 2). The aerobic sporeformers was detected only in Mrarch 2010 and the count was 21 400 cfu.mL^{-1}. Anaerobic sporeformers were only detected in December with a count of 390 cfu.mL^{-1}. *Staphylococcus* was not detected at site Eerste-1 in any samples over the 15 month sampling period (Table 2). The count for the intestinal enterococci ranged from 97 to 222 cfu.100 mL^{-1} water. The presence of index organisms suggests that potential pathogens like *Enterococcus faecalis* may possibly be present in the Eerste River. Their presence in the water could pose a health and food safety hazard if the water is used to irrigate fresh produce intended to be consumed raw since pathogens are known to have the ability to attach to plant tissue (Solomon & Sharma, 2009).

Salmonella and *Listeria* were present in 73% and 27% of the samples, respectively, over the 15 month sampling period (Table 2). The high *Salmonella* incidence in the river could pose a health issue since some of the Die Boord residences draw water from the river for gardening and recreational purposes. Therefore, contact with the water could lead to a serious health hazard. The high incidence of index organisms suggests the possible survival and build-up of related foodborne pathogens resulting from continuous faecal contamination of the river in previous years. Thus regular monitoring of our river systems is critical in preventing possible disease outbreaks since members of the genus *Salmonella* are food pathogens that have been associated with many disease outbreaks (Newell *et al*., 2010).

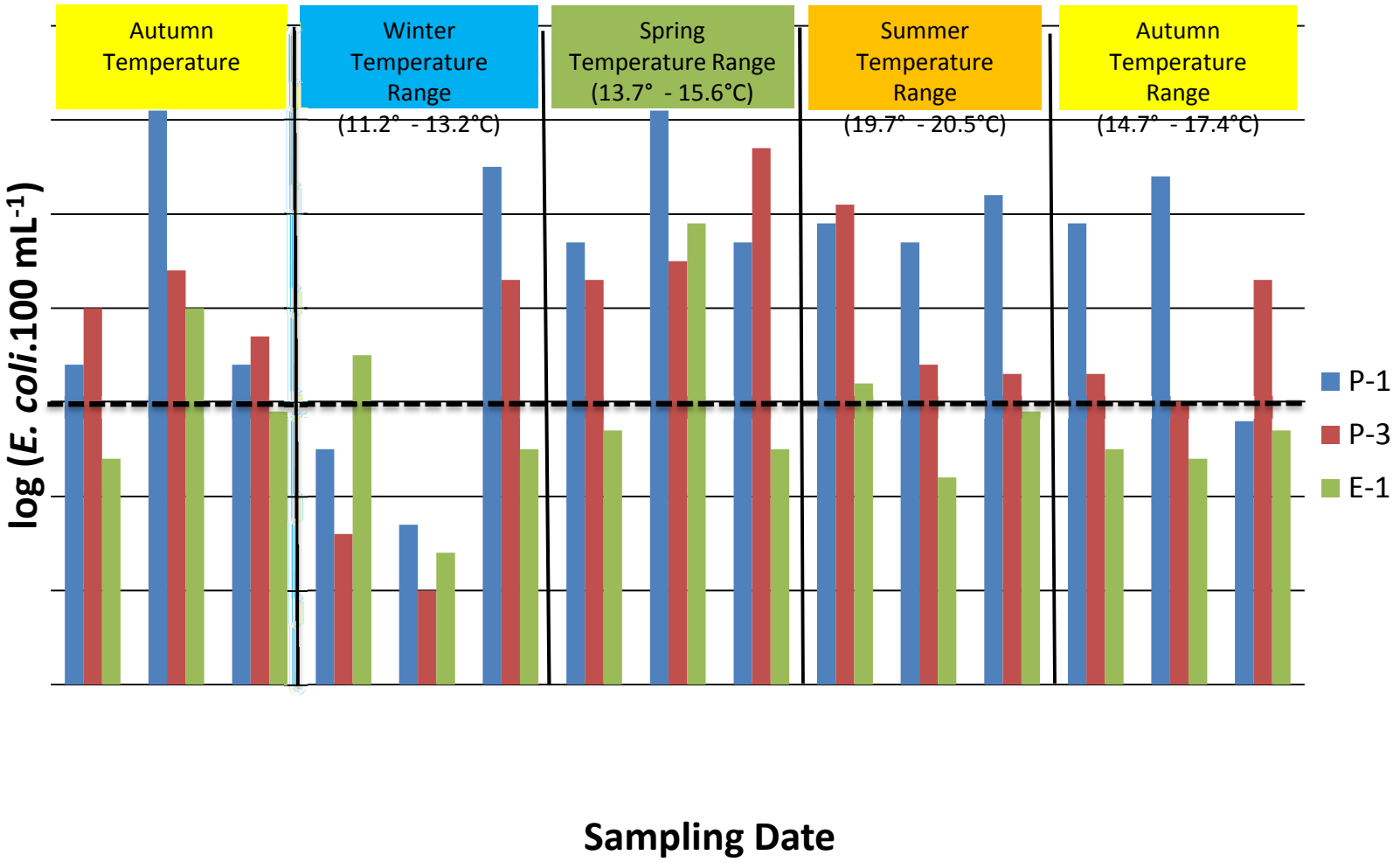

Figure 2 The *E.coli* loads in samples from Plank-1 (P-1), Plank-3 (P-3) and Eerste-1 (E-1). The dotted line represents the DWAF and WHO guidelines (1 000 *E. coli* 100 mL^{-1} water). Temperature ranges are based on data from Plank-1 and Plank-3 sites.

Table 2 Microbiological results (average of duplicates) of river water samples taken from the Eerste-1 site for the period March 2009 to May 2010.

Date	**Aerobic Colony Count** (cfu.mL^{-1})	**Aerobic Sporeformers** (cfu.mL^{-1})	**Anaerobic Sporeformers** (cfu.mL^{-1})	**Staphylococcus** (cfu.mL^{-1})	**Salmonella**	**Listeria**	**Enterococci** (cfu.100 mL^{-1})	**Coliforms** (MPN.100 mL^{-1})	**Faecal Coliform** (MPN.100 mL^{-1})
Mar-09	3 000	ND	ND	ND	TG	ND	169	230	230
Apr-09	22 600	ND	ND	ND	TG	ND	97	11 000	11 000
May-09	650	ND	ND	ND	TG	ND	107	790	790
Jun-09	3 160	ND	ND	ND	TG	ND	168	4 900	3 300
Jul-09	16 600	ND	ND	ND	TG	TG	ND	7 900	28
Aug-09	113	ND	ND	ND	TG	TG	ND	13 000	350
Sep-09	450	ND	ND	ND	TG	ND	ND	490	490
Oct-09	61 000	ND	ND	ND	TG	TG	ND	79 000	79 000
Nov-09	460	ND	ND	ND	TG	ND	ND	330	330
Dec-09	560	ND	390	ND	ND	ND	ND	4 900	1 700
Jan-10	39	ND	ND	ND	ND	ND	210	6 400	170
Feb-10	1 770	ND	ND	ND	ND	ND	ND	2 200	790
Mar-10	98 700	21 400	ND	ND	TG	ND	ND	3 300	330
Apr-10	2 830	ND	ND	ND	ND	ND	ND	3 100	230
May-10	190	ND	ND	ND	TG	TG	222	4 900	490

ND = Not Detected; TG = Typical Growth; MPN = Most Probable Number; cfu = colony forming units

The results for Eerste-1 (Table 2 and Fig. 2) showed lower faecal coliform loads than that found for Plank-1. The coliform counts were found to vary from 230 to 79 000 MPN.100mL^{-1} water. The faecal coliform ranged from 28 to 79 000 MPN.100 mL^{-1} water (Table 2) and the *E. coli* count (Fig. 2) reached a high of 79 000 MPN.100 mL^{-1} water which was lower than that of Plank-1 and Plank-3. Therefore it is possible that the Eerste River may have had a dilution effect to the Plankenburg River at the Plank-3 sampling site resulting in lower *E. coli* counts than found for the Plank-1 site. Although the *E. coli* counts were low in the Eerste-1 samples compared to Plank-1, in many cases they still exceeded the DWAF and WHO guidelines therefore it was concluded the Eerste River still poses a health and food safety hazard if used for irrigation of fresh produce intended to be consumed raw.

Plank-3

This site is situated about 2 kilometres downstream from the Plank-1 sampling site and is also a confluence point for the Plankenburg and Eerste Rivers. It is also an irrigation source point for nearby farmers. The ACC ranged from 676 to 240 000 cfu.mL^{-1} water (Table 3). Only two months showed counts for the aerobic and anaerobic sporeformers with 20 800 and 9 600 cfu.mL^{-1} water, respectively. Thus foodborne endospore forming pathogens (*Bacillus cereus* and *Clostridium botulinum)* may be present in the water from this site. It is interesting to note that these counts occurred during the warmer months of December 2009 and January 2010 (Table 3). The results are consistent with the previous study done by Ackermann (2010) where a high ACC of 150 000 cfu.mL^{-1} water was also reported for December 2007. Anaerobic and aerobic endospore formers were also reported for the warmer months of December 2006 and January 2007. The data suggests that the high temperatures over the summer months could possibly have an important impact on microbial loads in terms of growth. This was also shown in a study by Dukta & Kwan (1980).

No members of the genus *Staphylococcus* were detected at this site over the sampling period. This could suggest possible bacterial decay, dilution or settling between Plank-1 and Plank-3 considering the fact that *Staphylococcus* was detected at Plank-1. Counts for the intestinal enterococci ranged from 42 to 350 cfu.100 mL^{-1} water. This could indicate the presence of possible *Enterococcus faecalis* in the water.

The *Salmonella* and *Listeria* had a presence incidence of 53% and 47%, respectively, over the 15 month sampling period. The presence of these two index organisms poses a food safety hazard as they are indicative of the possible presence of related foodborne pathogens. Therefore possible contamination of fresh produce during irrigation can occur resulting in disease outbreaks.

Table 3 Microbiological results (average of duplicates) of river water samples from the Plank-3 site for the period March 2009 to May 2010.

Date	**Aerobic Colony Count** (cfu.mL^{-1})	**Aerobic Sporeformers** (cfu.mL^{-1})	**Anaerobic Sporeformers** (cfu.mL^{-1})	**Staphylococcus** (cfu.mL^{-1})	**Salmonella**	**Listeria**	**Enterococci** (cfu.100 mL^{-1})	**Coliforms** (MPN.100 mL^{-1})	**Faecal Coliform** (MPN.100 mL^{-1})
Mar-09	30 500	ND	ND	ND	ND	ND	42	9 500	9 500
Apr-09	87 200	ND	ND	ND	ND	ND	157	350 000	24 000
May-09	4 300	ND	ND	ND	TG	ND	185	4 900	4 900
Jun-09	8 900	ND	ND	ND	TG	TG	ND	4 900	36
Jul-09	11 900	ND	ND	ND	ND	TG	147	7 900	10
Aug-09	1 776	ND	ND	ND	TG	ND	43	790 000	21 000
Sep-09	676	ND	ND	ND	TG	TG	113	49 000	22 000
Oct-09	61 000	ND	ND	ND	TG	TG	ND	33 000	33 000
Nov-09	101 100	ND	ND	ND	TG	TG	ND	2 800 000	460 000
Dec-09	9 600	20 800	7 600	ND	ND	ND	350	230 000	130 000
Jan-10	12 500	19 500	9 600	ND	ND	ND	224	220 000	2 300
Feb-10	6 766	ND	ND	ND	TG	TG	ND	110 000	2 100
Mar-10	241 200	ND	ND	ND	ND	ND	ND	33 000	1 800
Apr-10	6 215	ND	ND	ND	ND	ND	245	33 000	1 100
May-10	81 400	ND	ND	ND	TG	TG	244	49 000	22 000

ND = Not Detected; TG = Typical Growth; MPN = Most Probable Number; cfu = colony forming units

The coliform counts ranged from 4 900 to 2 800 000 MPN.100 mL^{-1} water (Table 3). The faecal coliform count ranged from 10 to 460 000 MPN.100 mL^{-1} water (Table 3) and these were also confirmed as *E. coli* using the L-EMB plates. The highest *E. coli* count was 460 000 MPN.100 mL^{-1} water (Fig. 2).

The data shows that Plank-3 had generally lower counts for coliforms, faecal coliforms and *E. coli* compared to Plank-1 (Tables 1 and 3). Like the S*taphylococcus* results, the data could suggest a possible bacterial decay Dukta & Kwan (1980) or settling between Plank-1 and Plank-3. The study by (Dukta & Kwan,1980) showed that *Enterobacteriaceae* could survive and possibly multiply for a minimum period of eight days in a flowing stream. Ackermann (2010) also reported similar results suggesting possible decay or settling. The lower counts could also possibly be due to the dilution from the less contaminated Eerste-1 river water. Although the counts are lower than for Plank-1, irrigating with the water could still pose a food safety hazard according to DWAF and WHO guidelines (Fig. 2). In terms of temperature and *E. coli* loads, there seems to be no clear patterns with exceptions of the winter months.

Physico-Chemical Analysis

The results for the physico-chemical properties (temperature, pH, alkalinity, conductivity and COD) for Plank-1 and Plank-3 are presented in Table 4 and that for the Eerste-1 site in Table 5. The results will be discussed accordingly with Plank-1 and Plank-3 representing the Plankenburg River and Eerste-1 the Eerste River.

For this study the following parameter definitions were applied: The pH of the water measures the amount of dissolved hydrogen ions (DWAF, 1996); Alkalinity was taken as the measure of dissolved carbonates which is the measure of the rivers ability to resist changes in pH values (DWAF, 1996); The COD value was taken as the amount of oxidizable matter in the water susceptible to oxidation using a strong chemical oxidant (APHA, 2005); and conductivity is the measure of dissolved ions such as Na^+ and Cl^- which can conduct electricity (DWAF, 1996). There are DWAF guidelines for good quality water in terms of the physico-chemical properties (DWAF, 1996).

Plankenburg River

The alkalinity of the river water at both Plank-1 and Plank-3 ranged from 25 to 2 000 $mg.L^{-1}$ $CaCO_3$; the COD ranged from <5 to 421 $mg.L^{-1}$; the conductivity from 1 to 1 000 $mS.m^{-1}$ and the temperature from a low of 11.2℃ to a high of 20.5℃. In 80% of the sampling period the temperature data also reflects the effect that air temperature has on water temperature since the lower (11.2℃) and higher (20.5℃) temperatures occurred, respectively in the winter (July) and summer (February) months.

The COD values for the Plank-1 and Plank-3 were below within DWAF guidelines of <30 mg. L^{-1} $CaCO_3$ (DWAF, 1996) for 80% and 67% of the sampling period, respectively which means that in these instances the water was safe for irrigation purposes.

Table 4 The physico-chemical data (average of duplicates) for samples from the Plank-1 and Plank-3 sites.

Date	Temperature (℃)		pH		Alkalinity ($mg.L^{-1}$ as $CaCO_3$)		Conductivity ($mS.m^{-1}$)		COD ($mg.L^{-1}$)	
	P-1	**P-3**	**P-1**	**P-3**	**P-1**	**P-3**	**P-1**	**P-3**	**P-1**	**P-3**
Mar-09	16.6	17.6	7.14	6.57	750	750	4	24	69	121
Apr-09	15.3	15.6	6.65	6.39	25	500	20	31	<5	<5
May-09	15.0	15.3	6.78	6.90	125	1000	3	49	<5	38
Jun-09	12.3	12.6	6.87	7.06	750	875	42	43	<5	<5
Jul-09	12.1	11.2	6.16	5.77	2000	250	3	2	54	6
Aug-09	13.1	13.2	6.66	6.66	750	500	40	38	<5	<5
Sep-09	13.7	13.7	6.34	6.56	500	750	2	2	13	173
Oct-09	14.8	14.6	6.43	6.25	375	125	18	9	161	121
Nov-09	15.6	15.4	6.40	6.23	750	750	36	33	<5	<5
Dec-09	19.9	20.2	6.70	6.96	1000	500	3	33	<5	<5
Jan-10	20.4	19.7	6.75	6.85	1000	875	4	41	<5	<5
Feb-10	19.9	20.5	6.90	6.69	1125	35	55	1000	<5	421
Mar-10	17.4	17.0	7.17	7.01	1000	425	30	31	<5	<5
Apr-10	15.0	15.4	6.95	6.69	1000	625	4	1	<5	<5
May-10	14.7	15.0	7.01	6.95	750	750	3	40	<5	<5

The pH ranged from 5.77 to 7.17 and was within DWAF's Target Water Quality Range (TWQR) of 6.0 to 9.0 for pH (DWAF, 1996). The 1 000 $mS.m^{-1}$ which was outside DWAF's TWQR of 0 to 70 $mS.m^{-1}$ occurred only once in February 2009 (Table 4). Therefore it can be concluded that according to physico-chemical DWAF guidelines the Plankenburg River water will have no adverse effect on the crop yield production and based on this the water can be used for irrigation of fresh produce.

Eerste River

The alkalinity for the Eerste-1 samples ranged from 25 to 250 mL^{-1} $CaCO_3$ (Table 5). The COD ranged from 47 to 704 $mg.L^{-1}$ with 20% being above the DWAF guidelines. The conductivity ranged from 2 to 11 $mS.m^{-1}$ and is within DWAF's TWQR of 0 to 70 $mS.m^{-1}$ for conductivity (DWAF, 1996). The temperature data was similar to that for the Plankenburg and ranged from 11.2℃ to 20.9℃ (Tables 4 and 5). The pH ranged from 5.63 to 6.93 and was within DWAF's TWQR pH range of 6.0 to 9.0 (DWAF, 1996). Therefore because both pH and conductivity were within DWAF guidelines it can be concluded that irrigation of fresh produce with water from the Eerste-1 source will not reduce the crop yield production (DWAF, 1996).

CONCLUSIONS

The high aerobic colony counts for both the Plankenburg and Eerste Rivers indicate a high level of contamination and the possibility of potential pathogens being present in the water. The isolation of the indicator organism *E. coli* from the water confirms the type of pollution as being faecal. The presence of the index organism intestinal *Enterococci* also confirm the source of pollution as faecal. The fact that Plank-1 sampling site is downstream of an informal settlement confirms the pollution source as faecal. Therefore there is a possibility that enteric pathogens that may cause disease outbreaks are present in the water.

The high incidence of the index organisms *(Salmonella* and *Listeria)* confirms the presence of possible related enteric pathogens. The enumeration of aerobic and anaerobic also confirms the presence of possible pathogens such as *Bacillus cereus* and *Clostridium botulinum* which are known foodborne pathogens.

The data from this study clearly shows that the Plankenburg River was more polluted than the Eerste River as sampling sites Plank-1 and Plank-3 had higher *E. coli* loads than site Eerste-1. Intestinal *Enterococci* loads were also higher for the Plankenburg sites than for the Eerste River site. Furthermore *Staphylococcus* was absent from the Eerste River but present in the Plankenburg River.

Table 5 The physico-chemical data (average of duplicates) for the samples from the Eerste-1 site.

Date	Temperature (℃)	pH	Alkalinity ($mg.L^{-1}$ as $CaCO_3$)	Conductivity ($mS.m^{-1}$)	COD ($mg.L^{-1}$)
Mar-09	18.1	6.23	250	9	47
Apr-09	14.9	6.05	25	11	<5
May-09	15.0	6.74	125	11	<5
Jun-09	12.6	6.59	125	8	<5
Jul-09	11.2	5.65	250	2	<5
Aug-09	13.2	5.95	125	9	<5
Sep-09	13.3	6.22	125	7	<5
Oct-09	14.6	6.12	125	7	704
Nov-09	14.4	5.63	125	9	<5
Dec-09	20.5	6.48	125	9	<5
Jan-10	20.9	6.16	125	8	<5
Feb-10	20.8	6.07	125	7	295
Mar-10	18.4	6.93	125	9	<5
Apr-10	15.2	6.40	250	9	<5
May-10	15.2	6.79	125	8	<5

The water from both Plankenburg and Eerste Rivers join at the Plank-3 sampling site which would explain why it had lower *E. coli* loads than Plank-1. Although the Eerste River had lower *E. coli* loads, both rivers still exceeded DWAF and WHO guidelines of 1 000 cfu.100 mL^{-1} water (WHO, 1989; DWAF, 1996).

The high *E. coli* loads together with the high incidence of index organisms suggests both rivers to be highly contaminated with potential pathogens that can be transferred to crops through irrigation. Thus, it can be concluded according to the results and guidelines that both the Plankenburg and Eerste Rivers pose a health and food safety risk.

The data showed a large variation which suggests that there is a possibility that the water is not always highly contaminated. This also means that there is a possibility that during a single month the river may have low *E. coli* loads that are within guidelines making the river water suitable for irrigation of fresh produce intended to be consumed raw. Therefore the aim of the next research chapter was to assess the weekly, daily and hourly variation profiles for the faecal contamination of the Plankenburg River.

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CHAPTER 4

ASSESSMENT OF THE PLANKENBURG RIVER (SITE PLANK-3) FOR VARIATION IN MICROBIAL LOADS AS INDICATORS OF THE FAECAL CONTAMINATION

SUMMARY

In the previous study, monthly sampling of the Plankenburg and Eerste Rivers over a year period showed a wide load range variation in terms of faecal contamination, which could have lead to over or underestimations of the actual faecal contamination. Therefore, the aim of the study was to identify variation trends in faecal contamination at the Plank-3 site of the Plankenburg River. The Total coliforms (TC) and *E. coli* (EC) loads, and temperature and pH were monitored on samples from the Plank-3 site. The river was sampled every second week for eleven weeks from March to June 2010. Duplicate water samples (1 L) were collected every two hours over a twelve hour period every second day (excluding Saturdays) starting at 06h00 in the morning ending 18h00 in the evening. The weekly variation trend for TC showed a decrease over the entire sampling period with the highest count of 3 200 000 MPN.100 mL^{-1} during March. The EC counts showed a similar trend with the highest count of 440 000 MPN.100 mL^{-1} also in March. The daily variation trends were the same for both the TC and EC. The counts were found to increase from Monday to Thursday followed by a decrease to Sunday. The highest counts were on Thursday with average TC and EC counts of 1 900 000 and 160 000 MPN.100 mL^{-1}, respectively. The hourly variation trends were similar for both TC and EC with counts increasing from 06h00 to 12h00 followed by a decrease to 18h00. The weekly variation trend for the water temperature showed a decrease over the sampling period. The hourly variation trend showed an increase from 06h00 to 18h00. The trend for pH was the opposite with an increase over the sampling period. The analysis of covariance showed no correlation ($p < 0.05$) between the physico-chemical (temperature and pH) and the microbial variables (TC and EC). Therefore, it was concluded that temperature and pH had no impact on the both the total coliform and *E. coli* counts.

INTRODUCTION

South Africa is a developing country with 52% of its rivers used for the country's agricultural activities. Many of the rivers are considered to be the main source of water for irrigation purposes (Backeberg & Odendaal, 1998; Dalvie *et al.*, 2004). Previous studies have shown that many of the South African rivers have high faecal coliform loads (Obi *et al.*, 2002; Fatoki *et al.*, 2003; Germs *et al.*, 2004, Zamxaka *et al.*, 2004, Ackerman, 2010). It is known that polluted irrigation water leads to

carry-over of potential pathogens to crops being irrigated. This leads to waterborne pathogens entering the food chain.

Certain of the potential waterborne pathogens (DWAF, 1996a) have the ability to either adhere to or even penetrate fresh produce and subsequently lead to the infection of the consumers. A study by Chai *et al.* (2007) reported a 73% survival of *Campylobacter jejuni* on fresh vegetables irrigated with contaminated irrigation water. Other foodborne pathogens have been detected on fresh produce including lettuce, melon, seed sprouts, berries and apples (Sela & Fallik, 2009) that had been irrigated with contaminated water. It has been reported that once the pathogens have reached the fresh produce they can survive washing and disinfection post-harvest treatments (Frankel *et al*., 2009). More resistant strains of *E. coli* O157:H7 isolated from romaine lettuce and baby spinach were found to be able to survive irradiation and sodium hypochlorite washes. This was ascribed to their ability to form biofilms wherein they are protected from unfavourable environmental stresses (Niemera & Cook, 2010).

The foodborne pathogens *E. coli*, *Salmonella* and *Listeria* are known to be able to survive on fruit and vegetables and even proliferate in low pH and unfavourable growth temperature environments (Alegre *et al*., 2010). Therefore, contaminated fresh produce with microbial levels in excess of safe levels (DWAF, 1996b; WHO, 1989) can possibly reach and pose a risk to consumers. Prevention of pre-harvest contamination of fresh produce by irrigation water is important to the health of the consumer. Thus a safe and abundant supply of irrigation water is essential for the production of fresh produce.

The Plankenburg River in the Western Cape, South Africa is used by farmers for irrigation of fresh produce including fruit and vegetables (Ackermann, 2010). Previous studies have reported the Plankenburg River as being highly contaminated with faecal matter (Barnes & Taylor, 2004; Paulse *et al*., 2009; Lötter, 2010). In the previous chapter of this thesis (Chapter 3) a study over 15 months was done on two sites on the Plankenburg River and one on the Eerste River in order to observe microbial level variations over a longer period. Over the 15 month period faecal coliforms and *E. coli* counts were found to be present in consistently high loads varying from 10 000 to 1 400 000 MPN.100 mL^{-1}. Other indicator and index species were also found to be present.

The data from the monthly sampling as given in Chapter 3 showed a wide load range variation in terms of faecal contamination. Since sampling during the original 15 months (Chapter 3) was done on the same day of the week (Monday) and at about the same time (08h00-09h00), questions arose whether the contamination loads so far reported when sampling on a specific day and time represent an over or under-estimation of the microbial load. This led to the question as to what the daily and weekly variation in microbial load really was. In addition to this, was the fluctuation in temperature during the course of a day and what would the subsequent impact of temperature be on the faecal indicator loads. Previous studies have suggested that higher water temperatures may have a positive influence on the growth of faecal coliforms (Lötter, 2010) which

can result in higher numbers being reported. This also led to the question whether the contamination loads are that of *E. coli* or rather just other coliforms. Therefore information on the load profiles of total coliforms and *E. coli* present in polluted water is important for subsequent microbial safety of fresh produce and also for setting guidelines for the microbiological quality of irrigation water.

The aim of this study was to determine the total coliform and *E. coli* load profile variation over an eleven week period and to determine if the river water temperature and pH have an influence on the microbial load profiles.

MATERIALS AND METHODS

Sampling site

The Plank-3 sampling site along the Plankenburg River was selected because it is an irrigation source point for nearby farmers. It is also a confluence point between the Plankenburg and Eerste Rivers.

Sampling frequency

The river was sampled every second week for eleven weeks from March 2010 to June 2010. Duplicate water samples (1 L) were collected every two hours over a twelve hour period every second day (excluding Saturdays) starting at 06h00 in the morning ending 18h00 in the evening. The sampling of the water was done according to the SANS 5667-6 (2006) guideline. The samples were taken back to the lab for physico-chemical and microbiological analyses and analysed within 60 min after sampling.

Microbiological analysis

Total coliforms (TC) and E. coli (EC)

The Colilert-18 system (IDEXX Laboratories, Maine, USA) was used to determine both total coliforms and *E. coli* loads. The Colilert-18 system is more rapid and less labour intensive when compared to the Multiple Tube Fermentation (MTF) technique that was used in Chapter 3 of this thesis. Therefore, the Colilert-18 system was ideal for the enumeration of total coliforms and *E. coli* especially for the investigation of daily and hourly variation trends. A dilution series for each duplicate sample was prepared in sterile physiological saline solution (PSS). The Colilert-18 reagent was added to the dilution samples and left to completely dissolve. The 100 ml samples

were then poured into Quanti-trays (IDEXX Quanti-Tray/2000) and sealed as per the instruction manual (IDEXX). The sealed Quanti-trays were then incubated at 37°C for 18 h.

The total coliforms (TC) were enumerated by counting the number of yellow wells and *E. coli* (EC) was enumerated by counting the number of yellow (fluorescent) wells using a 365 nm UV lamp. The fluorescence is caused by the reaction between the β-glucuronidase enzyme in *E. coli* and the 4-methylumbelliferyl-β-D-glucuronide indicator (MUG) in the Colilert-18 reagent. Both the total coliforms (TC) and *E. coli* (EC) numbers were then estimated using the most probable number (MPN) chart provided by the manufacturer (IDEXX).

Physico-chemical analysis

The temperature and pH of the river samples was determined at the sampling point and the pH was determined using the microprocessor WTW320 model pH meter.

Statistical analysis

Main effect ANOVA using Statistica 9 (StatSoft) with date and time as the two effects, were determined. This was further extended to analysis of covariance where the effect of temperature and pH was also accounted for. The main purpose for doing the ANOVA's was not to determine significant differences between means, but rather to investigate mean trends over time of the day and also over subsequent days of sampling. The *r*-value (Spearman rank correlation coefficient) was used to assess if the water samples were representative duplicates in terms of the Total Coliform (TC) and *Escherichia coli* (EC) loads, and pH values (Clewer & Scarisbrick, 2001). The null hypothesis tested for all the variations was that the variables (TC, EC, water temperature and pH) stay constant over the sampling period. The *P*-value represents the null hypothesis and each estimate was calculated at a 95% confidence interval which means that the null hypothesis is rejected if the *P*-value is less than 5% (0.05).

RESULTS AND DISCUSSION

The results presented are for the Plank-3 site because this is the irrigation source point for nearby fresh produce farmers and thus the microbial quality of the water at this sampling site could have a direct impact on the food safety of the fresh produce being irrigated. The main reason for doing this study was to determine if the apparent variations in the faecal contamination load as observed in Chapter 3 during the monthly sampling of the Plankenburg River is found weekly, daily and hourly. The results for the weekly, daily and hourly variation trends in faecal contamination of the Plankenburg River are presented below.

Sampling accuracy

The sampling of the Plankenburg River was done in duplicate (2 (A and B) x 1 L samples). By sampling in duplicate it was assumed that the water in the two samples had the same variables (pH, temperature, TC and EC). In practice this assumption is not true since the river is flowing so it can thus be expected that each variable will have slightly different values. An *r*-value (Spearman rank correlation coefficient) was used to assess if the two river water samples are representative duplicates of one another in terms of the TC and EC loads, and pH values. The *r*-value indicates the closeness of the variables (TC, EC and pH) for each sample to a single value (Clewer & Scarisbrick, 2001). The closer the *r*-value is to +1, the more similar the variables values are to each other. The results of the *r*-value tests are presented in Figs. 1, 2 and 3.

The *r*-value for the three variables (TC, EC and pH) was 0.96, 0.82 and 0.97, respectively (Figs. 1, 2 and 3). The sign next to the *r*-value indicates either a positive or negative correlation between the samples. It was found that all the variables had a positive correlation and *r*-values close to +1. A positive correlation means the samples have the same characteristic and a negative correlation means the opposite. Thus, according to the results the two samples and their determined duplicates can be regarded as replicates and the variation trends generated are therefore valid and reproducible.

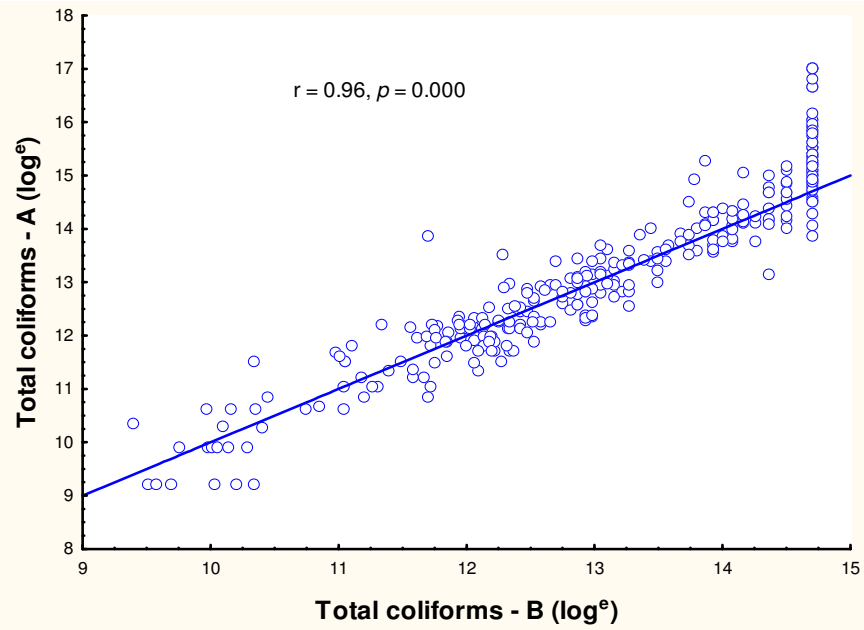

Figure 1 The analysis of covariance indicating the accuracy between the two water sample replicates (A and B) in terms of total coliform counts. The analysis was done at a significant level of 5% ($p = 0.05$).

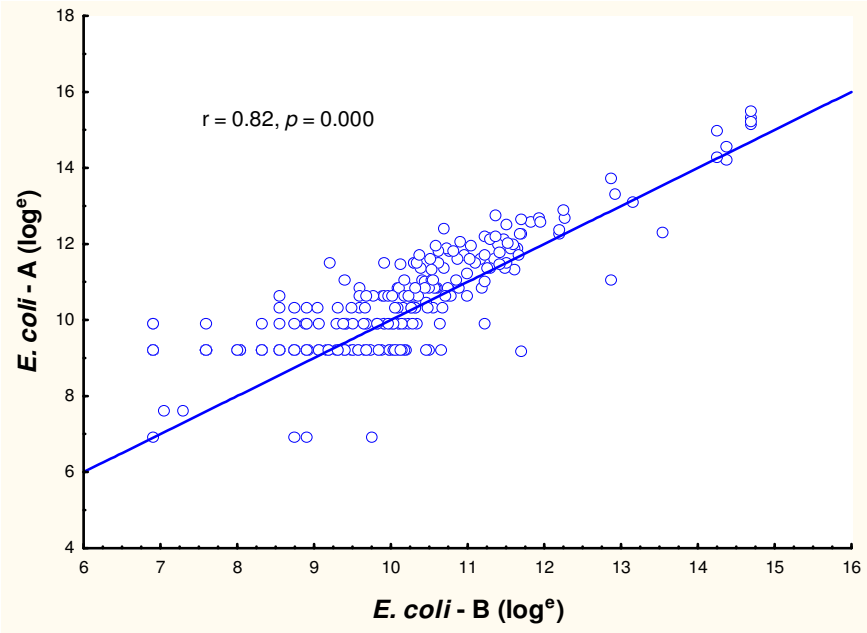

Figure 2 The analysis of covariance indicating the accuracy between the two water sample replicates (A and B) in terms of *E. coli* counts. The analysis was done at a significant level of 5% ($p = 0.05$).

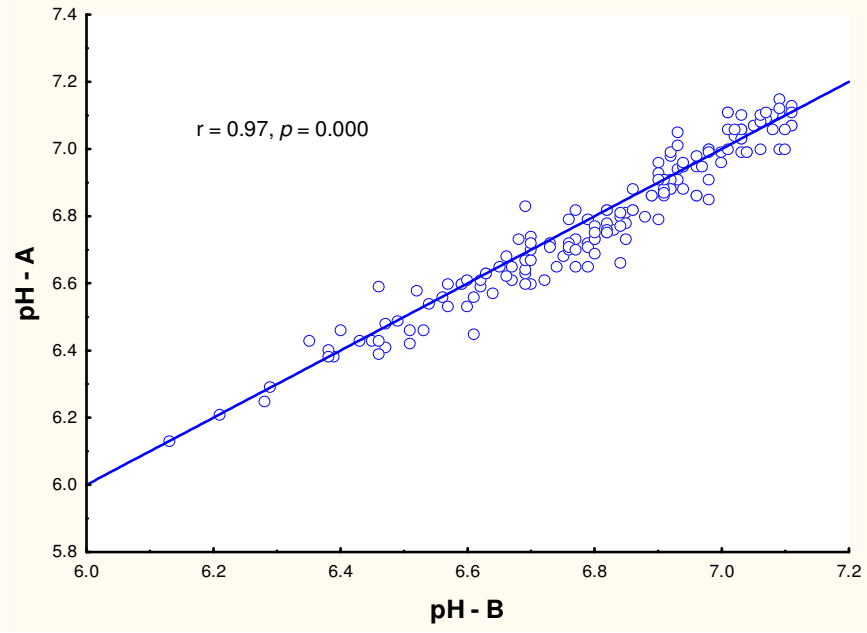

Figure 3 The analysis of covariance indicating the accuracy between the two water sample replicates (A and B) in terms of pH. The analysis was done at a significant level of 5% ($p = 0.05$).

Water temperature

Weekly variation profile

The weekly variation for the water temperature is presented in Fig. 4. The data showed that there was a decrease in water temperature ($p < 0.05$) over time during the sampling period. The temperature dropped from ±20℃ in week 1 (March) to ±14℃ in week 6 (June) indicating the change from the mid-summer to winter season.

Daily variation profile

It was found that there was also a change ($p < 0.05$) in the temperature of the water at the Plank-3 site over time during the sampling day (Fig. 5). The daily water temperature ranged from 16℃ at 06h00 to 17.5℃ at 18h00 (Fig. 5).

In the literature the importance of temperature on growth of *E.coli* in water has been has been clearly demonstrated (Vital *et al*., 2008) and specifically at temperatures below 20℃ where they survive, but have difficulty to grow. The optimum growth temperature of *E. coli* is 35°-37℃ under ideal conditions. Kovářová and co-workers (1996) also reported that *E.coli* is able to grow, albeit very slowly, at a temperature of 17.4℃ but only under ideal batch culture conditions.

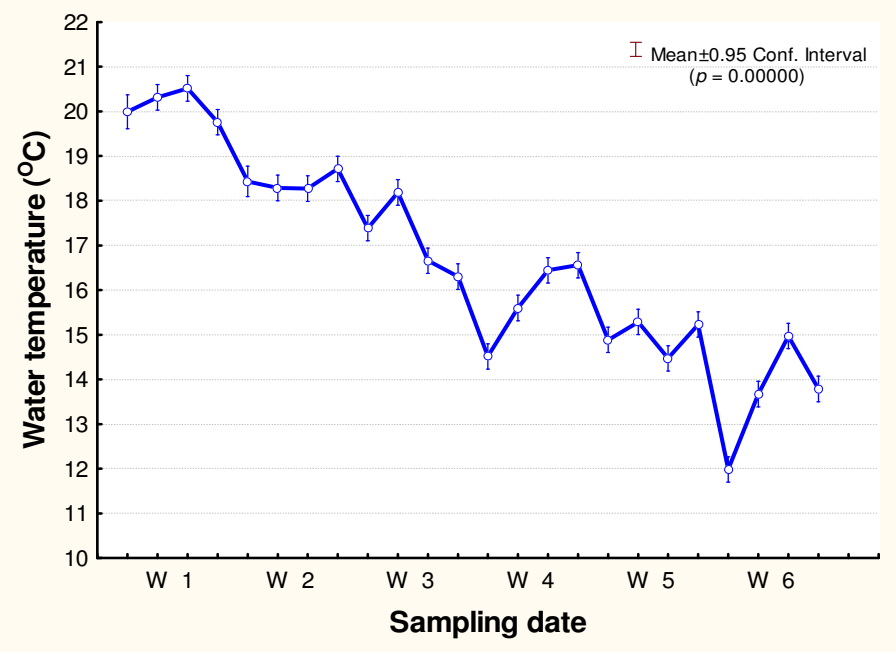

Figure 4 The weekly variation trend in water temperature from week 1 (March 2010) to week 6 (June 2010). The analysis was done at a significant level of 5% ($p = 0.05$). The average mean for each data point was calculated at a 95% confidence interval.

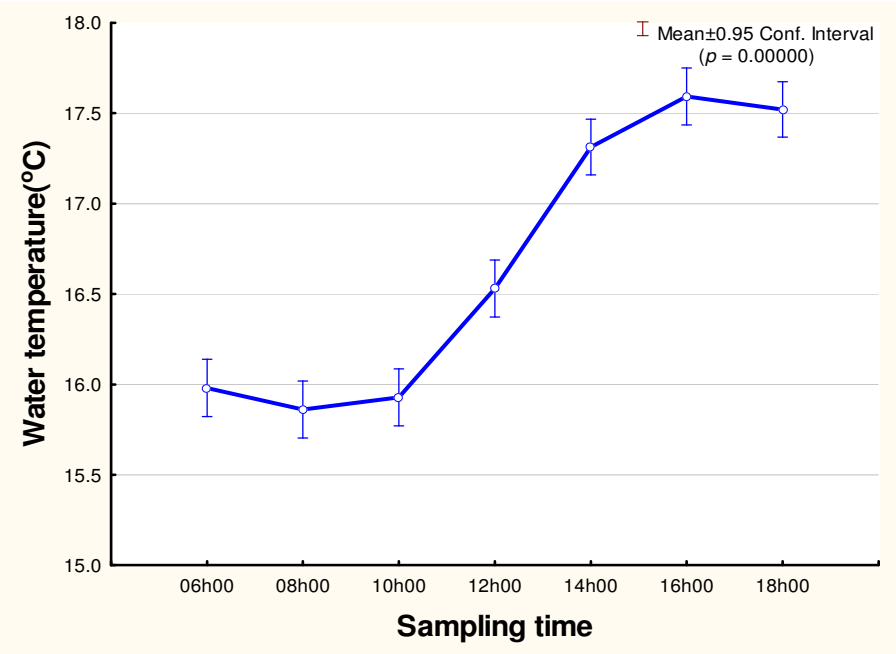

Figure 5 The hourly variation trend in water temperature from 06h00 to 18h00. The analysis was done at a significant level of 5% (p = 0.05). The average mean for each data point was calculated at a 95% confidence interval.

In another study by Dukta & Kwan (1980), the survival of *E. coli* at lower temperatures (between 10° and 21°C) in a flowing stream was examined. They reported survival but no growth of *E. coli* at temperatures between 10°C and 21°C in the flowing water.

The temperature range of the Plankenburg River at the Plank-3 site during the summer to winter sampling period for this study was in some instances about 15°C lower than the reported optimum growth temperature (35°-37°C) of *E. coli*. While this lower temperature range is more conducive for growth of mesophilic and psychrophilic bacteria, the temperature would probably have been high enough for survival (Leclerc *et al.*, 2001) but not to allow the enteric bacteria to proliferate. Leclerc *et al.* (2001) also reported that enteric bacteria grow optimally at 35°-37°C but specifically only in the presence of suitable and optimum concentrations of carbon, nitrogen and phosphorous sources. Thus the combination of temperature and optimal nutrient conditions in flowing water is important and will impact bacterial growth.

Water pH

Weekly variation profile

The average pH of the water changed over time ($p < 0.05$) during the sampling period (Fig. 6). The pH ranged from 6.3 to 7.1 between weeks 1 (March) and 6 (June). The trend shows a general increase in pH from week 1 to 6. The trend is different to that of the water temperature (Fig. 4) suggesting a negative correlation between the two. It was possible that the decrease in

temperature may indirectly have resulted in an increase in pH as a result of a slowing down in microbial metabolic activity at the lower temperatures. It is interesting to note that these results differ from previous studies where an increase in temperature resulted in an increase in pH (Ackermann, 2010). A point that must be taken into consideration is that the increase in pH was only 0.8 units and may have been due to a change in the chemical composition of the water.

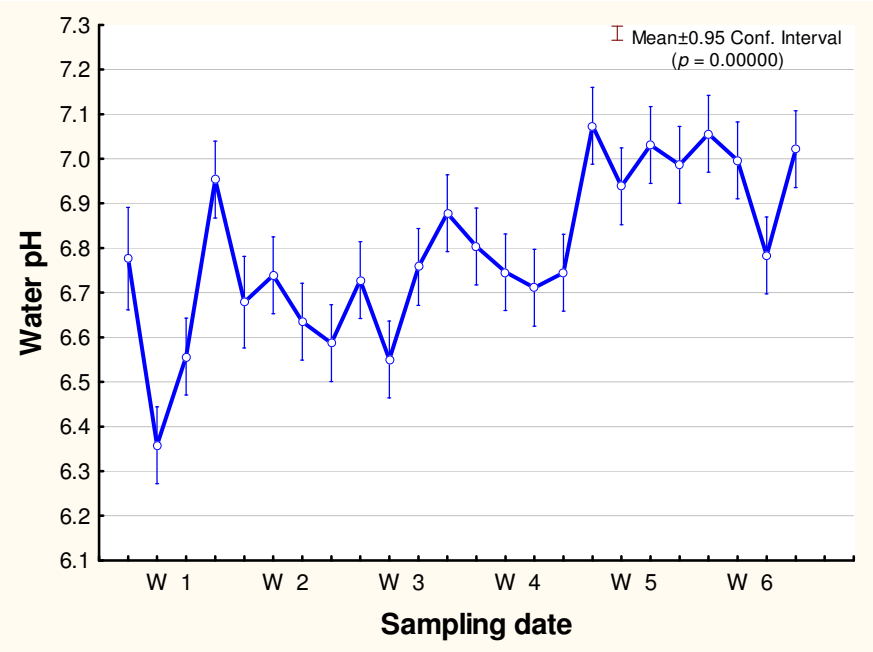

Figure 6 The weekly variation trend in water pH from week 1 (March) to week 6 (June). The analysis was done at a significant level of 5% (p = 0.05). The average mean for each data point was calculated at a 95% confidence interval.

Daily variation profile

The pH of the water also changed over time during a sampling week (Fig. 7). Some of the pH changes are very small but for others the variations was high. The trend showed a decrease in pH on Monday from 6.85 to 6.6 on Thursday. This was then followed by an increase in pH from 6.6 on Thursdays to 6.9 on Sundays.

Hourly variation profile

There was also a change in the pH over time during a sampling day (Fig. 8). The pH dropped from 6.8 at 06h00 to 6.68 by 18h00. In total this decrease of 0.12 in pH is small and is difficult to a give reason for this. However this variation trend is different to that of the water temperature where there was an increase in temperature over time during the day (Fig. 5). It is possible that the increase in pH may have been as a result of an increase in microbial metabolism activity at the higher temperatures. The optimum pH growth range for *E. coli* has been reported to be between the pH of 5.5 and 7.5 (Buchanan & Doyle, 1997) and thus it can be speculated that the pH

increases and decreases obtained during this study at the Plank-3 site might not have had a strong impact on the growth or survival of the coliforms.

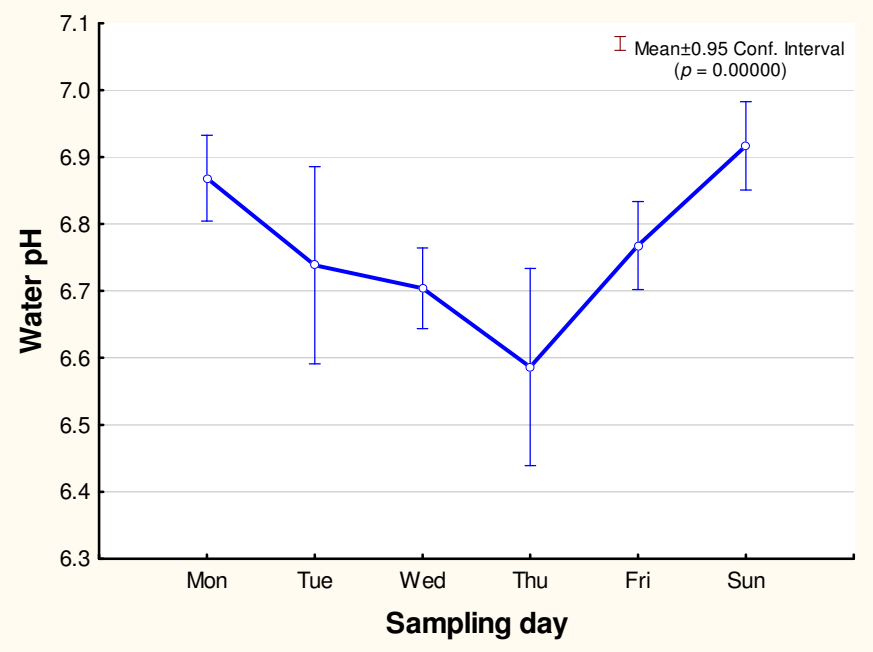

Figure 7 The variation trend in water pH from Monday to Sunday. The analysis was done at a significant level of 5% (p = 0.05). The average mean for each data point was calculated at a 95% confidence interval.

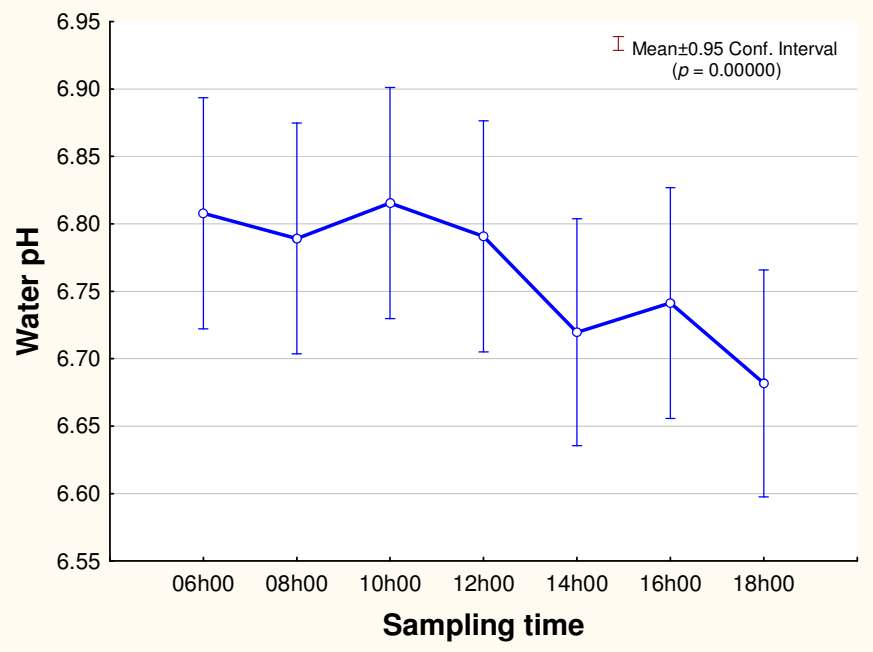

Figure 8 The variation trend in water pH from 06h00 to 18h00. The analysis was done at a significant level of 5% (p = 0.05). The average mean for each data point was calculated at a 95% confidence interval.

Total coliforms

Weekly variation profile

There was a change in the total coliform count ($p < 0.05$) over time for the entire sampling period (Fig. 9). The trend showed a decrease in the total coliform counts from week 1 (March 2010) to week 6 (June 2010). The highest average total coliform count was 3 200 000 MPN.100 mL^{-1} in week 3 (May 2010). The lowest average total coliform count was 20 000 MPN.100 mL^{-1} water in week 6 (June 2010). The observed decrease in temperature over time (Fig. 4) may have been a possible factor influencing the total coliform loads.

Although there was a decrease over time, the coliform count still exceeded the Target Water Quality Range (TWQR) of <1 000 faecal coliforms.100 mL^{-1} water (DWAF, 1996b). Thus there is a likelihood of contamination from vegetables and other crops that are eaten raw will result in the transmission of human pathogens. With the microbial loads found during this study there is additionally an increased risk of pathogens being carried over to the irrigated fresh produce? These results are concurrent with the findings from the monthly sampling (Chapter 3) whereby the faecal coliform counts also exceeded the recommended TWQR (DWAF, 1996b).

Daily variation profile

The data in Fig. 10 shows the daily variation profile of the total coliform counts. There was a change in the total coliform count ($p < 0.05$) over time during the sampling week. There was an increase in the average total coliform count from 440 000 MPN.100 mL^{-1} water on Monday to 1 900 000 MPN.100 mL^{-1} on Thursday. This was followed by a decrease in the average count from Thursday to Sunday. This profile shows an opposite trend to that of the pH profile (Fig. 7) where there was a decrease from Monday to Thursday followed by an increase to Sunday.

The highest coliform count averaged at about 1 900 000 MPN.100 mL^{-1} water on Thursdays and the lowest count averaged at about 162 000 MPN.100 mL^{-1} water on Sundays (Fig. 10). Therefore, considering the fact that sampling was done on Mondays for the monthly sampling (Chapter 3), it can be concluded that these results could represent an underestimation of the average daily contamination.

Although the counts on Sundays were low, they were still higher than that for the TWQR for total coliforms. Therefore, the increased risk of fresh produce contamination with spoilage organisms and possible pathogens was present throughout the week. For this study on the Plankenburg River, it was concluded that in terms of microbiological guidelines, the sampling time was less important as the counts were always above the TWQR. In cases of less polluted rivers where microbial pollution levels are much lower, weekly, daily and specific hourly variation would have to be considered.

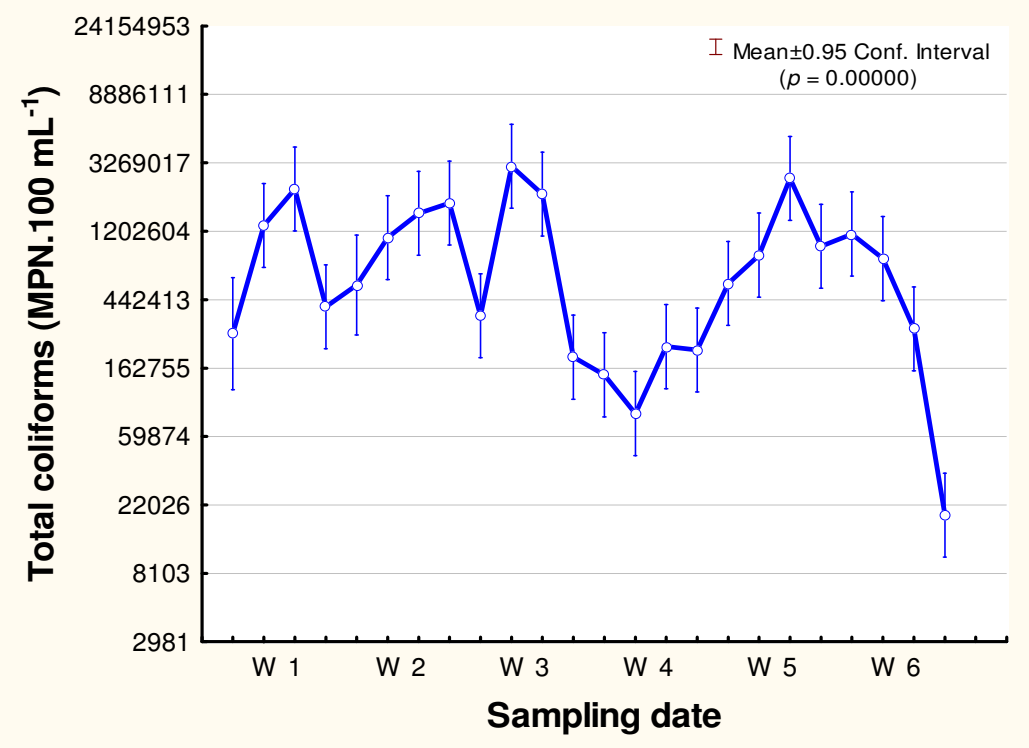

Figure 9 The variation trend in total coliform counts from week 1 (March 2010) to week 6 (June 2010). The analysis was done at a significant level of 5% ($p = 0.05$).The average mean for each data point was calculated at a 95% confidence interval.

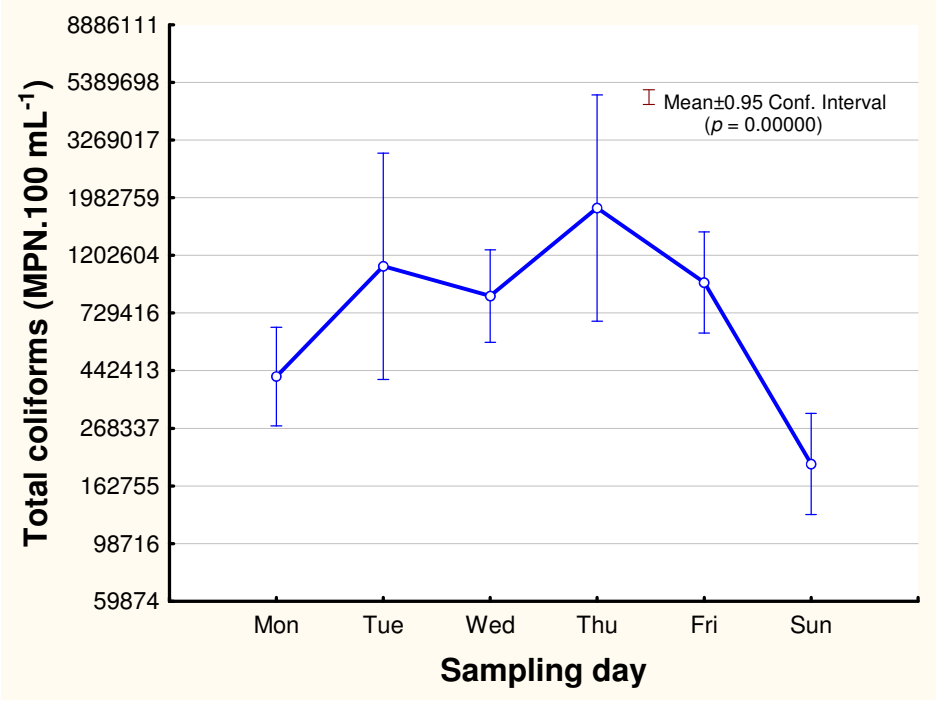

Figure 10 The variation trend in total coliform counts from Monday to Sunday. The analysis was done at a significant level of 5% ($p = 0.05$). The average mean for each data point was calculated at a 95% confidence interval.

Hourly variation profile

There was a change in the total coliform count ($p < 0.05$) over time during a sampling day (Fig. 11). The average total coliform count decreased from 440 000 MPN.100 mL^{-1} at 06h00 to 240 000 MPN.100 mL^{-1} at 08h00. This was then followed by an increase from 08h00 to 12h00, followed by a slight decrease from 660 000 at 12h00 to 540 000 MPN.100 mL^{-1} at 14h00. The counts then stayed fairly constant until 18h00. The highest coliform count averaged at about 660 000 MPN.100 mL^{-1} at 12h00 and the lowest count averaged at 240 000 MPN.100 mL^{-1} at 08h00. Therefore it can again be concluded that the findings of the monthly sampling (Chapter 3) are an underestimation of the average daily contamination since the sampling was always done between 08h00 and 09h00.

It is important to note that the total coliform count at 06h00 (540 000 MPN.100 mL^{-1} water) was higher than that at 08h00 (240 000 MPN.100 mL^{-1} water). This decrease can possibly be a continuation of the apparent decrease during the night. The counts were still above the TWQR for total coliforms therefore the risk of fresh produce contamination with spoilage organisms or possible pathogens was present throughout the study period.

E. coli

Weekly variation profile

The weekly variation profile of the average *E. coli* counts is presented in Fig. 12. There was a fairly large change in *E. coli* counts ($p < 0.05$) over time for the entire sampling period. In total there was a decrease from week 1 (March 2010) to week 6 (June 2010). The total profile is similar to that for the total coliforms (Fig. 9) and water temperature (Fig. 4). The highest count was in week 3 (May 2010) and the lowest count was in week 6 (June 2010). Thus, the change in temperature from higher during the summer period (20°C) to lower during the winter months could have influenced the microbial load.

The highest and lowest *E. coli* count averaged at 440 000 and 3 000 MPN.100 mL water, respectively. The fact that the *E. coli* counts were lower than that of the total coliforms (Fig. 9) was expected as the total coliform count includes *E. coli* as well as other *Enterobacteriaceae* strains such as *Klebsiella, Enterobacter, Citrobacter* and *Serratia* (DWAF, 1996b). The data clearly shows that the *E. coli* count throughout the sampling period exceeded both the DWAF and WHO guidelines of 1 000 *E. coli* per 100 mL for water used to irrigate fresh produce intended to be consumed raw (WHO, 1989; DWAF, 1996b). These results agree with the findings from Chapter 3 where the counts continuously exceeded the guidelines. Therefore, it was concluded that irrigation with the water from this sampling point in the Plankenburg River could result in carry-over of pathogens to contaminate of fresh produce with possible health implications for consumers.

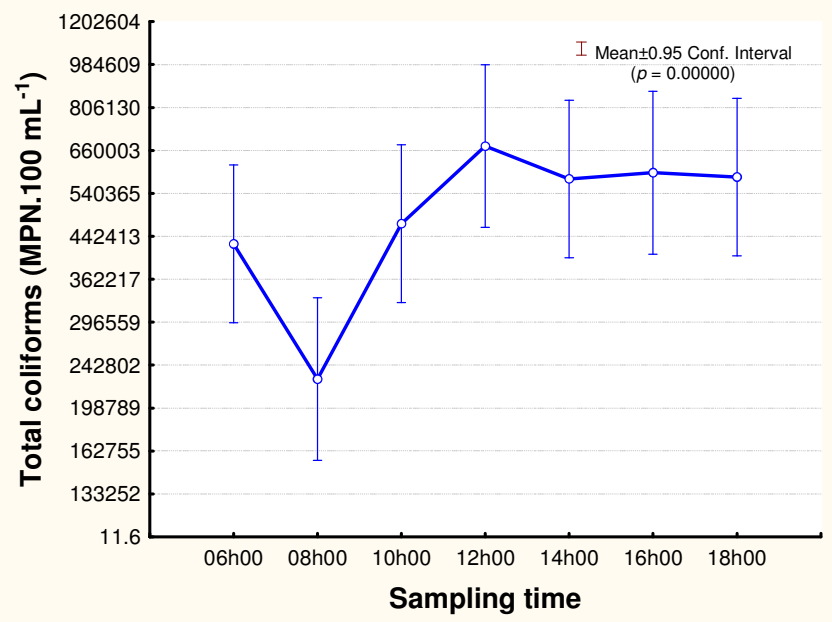

Figure 11 The variation trend in total coliform counts from 06h00 to 18h00. The analysis was done at a significant level of 5% ($p = 0.05$). The average mean for each data point was calculated at a 95% confidence interval.

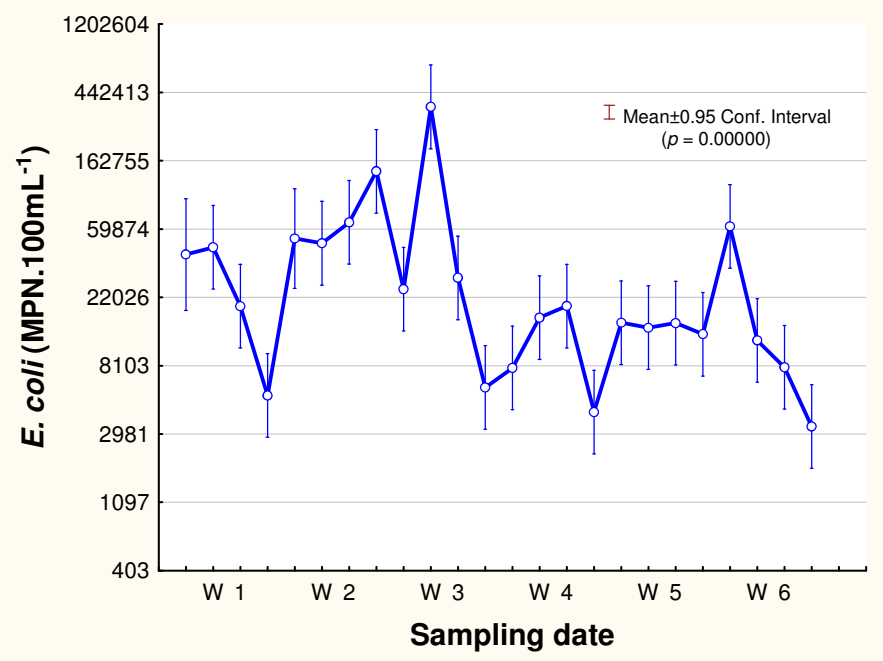

Figure 12 The variation trend in *E. coli* counts from week 1 (March 2010) to week 6 (June 2010). The analysis was done at a significant level of 5% ($p = 0.05$). The average mean for each data point was calculated at a 95% confidence interval.

Daily variation profile

There was a change in the *E. coli* count ($p < 0.05$) over time during a sampling week (Fig. 13). This was an increase from 22 000 MPN.100 mL^{-1} on Mondays to 160 000 MPN.100 mL^{-1} on Thursdays. This was then followed by a decrease from Thursday to Sunday reaching a low count of 5 000 MPN.100 mL^{-1}. The change in total coliform count during a sampling week (Fig. 10) showed a similar profile. The lower count on Sundays still exceeded DWAF and WHO guidelines but the profile as a whole still indicated an increased risk of fresh produce contamination with possible disease causing pathogens throughout the week. Therefore, the findings for the monthly sampling over a 15 month period as given in Chapter 3 could again be taken as an underestimation of the average daily contamination since the sampling was done on a Monday and the results in Fig. 12 show Thursdays as having the highest *E. coli* counts.

Hourly variation profile

There was also a change in the *E. coli* count ($p < 0.05$) over time during a sampling day (Fig. 14). The *E. coli* count increased from 8 000 MPN.100 mL^{-1} at 06h00 to 26 000 MPN.100 mL^{-1} at 12h00. This was then followed by an *E. coli* decrease to 18 000 MPN.100 mL^{-1} at 16h00 where after the count stayed constant at 8 000 MPN.100 mL^{-1} until after 18h00. This profile is similar to that found for the total coliforms. When the data from this study is compared with the findings from the monthly sampling (Chapter 3) it can again be concluded that the monthly sampling is an underestimation of the daily average *E. coli* loads (Fig. 13).

The *E. coli* count at 06h00 was lower than the average at 18h00 (Fig. 14) which suggests a further decrease during the night. Although lower at 06h00, the counts still exceeded the DWAF and WHO guidelines. Thus again there is an increased risk of fresh produce contamination with possible disease causing pathogens if the water is used for irrigation throughout the day.

Covariate analysis for correlations

The weekly and hourly variations of water temperature (Figs. 4 and 5) were similar to that of total coliforms (Figs. 9 and 11) and *E. coli* (Figs. 12 and 14). This raises the question of whether temperature influences the growth and/or survival of the microbes present. This is important as microbial growth at higher temperatures will result in higher counts giving an overestimation of the average daily contamination. This then affects the validity of the data because the higher counts give a false impression of the actual size of the contamination.

The same is true for pH because when there was an increase in the total coliform (Figs. 9 and 11) and *E. coli* counts (Figs. 12 and 14), there was a smaller decrease in the pH (Figs. 6 and 7). Therefore a higher pH may have had a positive effect on microbial activity and subsequent growth which then may have resulted in an overestimation of the actual contamination.

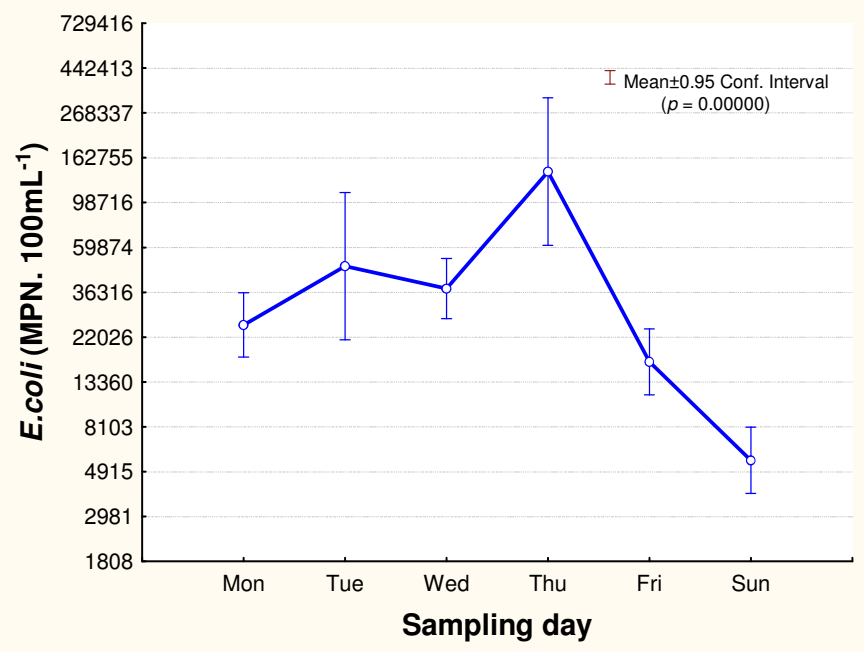

Figure 13 The variation trend in *E. coli* counts from Monday to Sunday. The analysis was done at a significant level of 5% ($p = 0.05$). The average mean for each data point was calculated at a 95% confidence interval.

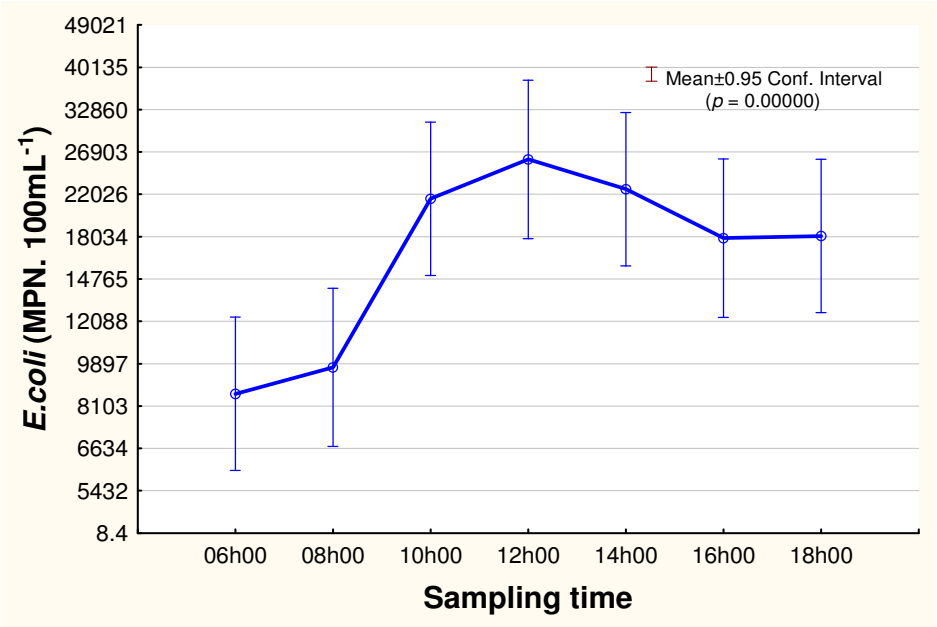

Figure 14 The variation trend in *E. coli* counts from 06h00 to 18h00. The analysis was done at a significant level of 5% ($p = 0.05$). The average mean for each data point was calculated at a 95% confidence interval.

To assess possible correlation between the physico-chemical variables (temperature and pH) and the microbial variables (total coliforms and *E. coli*), analysis of covariance was performed on the data using Statistica9 (StatSoft). The analysis was computed under the null hypothesis that temperature and pH did have an impact on the total coliform and *E. coli* counts. The data showed that the null hypothesis could be rejected at the 5% level ($p < 0.05$).

Total coliforms

There was no correlation ($p < 0.05$) found between the physico-chemical variables (temperature and pH) and total coliforms (Fig. 15) as the resulting hourly variations of the total coliform showed a similar profile to the one without covariate analysis. This is so because the analysis of covariance removes the effect that temperature and pH has on the total coliform data. Therefore, if the shape of the variation profile does not change, it means there was no effect on the data. Thus temperature and pH as found under the environmental conditions present in this study had no direct impact on the total coliform counts.

E. coli

The results for the analysis of covariance between the physico-chemical variables (temperature and pH) and *E. coli* are presented in Fig. 16. Again there was no correlation ($p < 0.05$) found between the physico-chemical variables (temperature and pH) and *E. coli* numbers as the resulting profile was similar to that where no covariate analysis had been performed. Therefore it was concluded that temperature and pH as found under the environmental conditions as found in this study on this sampling point of the Plankenburg River had no impact on the *E. coli* counts.

Conclusions

The weekly variations for both the total coliform and *E. coli* counts showed a decrease in the level of contamination. Although the counts decreased, the level of contamination still exceeded guidelines and these findings concur with the monthly sampling (Chapter 3) where levels also exceeded guidelines. The results also confirm that the observed decrease in microbial contamination loads over the winter months during the monthly sampling (Chapter 3) also occurs on a weekly basis.

There were also daily variations in the total coliform and *E. coli* counts within the sampling week. The level of microbial contamination increased from Monday to Thursday and decreased again until Sunday. The highest level of contamination was on Thursdays and the lowest was on Sundays. These findings suggest a possible underestimation of the level of microbial contamination with the results from the monthly sampling (Chapter 3) since sampling took place on Monday of every month.

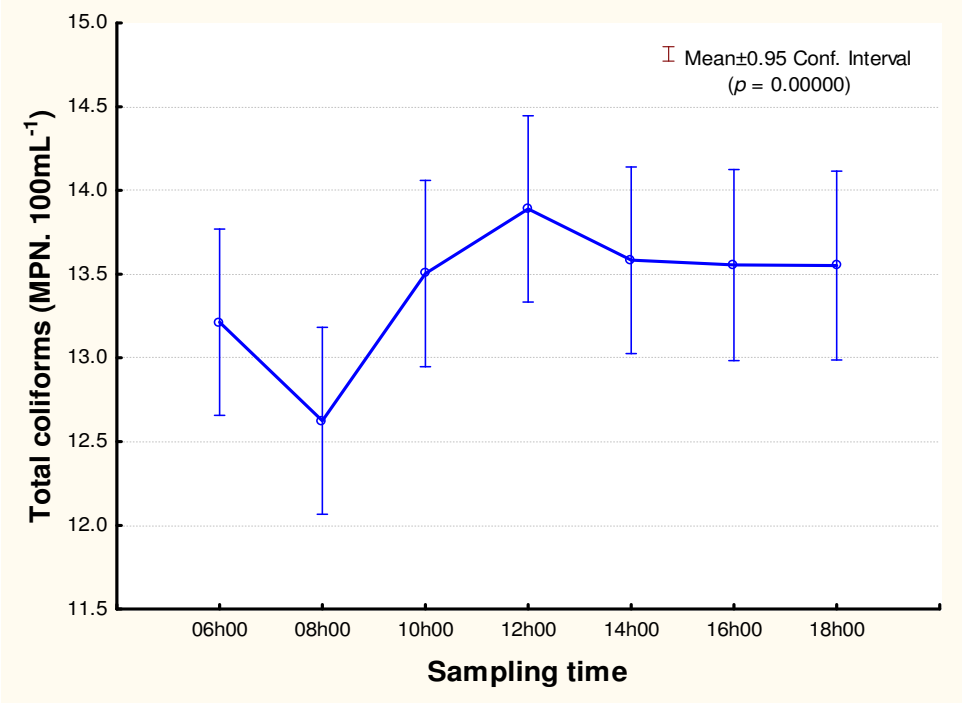

Figure 15 The analysis of covariance for the correlation between total coliforms and the physico-chemical variables (temperature and pH). The analysis was done at a significant level of 5% ($p = 0.05$).

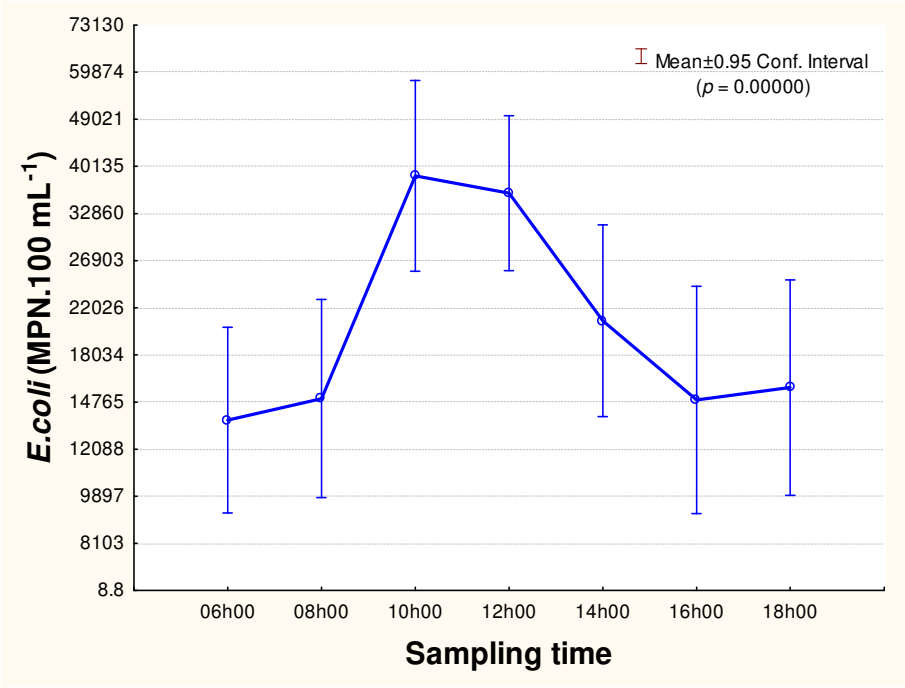

Figure 16 The analysis of covariance for the correlation between *E. coli* and the physico-chemical variables (temperature and pH). The analysis was done at a significant level of 5% ($p = 0.05$).

The levels of contamination also varied from hour to hour within a sampling day. The hourly variation profiles for total coliforms and *E. coli* both showed an increase in the level of contamination from 06h00 to 12h00. This was then followed by a decrease from 12h00 to 18h00. These results again suggest an underestimation of the average daily/weekly/monthly level of the contamination for the monthly sampling results (Chapter 3) because the sampling was done between 08h00 and 09h00 on Mondays.

Results from the analysis of covariance confirmed that temperature and pH had no impact ($p < 0.05$) on the total coliform and *E. coli* counts. Therefore, it can be concluded that the results from the monthly sampling (Chapter 3) when compared with the weekly/daily values should be considered an underestimation of the average level of contamination in the river water. It can also be concluded that the monthly profile observed in the previous research chapter (Chapter 3) also occurs on a weekly and daily basis. Therefore, a detailed preliminary study of a river needs to be done before a decision can be made about the sampling frequency for the baseline study and monitoring of a river.

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CHAPTER 5
GENERAL DISCUSSION AND CONCLUSIONS

The increased consumption of fresh fruit and vegetables is partly due to their recommendation as part of a healthy diet (WHO, 1990). However, the increased consumption of fresh produce has also been shown to be related to increases in foodborne disease outbreaks (CDC, 2010). This has in many cases been shown to be directly due to carry-over of pathogens such as *Salmonella*, *E. coli, Staphylococcus* and *Listeria* (Roberts *et al*., 2009) from contaminated irrigation water. In South Africa, rivers are the main source of irrigation water but many of our rivers have been found to be unsuitable for irrigation of fresh produce mainly because of the unacceptably high levels of faecal contamination (Olaniran *et al*., 2009; Paulse *et al*., 2009).

For this reason the South African Water Research Commission initiated a research project in 2007 to investigate the link between the quality of irrigation water and the safety of produce (Backeberg, 2006). The main objective of the research was to investigate which bacterial contaminants are found in polluted irrigation water sources and highlight their potential risks and carry-over potential to crops cultivated using such water sources. Studies done on the Plankenburg River as part of the WRC project found the river to be unsuitable for irrigation of fresh produce because of the high levels of indicator and index organisms. Although no disease outbreak as a direct or indirect use of the Plankenburg River has been reported, it was considered important to monitor the river for faecal pollution over a longer period so that corrective actions to prevent disease outbreaks can be implemented based on longer term data. Thus one of the objectives of this study was to continue monitoring the microbial quality of the Plankenburg and Eerste Rivers for a further 15 months and also to study the daily variation in microbial load profiles.

As part of the ongoing WRC project the baseline study of the Plankenburg (sites Plank-1 and Plank-3) and Eerste Rivers (Eerste-1) was continued for 15 months. The data obtained showed that all three sites continuously had high faecal contamination levels that exceeded DWAF and WHO guidelines of 1 000 *E. coli* per 100 mL water for irrigation of fresh produce intended to be consumed raw (DWAF, 1996; WHO, 1989). The Plankenburg River had higher faecal contamination levels (*E. coli* = 1 400 000 MPN 100 mL^{-1}) than the Eerste River (*E. coli* = 79 000 MPN 100 mL^{-1}). The data from this study clearly shows that the Plankenburg River was more polluted than the Eerste River as both sampling sites Plank-1 and Plank-3 always had higher *E. coli* loads than site Eerste-1. Intestinal *Enterococci* loads were also higher for the Plankenburg sites than for the Eerste River site. Furthermore *Staphylococcus* was absent from the Eerste River but always present in the Plankenburg River. The Kayamandi informal settlement just above the Plank-1 site is suspected to be the main source of the faecal pollution as the highest faecal contamination levels were present at this sampling site. Therefore because of the extremely high coliform, faecal coliform and *E. coli* levels which exceeded the DWAF and WHO guidelines of 1

000 cfu.100 mL^{-1} water (WHO, 1989; DWAF, 1996), and the presence of other index organisms both rivers must be considered to be unsuitable as water sources for the irrigation of fresh produce intended to be consumed raw.

Although the Eerste River had in most cases lower faecal levels, it showed the highest incidence of index microorganisms (*Salmonella* and *Listeria*) at the Eerste-1 sampling site. There were also incidences of intestinal enterococci, *Staphylococcus* and endosporeformers for both rivers. Therefore, the high occurrence of faecal indicator and index organisms suggest the presence of potential pathogens that can be carried-over to fresh produce during irrigation. Pathogens are known to survive minimal processing and the likelihood of contaminated fresh produce reaching the consumer is very possible. Also the consumption of contaminated fresh produce may lead to disease outbreaks that can result in death for children, the elderly and those that are medically stressed. Therefore, it was concluded that irrigation with water from both rivers poses a health hazard and should not be allowed until preventative measures have been implemented to minimize faecal pollution of the rivers.

The results of this baseline study as well as other studies by Lötter (2010) and Ackermann (2010) showed a lot of variation from month to month in terms of total coliforms, *E. coli,* and even the APC counts, even though the sampling was done at the same sites and at a fixed time and day of the week. As a result several important questions were raised. The first was which data (high or low counts) should be reported as being the true representation of the river water quality seeing that the rather wide variation was present for the entire sampling period of 15 months and those found by other researchers (Ackermann, 2010; Lötter 2010). This led to the question whether the observed monthly variation in faecal contamination levels was also present in a weekly, daily and hourly basis. A further environmental factor was the water temperature which showed similar varying trends. Together with this fact, the question of whether temperature has a direct impact on the microbial counts has always been an issue. It is a very important question especially for setting guidelines because the resulting data may be an overestimate or underestimation of the average contamination level due to the impact of temperature on the survival and growth of microbial organisms. As a result an assessment of weekly, daily and hourly variation trends was conducted on the Plank-2. This site was specifically used as it is an irrigation source point for nearby fresh produce farmers and is about 2km further downstream from the Kayamandi informal settlement. It has on several occasions been shown that the contamination levels 1km before this informal settlement are absent or when present well below the recommended guidelines.

The results for assessment of the variation in microbial loads showed similar weekly, daily and hourly variation trends for both total coliforms and *E. coli* counts. The weekly variation trend showed a decrease in counts from week 1 (Late summer = March) to week 6 (early winter = June). The daily variation trend showed an increase from Monday to Thursday and a decrease from Thursday to Sunday. The lowest faecal contamination levels were present on Sundays. The hourly

variation trend showed an increase from 08h00 to 12h00 and a decrease until 18h00 and then stabilized to the next day. Based on the data obtained it was also concluded that the data for the monthly baseline sampling over 15 months was a large underestimate of the average microbial load. This is because the sampling was done on Monday mornings between 08h00 and 09h00 but the variation trends showed the highest counts on Thursday between 11h00 and 12h00. Although the actual counts were an underestimate they still exceeded guidelines. Even though there was an underestimation of the microbial loads, the quality of the water from this site is still not suitable for irrigation of fresh produce intended to be eaten raw. Thus, from the data obtained it is recommended that when river microbial load studies are being planned, a preliminary study of the river should always be done beforehand to help decide on the most optimal sampling time so as to yield the correct data that can be used for guideline purposes.

The question of whether temperature has a direct impact on the microbial counts has always been an issue. It is a very important question especially for setting guidelines because the resulting data may be an overestimate or underestimation of the actual contamination level due to the impact of temperature on the survival and growth of microbial organisms. In this study the highest total coliform (3 200 000 MPN.100mL^{-1}) and *E. coli* (440 000 MPN.100mL^{-1}) counts were found in week 3 (May). The change in weather which affects the water temperature was suspected to be the main cause of the decreasing trends since the highest water temperature was also during week 3. However, this was not the case as the analysis of covariance showed no correlation ($p < 0.05$) between the microbial (total coliforms and *E. coli*) and physico-chemical variables (water temperature and pH). Therefore it was concluded that temperature as measured during this study had no major impact on the microbial counts and that the counts are actual contamination levels. This conclusion is based on statistical analysis and no experiments were done to show what the survival and growth of microbes is at different temperatures. Therefore actual growth kinetics studies still need to be done to properly investigate the impact of temperature and other physico-chemical variables have on the survival and growth of microbial organisms.

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