

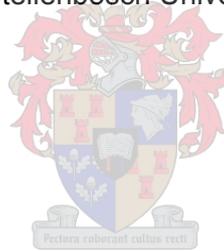
**INVESTIGATING THE EFFICACY OF MEDIUM PRESSURE UV AND HYDROGEN PEROXIDE  
AS ON-FARM TREATMENT METHODS TO REDUCE THE MICROBIAL LOAD OF IRRIGATION  
WATER**

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### **Declaration**

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## Abstract

Many South African farmers are forced to use water from nearby rivers for crop irrigation, since it is the most affordable and sometimes only source of water available to them. During this research project, a baseline study was performed on a farm irrigating fresh produce with water obtained from the Eerste River. The baseline study was done over a five month period, at six preselected sampling points, to determine the microbial and physico-chemical parameters of the water so a baseline could be established to compare the results to when the ultraviolet (UV) apparatus was installed (February 2013). Aerobic colony count (ACC), total coliforms (TC) and *Escherichia coli* (*E. coli*) were tested for during the microbiological study, while the physico-chemical analysis comprised of temperature, pH, conductivity, chemical oxygen demand (COD), alkalinity and total soluble solids (TSS). The UV treatment study was also performed over a five month timeline, at eight different sampling points (original six sampling points, with additional sampling points before and after UV). The same microbiological tests were performed during the UV treatment study, but turbidity and percentage ultraviolet transmittance (% UVT) were performed additionally during physico-chemical analysis.

During the baseline study ACC, TC and *E. coli* counts as high as 9 600 cfu.mL<sup>-1</sup>, 13 799 MPN.100 mL<sup>-1</sup> and 2 098 MPN.100 mL<sup>-1</sup> were isolated at the river (Sampling Point 1), respectively. While performing the UV treatment study ACC, TC and *E. coli* counts as high as 142 000 cfu.mL<sup>-1</sup>, 241 960 MPN.100 mL<sup>-1</sup> and 6 867 MPN.100 mL<sup>-1</sup> were isolated at the river, respectively. As a result it was concluded that the Eerste River was mostly unsuitable for irrigation of fresh produce that are consumed raw. The higher counts in the river, during the UV treatment study might be attributed to the increase in rainfall that occurred in the sampling months (March to July 2013).

The counts as measured at the point of irrigation are considered of greater importance, since the counts present in the river might still decrease to below the guideline levels after passing through sand filters and the addition of hydrogen peroxide (current mode of treatment) or after passing through the UV in the UV treatment study. The ACC, TC and *E. coli* counts during the baseline study were as high as 8 800 cfu.mL<sup>-1</sup>, 24 196 MPN.100 mL<sup>-1</sup> and 85 MPN.100 mL<sup>-1</sup> at the point of irrigation (Sampling Point 6), respectively. After hydrogen peroxide addition average log-reductions ranging between 0.65 and 1.13 were seen, but reduction was never constant.

The counts at the point of irrigation remained more or less constant compared to the river due to contamination that occurred at the sand filters, making the water unsuitable for irrigation of fresh produce in terms of ACC and TC counts. In the UV treatment study ACC, TC and *E. coli* counts were as high as 35 000 cfu.mL<sup>-1</sup>, 10 462 MPN.100 mL<sup>-1</sup> and 63 MPN.100 mL<sup>-1</sup> at the point of irrigation (Sampling Point 8), respectively. Average log-reductions in the range of 0.90 to 1.25 were achieved, but it was inconsistent. After treatment with chlorine and re-sanding of the sand filters, no further contamination occurred and counts decreased to below guideline limits, making the water safe for irrigational use in terms of all of the microbiological parameters. Not only is UV

treatment more effective in reducing microbiological counts than  $H_2O_2$ , it is also relatively less expensive in the long term. Hydrogen peroxide treatment of water amounts to a very high capital expense every month, whereas UV may seem expensive when starting up, but the monthly operating cost thereafter is marginally less than for  $H_2O_2$ .

It is of great importance to farmers to find a treatment that would reduce the counts in the river water to below the guideline limits required for safe irrigation since pathogens can be carried over from water onto fresh produce, resulting in an increase in produce-associated foodborne outbreaks and loss of consumer trust.

## Opsomming

Menigte Suid-Afrikaners is afhanklik van nabygeleë riviere om hulle oeste te besproei aangesien dit meestal die mees bekostigbare en soms enigste bron tot hul beskikking is. Tydens hierdie projek is 'n grondslag sowel as 'n UV behandelingsmetode studie uitgevoer op 'n plaas wat vars vrugte en groente besproei met water wat hul vanuit die Eersterivier verkry. Die grondslagstudie is oor 'n tydperk van vyf maande uitgevoer by ses voorafgekose punte. Dit is gedoen om die mikrobiologiese sowel as chemiese parameters van die water te bepaal sodat 'n grondslag beskikbaar kon wees om met resultate te vergelyk wat met behulp van die ultravioletmasjien verkry is (in Februarie 2013 geïnstalleer). Tydens die mikrobiologiese studie is daar vir aerobiese koliform tellings (ACC), totale koliforme (TC) en *Escherichia coli* (*E. coli*) getoets. Tydens die chemiese analise is temperatuur, pH, konduktiwiteit, chemiese suurstof benodiging, alkaliniteit en totale oplosbare vastestowwe in die water getoets. Die UV behandelingsmetode studie is ook oor 'n tydperk van vyf maande uitgevoer, met twee addisionale toetspunte by. Presies dieselfde mikrobiologiese analises as wat tydens die grondslag studie uitgevoer is, is tydens die UV behandelingsmetode studie uitgevoer, maar vir die chemiese analise het turbiditeit en persentasie ultraviolet transmissie van die water bygekom.

Gedurende die grondslag studie was ACC, TC and *E. coli* tellings so hoog as 9 600 cfu.mL<sup>-1</sup>, 13 799 MPN.100 mL<sup>-1</sup> en 2 098 MPN.100 mL<sup>-1</sup> onderskeidelik uit die rivier geïsoleer (Punt 1). Tydens die UV behandelingsmetode studie was ACC, TC en *E. coli* tellings so hoog as 142 000 cfu.mL<sup>-1</sup>, 241 960 MPN.100 mL<sup>-1</sup> en 6 867 MPN.100 mL<sup>-1</sup> onderskeidelik by die rivier geïsoleer. Gevolglik is daar afgelei dat die Eersterivier se water meestal ongeskik is om te gebruik vir die besproeiing van vars groente en vrugte wat rou geëet word sonder dat enige verdere behandeling plaasvind. Die hoër tellings wat tydens die UV behandelingsmetode in die rivier sigbaar was kan hoofsaaklik toegeskryf word aan die toename in reënval in daardie tyd (Maart tot Julie 2013).

Tellings soos gemeet by die punt van besproeiing is wel van groter belang as die wat aangeteken is by die rivier; aangesien die tellings wat in die rivier aangeteken is steeds kan afneem tot onder aanvaarbare hoeveelhede soos in die standaard uiteengesit, want die water moet steeds deur sandfilters beweeg en word ook huidiglik deur waterstofperoksied behandel tydens die die grondslagstudie of beweeg deur die UV apparaat in die UV behandelingsmetode studie. Die ACC, TC en *E. coli* tellings soos gemeet by die besproeiingspunt (Punt 6) was so hoog as 8 800 cfu.mL<sup>-1</sup>, 24 196 MPN.100 mL<sup>-1</sup> en 85 MPN.100 mL<sup>-1</sup>, onderskeidelik. Na waterstofperoksied byvoeging was die gemiddelde log-reduksies sigbaar, tussen 0.65 en 1.13, maar afnames was nooit konstant nie. Die tellings by die punt van besproeiing het ongeveer konstant gebly in vergelyking met die tellings wat by die rivier aangeteken is; moontlik as gevolg van die hoë kontaminasie vlakke in die sandfilters. Kontaminasie van sandfilters het veroorsaak dat die water ongeskik was vir die gebruik van besproeiing van vars groente as gevolg van die hoë

ACC en TC vlakke. Tydens die UV behandelingsmetode studie is ACC, TC en *E. coli* tellings so hoog as 35 000 cfu.mL<sup>-1</sup>, 10 462 MPN.100 mL<sup>-1</sup> en 63 MPN.100 mL<sup>-1</sup>, onderskeidelik aangeteken (Punt 8). Gemiddelde log-reduksies tussen 0.90 tot 1.25 was verkry, maar behandeling en afnames in tellings was nie konstant nie. Nadat die sandfilters met chloor behandel is en die sand daarin vervang is, het geen verdere kontaminasie by die punt voorgekom nie. Nadat al die voorafgenoemde behandelings afgehandel is, het die tellings tot laer as die van die standaard gedaal en dus was die water nou veilig om te gebruik vir besproeiingsdoeleindes in terme van die mikrobiologiese parameters. Die UV behandelingsmetode is nie net meer effektief in die verlaging van mikrobiologiese tellings as waterstofperoksied nie, dis ook heelwat goedkoper in die langtermyn. Waterstofperoksied behandeling van water lei tot 'n baie hoë kapitale onkoste per maand, terwyl UV baie duur mag voorkom in die beginfase, maar die maandelikse kostes is aansienlik laer as die van waterstofperoksied en maak sodoende op daarvoor.

Dit is van uiterste belang vir boere om 'n water behandelingsmetode te vind wat die hoë tellings in die rivier sal afbring tot laer as Suid-Afrikaanse en Kanadese riglyne; aangesien patogene oorgedra kan word van vars vrugte en groente. Laasgenoemde kan tot 'n drastiese toename in vars voedsel geassosieerde siektes en gevolglik 'n afname in die vertrouwe wat 'n kliënt in 'n produk plaas, lei.

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### List of Abbreviations

% UVT	- Percentage ultraviolet transmittance
ACC	- Aerobic colony count
AOP	- Advanced oxidation process
CDC	- Centers for Disease Control and Prevention
CFU	- Coli-forming units
cm	- Centimetre
COD	- Chemical oxygen demand
<i>C. parvum</i>	- <i>Cryptosporidium parvum</i>
CSIR	- Council for Scientific and Industrial Research
DAFF	- Department of Forestry and Fisheries
DBPs	- Disinfection by-products
DNA	- Deoxyribonucleic acid
DRC	- Democratic Republic of Congo
DWAF	- Department of Water Affairs and Forestry
<i>E. coli</i>	- <i>Escherichia coli</i>
F <sup>-</sup>	- Fluorine
FAO	- Food and Agriculture Association
FDA	- Food and Drug Administration
GAP	- Good agricultural practices
GDP	- Gross domestic product
GMP	- Good manufacturing practises
<i>G. lamblia</i>	- <i>Giardia lamblia</i>
H <sub>2</sub> O <sub>2</sub>	- Hydrogen peroxide
H <sup>+</sup>	- Hydrogen ion
HACCP	- Hazard Analysis Critical Control Points
HCl	- Hydrochloric acid
HOCL	- Hypochlorous acid
HUS	- Haemolytic uremic syndrome
IC	- Ion chromatography
ICP-AES	- Inductively coupled plasma atomic emission spectrometry
kHz	- Kilohertz
kPa	- kilopascal
L	- Litre
mg	- Milligram
MHz	- Megahertz
mJ	- Millijoules

mL	- Millilitre
MPN	- Most probable number
mS	- milliSiemens
MUG	- 4-methylumbelliferyl- $\beta$ -D-glucuronide
NM	- Nanometres
NTU	- Nephelometric turbidity units
OCl <sup>-</sup>	- Hypochlorite
PCA	- Plate count agar
PDC	- Provincial Development Council
<i>P. infestans</i>	- <i>Phytophthora infestans</i>
PPM	- Parts per million
QMRA	- Quantitative microbial risk analysis
RNA	- Ribonucleic acid
SAPA	- South African Press Association
SAWQG	- South African water quality guideline
TC	- Total coliforms
TSS	- Total suspended solids
TWQR	- Target water quality range
UNEPFI	- United Nations Environment Programme Finance Initiative
USA	- United States of America
UV	- Ultraviolet
<i>V. cholerae</i>	- <i>Vibrio cholerae</i>
WHO	- World Health Organisation
WRC	- World Research Commission

# CONTENT

	Page
Abstract	iii
Opsomming	v
Acknowledgements	vii
List of abbreviations	viii
Chapter 1: Introduction	1
Chapter 2: Literature review	6
Chapter 3: Scoping study on different on-farm treatment options to reduce the high microbial contaminant loads of irrigation water to reduce the related food safety risk	66
Chapter 4: General discussion and conclusions	110

This thesis/dissertation is presented in the format prescribed by the Department of Food Science at Stellenbosch University. The structure is in the form of one or more research chapters (papers prepared for publication) and is prefaced by an introduction chapter with the study objectives, followed by a literature review chapter and culminating with a chapter for elaborating a general discussion and conclusion. Language, style and referencing format used are in accordance with the requirements of the *International Journal of Food Science and Technology*. This thesis/dissertation represents a compilation of manuscripts where each chapter is an individual entity and some repetition between chapters has, therefore, been unavoidable.

## CHAPTER 1

### INTRODUCTION

Water is an indispensable natural resource. It is fundamental to life and a crucial component in the environment. It is utilised on large scale in food production, in industrial areas, for hygiene and sanitation purposes and for power generation (Walmsley *et al.*, 1999; Steele & Odumeru, 2004; Paulse *et al.*, 2009).

South Africa is a water scarce country facing an undeniable national water crisis, not only in terms of availability, but also in terms of the quality of its fresh water resources. Fresh produce production is an important component of Western Cape agriculture as well as the economic viability of the country (Davies *et al.*, 1993; Gemmell & Schmidt, 2012; Van der Laan *et al.*, 2012).

In the past few years consumers from all over the world have started consuming more fruits and vegetables as they became increasingly aware of their health and as a result there has been a visible increase in produce-associated foodborne outbreaks (Brackett, 1999; Pollack, 2001, Buck *et al.*, 2003; Lynch *et al.*, 2009; Panigrahy *et al.*, 2011). Higher incomes, increased domestic production, consumer awareness of the importance of consuming fresh produce and greater availability are just a few factors that contributed to an increase in fruit and vegetable consumption (Brackett, 1999; Pollack, 2001, Buck *et al.*, 2003; Heaton & Jones, 2008). Reported foodborne outbreaks due to the consumption of fresh produce will thus vary between developed and developing countries (Ijabadeniyi, 2010). According to literature, faecally polluted irrigation water has often been identified as the main source of contamination of fresh produce implicated in foodborne outbreaks (Beuchat, 1996; Brackett, 1999; Okafu *et al.*, 2003; WHO, 2004).

Recycling of wastewater in the future may no longer be an option but a requirement because of water shortages (Song *et al.*, 2006; FAO & WHO, 2008; Gemmell & Schmidt, 2012). The demand for water is currently in excess of water available in river basins. South Africa has a mean annual rainfall of approximately 490 mm, which is half the world average (SAPA, 2010). Only 9% of the annual rainfall is converted to river runoff (UNEPFI, 2010). Most of the available fresh water resources in South Africa are almost fully utilised and under stress. To ensure a future for this country, no unnecessary waste of water should occur (Paulse *et al.*, 2009).

Most of South Africa's water resources are stored in dams, and water abstraction schemes. This water allows for the adequate functioning of industry, for domestic as well as agricultural uses (Paulse *et al.*, 2009). Commercial and small-scale farmers generally irrigate their crops with water from nearby dams, ponds, rivers, streams and wells (Ijabadeniyi *et al.*, 2011). Irrigation water of an acceptable quality is required for profitable and sustainable crop production (Van der Laan *et al.*, 2012).

Several studies performed in the last few years found that the water quality of many South African rivers declined dramatically due to an increase in pollution levels (Paulse *et al.*, 2009;

Ijabadeniyi, 2010; Kikine, 2011; Britz & Sigge, 2012; Gemmell & Schmidt, 2012). Water can be a vector for many microorganisms, including pathogenic strains such as *Escherichia coli*, *Vibrio cholerae*, *Cryptosporidium* and *Giardia* which are most often associated with waterborne and food related diseases (Leclerc *et al.*, 2002; Coetzer, 2006; Wilkes *et al.*, 2009). Irrigation water is also frequently contaminated with the plant pathogen, *Phytophthora*, which is able to cause fruit rot (Yamak *et al.*, 2002; Hausbeck *et al.*, 2012). Little is known about the entire microbial quality profile of South African rivers, but the data available shows worrying results. Kikine (2011) performed a baseline study on the Eerste River near Stellenbosch to determine the microbiological quality of the water. The coliform counts at the Eerste River site ranged between 230 and 79 000 MPN.100 mL<sup>-1</sup>. Huisamen (2012) also examined the microbial loads of the Eerste River and found high faecal coliforms and *E. coli* concentrations, ranging from 230 to 7 000 000 cfu.100 mL<sup>-1</sup>.

Several factors are known to contribute to the condition of South Africa's rivers. These include pollution with improperly treated human, industrial and municipal wastes due to improperly functioning or damaged sewage treatment plants, storm water overflows and agricultural effluent run-off (Schultz-Fademrecht *et al.*, 2008; Lötter, 2010). Informal settlements are yet another major source of source water contamination in South Africa, since they are mostly located upstream from areas of a river used for irrigation, thus all the waste and effluents produced wind up polluting the natural water sources and contribute to crop contamination (PDC, 2005; Lötter, 2010). Many farmers in South Africa's agricultural community use water from nearby rivers for crop irrigation, since it is the most affordable and sometimes only source of water available to them. These rivers are often contaminated with high microbial loads and are thus of questionable quality for irrigation. Therefore if possible, contaminated water should not be used to irrigate fresh produce. It is thus of utmost importance that the farmers know the quality of the water they use to irrigate crops, since pathogens can be carried over from water onto fresh produce (Ijabadeniyi *et al.*, 2011).

Disinfection of water is of great importance since it controls growth of microbiological pathogens in the irrigation system and reduces the risk of introducing disease to the farm and crops through irrigation water (Yiasoumi *et al.*, 2005; Pehlivanoglu-Mantas *et al.*, 2006). There are a wide range of disinfectants available in treating water used for irrigational purposes. Not only river water, but also waste- or reclaimed water can be disinfected to meet microbiological requirements (Parker, 2012). A long term solution for these farmers would be to apply on-farm treatments to the water they use for irrigation.

Therefore the overall objective of this research study was firstly to investigate the change in water quality (in terms of microbial and physico-chemical parameters) over the entire on-farm irrigation system using water from the Eerste River (referred to as the baseline study) and secondly, to investigate the efficacy of an ultraviolet (UV) on-farm treatment system to reduce the microbial load in the irrigation water prior to irrigation (UV treatment study). Both the baseline and UV treatment study was performed over a five month period.

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## CHAPTER 2

### LITERATURE REVIEW

#### BACKGROUND

Water is an indispensable natural resource. It is fundamental to life and a crucial component in the environment. It is utilised on large scale in food production, in industrial areas, for hygiene and sanitation purposes and for power generation (Walmsley *et al.*, 1999; Steele & Odumeru, 2004; Paulse *et al.*, 2009; CDC, 2014).

The nature and rate of economic growth in South Africa, has an enormous impact on water abstraction and discharge. It is important that the water sector align the provision of water with the spatial and sectoral growth of the economy. Economic change should be taken into account since it can influence water requirements in certain areas. Currently social change is bringing to light a wide range of challenges such as circular migration between rural and urban areas, growing informal settlements on the margins of towns and questions about how to provide these consumers with free water in the most cost effective way (CSIR, 2012).

Another problem water managers are faced with, is that much of South Africa's water storage, distribution and monitoring, treatment and wastewater collection infrastructure is handling loads above its capacity, causing it to become outdated and in need of reparation or being replaced completely (Ijabadeniyi *et al.*, 2011; CSIR, 2012). Effective infrastructure maintenance can result in sustainable water services and more efficient distribution and use of water which will help carry some of the increased demand for water brought on by economic growth and social changes (CSIR, 2012).

South Africa is a semi-arid region, where water is scarce when compared to other countries (Davies *et al.*, 1993; Gemmell & Schmidt, 2012; Van der Laan *et al.*, 2012). Recycling of wastewater in the future may no longer be an option but a requirement because of water shortages (Song *et al.*, 2006; FAO & WHO, 2008b; Gemmell & Schmidt, 2012). The demand for water is currently in excess of water available in river basins. The country has a mean annual rainfall of approximately 490 mm, which is half the world average (SAPA, 2010). Only 9% of the annual rainfall is converted to river runoff (UNEPFI, 2010). Most of the available fresh water resources in South Africa are almost fully utilised and under stress. To ensure a future for this country, no unnecessary waste of water should occur (Paulse *et al.*, 2009).

Most of South Africa's water resources are stored in dams, and water abstraction schemes. This water allows for the adequate functioning of industry, for domestic as well as agricultural uses (Paulse *et al.*, 2009). Commercial and small-scale farmers generally irrigate their crops with water from nearby dams, ponds, rivers, streams and wells (Ijabadeniyi *et al.*, 2011). Irrigation water of an acceptable quality is required for profitable and sustainable crop production (Van der Laan *et al.*, 2012).

There are three main sources of irrigation water available; these include ground-, surface- and wastewater (Steele & Odumeru, 2004). Groundwater is primarily located in aquifers under the earth's surface. Surface water consists of various fresh water sources such as ponds, lakes, rivers and creeks. Wastewater is commonly referred to as human or animal sewage and is increasingly used for irrigation purposes due to a rapid increase in population growth, urbanisation and climate change (Steele & Odumeru, 2004; WHO, 2006a; Gemmell & Schmidt, 2012). Irrigation with wastewater can increase the available water supply in a country dramatically and is able to provide important nutrients for crops thereby saving fertiliser costs (Gemmell & Schmidt, 2012). However, the application of wastewater should be carefully considered since improperly treated wastewater can contain high levels of foodborne pathogens (Steele & Odumeru, 2004; Battilani *et al.*, 2010; Gemmell & Schmidt, 2012).

Since groundwater availability is limited by predominantly hard rock geology in South Africa, surface water is a more widely available resource. In areas where groundwater is available, it is frequently over exploited (UNEPFI, 2010).

The Cape Town region experiences rainfall throughout the year, but most precipitation occurs during winter (UNEPFI, 2010). In comparison, Johannesburg's rainfall season is in the summer months. As can be derived from the different rainfall seasons, the Western Cape's agriculture is significantly different from that of the rest of South Africa. This significant difference can be ascribed to the large differences in physical resources. The winter rainfall region of the Boland and the year-round rainfall of the Southern Cape provide an unique crop mix and productive potential due to agricultural conditions. The Boland region has always been known for its stability in agriculture production (PDC, 2005). Aforementioned can be attributed to a stable and relatively adequate winter rainfall and is supported by a well-developed infrastructure for both input supply and output processing (PDC, 2005; UNEPFI, 2010).

Primary agriculture is a very important sector in the South African economy despite its relatively small share of the total gross domestic product (GDP) (DAFF, 2011; Huisamen, 2012). Agriculture is not only a major earner of foreign exchange, it also contributes significantly to providing job opportunities, especially in rural areas (DAFF, 2011). Agriculture in the Western Cape contributes substantially to the amount of available job opportunities which include approximately 8 500 commercial and 2 500 small scale farmers and more or less 220 000 jobs for farm workers (Huisamen, 2012).

The total estimated value of agricultural production in South Africa in 2010 was R138 904 million, while it contributed approximately R60 billion to the GDP. Since 1970 the primary agricultural sector has grown by an average of approximately 11.8% per annum. In the same time period South Africa's total economy grew by 14.9% per annum, resulting in a drop in agriculture's share of the GDP from 7.1% in 1970 to 2.5% in 2010. The gross farming income from all agricultural products was estimated at R131 699 million for the time period 31 July 2010 to 30 June

2011. For the same time period the gross income from horticultural products rose by 0.7% (DAFF, 2011).

Fruit farming is one of the largest contributors to agriculture in the Western Cape. Growing conditions in this region are ideal for both soft citrus and deciduous fruit. Since 1990, the total value of citrus production has increased by 9.9% a year and the trend is expected to continue in years to come (PDC, 2005).

The Western and Eastern Cape provinces are the main deciduous fruit producing areas in South Africa. Deciduous fruits are mainly grown in areas with warm, dry summers and cold winters. It was estimated that approximately 75 025 hectares of land was covered with deciduous fruits in the 2010 season in these two provinces. The production of deciduous fruit decreased by 6.3%, from 1 679 million tons in 2009 to 2010 to 1 573 million tons in 2010 to 2011 (DAFF, 2012). With more or less 2500 deciduous fruit growers, the Western Cape is currently the country's largest producer of deciduous fruit, accounting for approximately 85% of the total agricultural exports in South Africa (PDC, 2005). During 2010 to 2011, deciduous fruit contributed approximately 24.1% to the total value of horticultural products. The exporting of deciduous fruits is a major contributor to South Africa's foreign exchange. Approximately 48.2% of all the deciduous fruits produced during the 2010/2011 season was exported and as a result contributed to 75.2% of the total foreign exchange export earnings. Between October 2010 and September 2011 the total amount of horticulture produce exported amounted to 758 760 tons (DAFF, 2012).

Citrus fruit is grown in areas with subtropical conditions such as the Limpopo, Eastern Cape, Mpumalanga, Western Cape and KwaZulu-Natal provinces. It was estimated that approximately 58 101 hectares of land was covered with deciduous fruits in the 2010 season in these provinces. Citrus fruit production increased slightly, from 2 151 395 tons in 2009/2010 to 2 151 652 tons in the 2010/2011 season. South Africa is one of the major citrus fruit exporters in the world. In the 2010/2011 season, South Africa exported 1 321 369 tons of citrus fruits to the Netherlands (DAFF, 2012).

Vegetables are produced in most parts of the country. However, in certain areas farmers tend to concentrate on specific crops. The total production of vegetables, excluding potatoes, increased by 1.0% from 2 520 724 tons to 2 550 121 tons between the time periods 2009/2010 to 2010/2011. Approximately 48.0% of all vegetables produced from July 2010 to June 2011 were sold at major fresh produce markets around South Africa. Only 3.0% of all the vegetables produced in this time period were exported (DAFF, 2012).

Vegetable production is an important component of Western Cape agriculture, due to the suitability of the regional climate. Most of the fresh produce that gets exported is either from urban fresh produce markets or through farmers. More than 150 million tons of fresh vegetables go through the Epping Fresh Produce Market in Cape Town annually. Of this more or less 50% of produce gets sold via the informal sector, produced under contract for major supermarket chains, for example WoolWorths, or exported, largely to the European Union. A vast amount of different

vegetables are produced annually in the Western Cape, in addition to this the region is also responsible for the production of 80 - 90% of the national vegetable seed production (PDC, 2005).

The Western Cape has the highest rate of growth and development of fresh produce of all nine provinces. Agriculture is one of the largest contributors to the Western Cape's economy. The province contributes approximately 14.0% to the country's GDP and generates approximately 23.0% of the total value added in the agriculture sector in South Africa. Fruit, poultry and eggs, winter grains, viticulture and vegetables together comprise more than 75% of the total agricultural output in the province. The aforementioned commodities are only a few of the contributors. In total the Western Cape has as many as 11 commodities that contribute significantly to agriculture production. As a result, the diversity of agriculture enterprises available in the province also contributes to agriculture's general stability (Ijabadeniyi, 2010).

Since fruit and vegetable export not only from the Western Cape, but also the whole of South Africa, makes such a large contribution to the country's economy, it is important to insure that all fresh produce that are exported are safe for consumption (PDC, 2005).

## POTENTIAL SOURCES OF CONTAMINATION

Contamination of fresh produce can occur before or during harvest, while processing as well as during distribution (Brackett, 1999; Panigrahy *et al.*, 2011). Almost every step from 'farm-to-fork' can have an impact on the microbiological safety of food, especially fresh produce. For many years the responsibility of ensuring safe food rested on the processor, but in the case of fresh produce, events which occurred years before the crop was planted can have an effect on bacteriological quality and safety of the final product produced (Brackett, 1999).

Contamination of fruits and vegetables can be divided into pre-harvest and postharvest sources of contamination (Beuchat, 2002). Potential pre-harvest sources of fresh produce contamination include domestic and wild animals, dust, faeces, inadequately composted manure, human handling, insects, irrigation water as well as water used to apply insecticides and fungicides. The main source of pre-harvest contamination of fresh produce happens in the field and is due to the use of water of questionable microbial quality for irrigation purposes (Beuchat & Ryu, 1997; Steele & Odumeru, 2004; Beuchat, 2006; Johnston *et al.*, 2006; Bourquin, 2009; Panigrahy *et al.*, 2011).

A field on which livestock and wild animals had access, is more likely to be contaminated with enteric pathogens than fields' animals could not access (Tauxe, 1997; Panigrahy *et al.*, 2011). Other important considerations include fields prone to flooding, since waters that covered areas where animals grazed are capable of gaining access to crop fields and contaminating the soil and produce as well as nearby rivers (Brackett, 1999). Thus farmers should not make use of untreated manure for fertiliser (Panigrahy *et al.*, 2011). Potential postharvest sources of fruit and vegetable contamination include faeces, human handling, harvesting equipment, transport containers, domestic and wild animals, insects, dust, wash and rinse water, improper storage and cross

contamination just to name a few examples (Duffy *et al.*, 2005; Ijabadeniyi, 2010; Panigrahy *et al.*, 2011).

### CONTAMINATION LEVELS OF SOUTH AFRICAN RIVERS

Several studies performed in the last decade found that the water quality of many South African rivers declined dramatically due to an increase in pollution levels (Paulse *et al.*, 2009; Ackermann, 2010; Ijabadeniyi, 2010; Lötter, 2010; Kikine, 2011; Gemmell & Schmidt, 2012; Huisamen, 2012). Several factors contribute to the condition of South Africa's rivers including pollution with improperly treated human, industrial and municipal wastes due to improperly functioning or damaged sewage treatment plants, storm water overflows as well as agricultural effluent run-off (Schultz-Fademrecht *et al.*, 2008; Lötter, 2010). Another major source of source water contamination in South Africa is because of informal settlements that are present upstream from areas of a river used for irrigation, thus all the waste and effluents produced wind up polluting the natural water sources and contribute to crop contamination (PDC, 2005; Lötter, 2010). As a result of water source contamination, not only the water source, but also the type of irrigation system used can have an effect on the amount of pathogens present on crops (Brackett, 1999; Bourquin, 2009).

Many farmers in South Africa's agricultural community use water from nearby rivers for crop irrigation, since it is the most affordable and sometimes only source of water available to them. It is thus of utmost importance that the farmers know the quality of the water they use to irrigate crops, since pathogens can be carried over from water onto produce (Ijabadeniyi *et al.*, 2011).

Paulse *et al.* (2009) investigated and compared the microbiological contamination levels from June 2004 till June 2005 on the Plankenburg River as well as from March 2005 till November 2005 on the Berg River. They tested samples from various sites. The average faecal coliforms and *E. coli* counts recorded in the Plankenburg River were both 3 500 000 cfu.100 mL<sup>-1</sup>. The average faecal coliforms and *E. coli* counts recorded in the Berg River was 17 000 000 cfu.100 mL<sup>-1</sup> and 2 500 000 000 cfu.100 mL<sup>-1</sup>, respectively (Paulse *et al.*, 2009).

In an exploratory study Ackermann (2010) tested the microbiological and water chemistry of the Berg and Plankenburg Rivers at different sites. Faecal coliform counts ranging from 540 to 1 700 000 cfu.100 mL<sup>-1</sup> and 490 to 160 000 cfu.100 mL<sup>-1</sup> were found for the Berg and Plankenburg Rivers, respectively. Potential human pathogens such as *Salmonella*, *Staphylococcus*, *Listeria*, endospore-formers, *E. coli* and intestinal *Enterococci* were frequently isolated from all the sites that were sampled.

Ijabadeniyi (2010) tested the bacteriological quality as well as physico-chemical parameters on water from an irrigation canal from the Loskop Dam and the two rivers, Olifants and Wilge, which fed the dam. *Staphylococcus aureus* was found in 25.0%, 33.0% and 58% of the water samples taken from the Olifants River, Wilge River and the Loskop Dam canals respectively. Coliform and faecal coliform levels of the rivers were determined and only met the international

standard (1 000 MPN.100 mL) once during all the times Ijabadeniyi (2010) tested the water samples. Several of the water samples Ijabadeniyi (2010) tested, were also positive for the presence of *E. coli*, intestinal *Enterococcus* as well as *Salmonella*.

While testing river water samples, for agricultural purposes in the Western Cape, Lötter (2010) found faecal coliform counts as high as 160 000 cfu.100 mL<sup>-1</sup> in the Plankenburg River, while counts as high as 460 000 cfu.100 mL<sup>-1</sup> were observed in the Mosselbank River. Apart from this, high numbers of *Staphylococci* and intestinal *Enterococci* were often found, while *E. coli*, *Listeria* and *Salmonella* were always present in all samples taken from both of these rivers.

Kikine (2011) performed a baseline study on the Plankenburg and Eerste Rivers to determine the microbiological quality of the water. The Plankenburg River had much higher coliform counts, ranging from 1 200 to 13 000 000 MPN.100 mL<sup>-1</sup>, than the Eerste River site where the counts ranged between 230 and 79 000 MPN.100 mL<sup>-1</sup>. He also found high levels of *Salmonella*, *Staphylococcus*, *Listeria* and endospore formers in the river water samples.

Gemmell en Schmidt (2012) conducted a study on the Baynespruit River in Sobantu, a suburban area in Pietermaritzburg. They tested the physico-chemical and microbiological parameters of the river water to determine its acceptability for crop irrigation. They found faecal coliform counts of up to 1 600 000 cfu.100 mL<sup>-1</sup> in the river water samples and 160 000 per gram on the produce that were tested.

Huisamen (2012) examined the microbial loads of the Plankenburg and Eerste Rivers and found high faecal coliforms and *E. coli* concentrations, ranging from 310 to 7 000 000 cfu.100 mL<sup>-1</sup> and 230 to 7 000 000 cfu.100 mL<sup>-1</sup>, respectively.

The recommended irrigation water guidelines of  $\leq 1000$  (WHO, 1989) and  $\leq 4\ 000$  cfu.100 mL<sup>-1</sup> (DWAF, 2002) for faecal coliforms and *E. coli*, respectively were mostly exceeded in all the tested water samples, over the years, indicating faecal pollution and thus a high health risk (Gemmell & Schmidt, 2012). Faecally polluted water is of great concern to farmers, field workers, fresh produce retailers and consumers, because the contaminated water source is often utilised for irrigation of fresh or minimally processed fruits and vegetables (Tauxe, 1997; Warrington, 2001; WHO, 2006).

From the studies done in previous years (Paulse *et al.*, 2009; Ackermann, 2010; Ijabadeniyi, 2010; Lötter, 2010; Kikine, 2011; Gemmell & Schmidt, 2012; Huisamen, 2012), it can be concluded that the water from all of the different river sites were not suitable for agricultural irrigation purposes as they regularly exceeded the guidelines for faecal coliforms and *E. coli* as set out by South African guidelines (WHO, 1989; DWAF, 2002).

### **Different irrigation systems**

Irrigated agriculture plays an important role in South Africa. In 2011 irrigated agriculture was the largest user of runoff water in South Africa. The government wants the agriculture sector to become more efficient and as a result reduce water consumption in order to increase the amount

of water available for domestic use. Currently more than 1 600 000 hectares of land is irrigated in South Africa. Years of research showed that the type of irrigation system used can have a major influence on the amount of water used annually for irrigation purposes (Reinders, 2011).

The purpose of an irrigation system is to apply the desired amount of water, at the correct application rate and uniformly to the whole field, at the right time, with the least amount of water losses and as economically as possible (Reinders, 2011).

Three very distinct groups of irrigation systems are used in South Africa, namely flood, mobile and static irrigation systems (WHO, 2006; Reinders, 2011). The most common type of flood system is furrow where water infiltrates the land, by means of gravity, while flowing over the soil other types include basin and border. Centre-pivot, linear and travelling-gun systems are a means of mobile irrigation and move over field surfaces, without help, while irrigating the crop from above. Static systems are defined as a system that remains stationary throughout the irrigation process and two types are primarily used namely sprinkler and micro-systems (i.e. permanent or portable like quick-coupling, drag-line, hopalong, big-gun, side-roll and boom irrigation systems, micro-sprayers, minisprinklers and drip-irrigation systems) (Reinders, 2011). All of the irrigation systems have distinct advantages and disadvantages and in some cases special measures have to be taken to protect consumers, farm workers, animals as well as the public that might have access to crop fields (Table 1) (Warrington, 2001; WHO, 2006).

Choosing a specific irrigation system is a difficult decision since the various systems each have a wide field of application. Many factors play a role in choosing the correct system, for instance water quality, the type of soil to be irrigated, the slope of the land to be irrigated, the crop to be irrigated, the number of labourers that are available and, of course, the amount of money the farmer is able and or willing to spend (Koegelenberg, 2007).

The location and composition of the field where crops are grown, the type of irrigation system used and the surface of the irrigated produce are only a few of the factors contributing to the contamination of fresh produce (Gerba & Choi, 2006). If the edible part of a crop grows in or near soil, contamination is more likely to occur than for fruits growing further up from the ground (Battilani *et al.*, 2010). Some fruits or vegetables have open or grooved structures that may retain water and as a result contaminate the plant (Gerba & Choi, 2006). Enteric pathogens are extremely resistant to environmental conditions and can survive for extended periods on crops, in water and in or on soil (Song *et al.*, 2006). It is thus of great importance to choose carefully which irrigation system type to apply to specific produce to prevent unnecessary contamination (Gerba & Choi, 2006). If wastewater is the only source available for irrigation, subsurface drip irrigation could be used to prevent or reduce contamination of crops, it can increase crop yield and reduce health risks through minimum exposure of contaminated water to people or crops being irrigated (Song *et al.*, 2006). Even though drip irrigation is one of the most efficient irrigation systems, a World Research Commission (WRC) supported project found evidence that even this system is fallible if mismanagement and maintenance problems are evident (Reinders, 2011).

## Chapter 2

**Table 1** Different irrigation water application systems (WHO, 2006; Koegelenberg, 2007; Reinders, 2011)

<b>Irrigation technique</b>	<b>Advantages</b>	<b>Disadvantages and special measures needed in wastewater irrigation</b>
<b>Flood</b>	Lowest capital cost Low energy Plant self does not get wet, preventing contamination Exact levelling not required Irrigation is not affected by climatic and water quality characteristics	Great water losses may occur if the system is not well designed and maintained May lead to waterlogging and soil salinity if there are no provisions for adequate drainage The system is labour intensive Fieldworkers, crop handlers and consumers need protection against water
<b>Furrow</b>	Low cost Low energy Plant self does not get wet, preventing contamination	Great water losses may occur if the system is not well designed and maintained May lead to waterlogging and soil salinity if there are no provisions for adequate drainage The system is labour intensive Levelling may be needed Fieldworkers needs to wear protective gear to prevent contamination
<b>Spray and sprinkler</b>	Medium water use efficiency Levelling not required Low labour need Advanced sprinklers capable of reducing exposure to pathogens by 1 log unit have been developed New technologies prevent spray drift and might be able to reduce crop contamination by better targeting Permanent systems is not so sensitive to wind as movable systems Able to leach out salts from the soil	High cost High energy requirement Some crops are prone to more contamination Irrigation system should be at least 50 - 100 metres from houses and roads Moving of pipes of movable systems may damage crops
<b>Subsurface and localised (drip, trickle and bubbler)</b>	Most water-efficient method of irrigation Higher yields Potential for significant reduction of crop contamination Localised and subsurface irrigation systems can reduce exposure to pathogens by 2 – 6 log units	Highest cost Reliable filters are necessary to prevent the system from becoming clogged Systems must be properly managed to insure successful irrigation

**Irrigation water standards**

Irrigation water standards for crops were initially created to protect consumers, farm workers, animals as well as the public that might have access to crop fields. The type of irrigation system used, the crop that is grown and how the crop is consumed, raw or cooked, all plays a role in how strict irrigation standards are (Warrington, 2001; WHO, 2006). As a result, to insure the safety of

others, it was of utmost importance to construct a set of water quality guidelines for irrigation water to ensure that the water used is safe for its intended use (Ackermann, 2010). Parameters that may influence water quality and have a negative effect on the environment include pathogens, coliforms, salts, metals, toxic organic compounds, nutrients (i.e. nitrogen, phosphorous and potassium), organic matter, suspended solids as well as pH (Asano, 1987; Freese *et al.*, 2003; DWAF, 2004; WHO, 2006; McCaffrey, 2011).

Salinity is a measure of the dissolved salts that are present in water and usually increases as water levels decrease. Salinity is measured as either total dissolved solids or as electrical conductivity (McCaffrey, 2011). Wastewater use will always increase the salinity of soils and groundwater, because it contains a lot more salts than fresh water sources (WHO, 2006). For wastewater irrigation in South Africa, the electrical conductivity of the water may range between 70 to 200 milliSiemens (DWAF, 2004). Excessive irrigation and runoff containing water from agriculture may increase water's salinity levels (Bellingham, 2009; McCaffrey, 2011). It is important that the salt content of water used to irrigate crops is not too high, since it might damage crops or in some cases even cause soil permeability problems. It was found that water containing more than 500 mg.L<sup>-1</sup> total dissolved solids is unsuitable for irrigation of many plants and might impart an unpleasant taste on the water (McCaffrey, 2011).

Water's pH is measured to determine its acidity and alkalinity and may vary within different water sources (McCaffrey, 2011; Elqert, 2012). The generally accepted range for pH in municipal water is 6.5 to 8.5 with an upper limit of 9.5, but these ranges may vary between 5.5 and 9.5 in South African wastewater sources (Asano, 1987; DWAF, 2004; Elqert, 2012). It is important to take the pH of water into consideration when it is used for irrigation since certain crops require specific pH ranges for optimum growth (WHO, 2006).

Turbidity is a measurement of how light scatters when it is aimed at water and bounces off the suspended particles such as clay, silt, finely divided organic and inorganic matter, plankton and other microscopic organisms which are naturally suspended in irrigation water; it is not a measurement of the particles themselves. Measuring turbidity gives an estimate of suspended solids in the water and is measured in nephelometric turbidity units (NTU) (McCaffrey, 2011; Elqert, 2012). Though high turbidity is often a sign of poor water quality and land management, crystal clear water does not always guarantee healthy water. Extremely clear water can signify very acidic conditions or high levels of salinity (McCaffrey, 2011).

Chemical oxygen demand (COD) is a measurement of the amount of organic pollutants that are present in irrigation water (Ackermann, 2010). If the oxygen levels in irrigation water are high, it can be presumed that pollution levels in the water are low and the opposite is also true (McCaffrey, 2011). The COD is calculated by measuring the rate at which the organic matter, consumes the oxygen present in the water and is expressed in terms of milligram oxygen per litre of water (Ackermann, 2010). When wastewater is used for irrigational purposes in South Africa, the COD level is not allowed to exceed 75 mg.L<sup>-1</sup> when irrigating an area with up to 2 000 cubic

**Chapter 2**

metres of water. The COD value increases as the amount of water to be irrigated, decreases (DWAF, 2004).

Faecal coliforms are naturally occurring bacteria found in the intestines of all warm blooded animals and humans as well as birds. The presence of faecal coliforms in water is an indicator of faecal contamination (McCaffrey, 2011; Elqert, 2012). Coliforms are useful indicators of the possible presence of pathogenic bacteria and viruses (Elqert, 2012). When up to 2 000 cubic metres of wastewater are used to irrigate crops, in South Africa, faecal coliforms are not allowed to exceed 1 000 cfu.100 mL<sup>-1</sup> (DWAF, 2004).

Fruits and vegetables that are consumed raw can sometimes not be washed to remove all pathogens and they often do not undergo any processing steps to kill pathogens later, before consumption (Warrington, 2001; Gerba & Choi, 2006; Battilani *et al.*, 2010). Treated wastewaters can be used to irrigate crops in areas where relatively clean water is not available for crop irrigational purposes. The World Health Organization (WHO) as well as the Department of Water Affairs and Forestry (DWAF) published guidelines for the microbiological quality of treated wastewaters for use in agriculture and aquaculture (WHO, 1989; DWAF, 2004).

The guidelines were for restricted and unrestricted irrigation (Lazarova & Bahri, 2005; Mara *et al.*, 2007). Restricted irrigation guidelines were applied for all crops that are cooked before consumption. Unrestricted irrigation guidelines included parameters applicable to the irrigation of all fruits and vegetables that are consumed raw. According to the guidelines for the unrestricted irrigational use of treated wastewater, water may only contain  $\leq 1$  human intestinal nematode egg and faecal coliforms should be less than 1 000 cfu.100 mL<sup>-1</sup> (WHO, 1989; DWAF, 2004).

In later years research in quantitative microbial risk analysis (QMRA) and epidemiological based studies contradicted the WHO guidelines for coliforms and proposed that faecal coliform counts should be undetectable or  $\leq 2.2$  total coliforms per 100 mL since irrigation with improperly treated wastewater could lead to illness (Mara *et al.*, 2007).

**FRESH PRODUCE RELATED FOODBORNE OUTBREAKS**

In the past three decades consumers has started consuming more fruits and vegetables as they became increasingly aware of their health (Brackett, 1999; Pollack, 2001; Buck *et al.*, 2003; FDA, 2008; Gravani, 2009; Lynch *et al.*, 2009; Panigrahy *et al.*, 2011). Higher incomes, increased domestic production, product convenience, consumer awareness of the importance of consuming fresh produce, technological improvements that maintain the quality of fresh fruits and vegetables for a longer time and greater availability and diversity of products due to trade are additional factors that contributed to an increase in fruit and vegetable consumption (Brackett, 1999; Pollack, 2001; Buck *et al.*, 2003; Heaton & Jones, 2008). An increase in at risk populations (children, immune-compromised individuals, pregnant and elderly), enhanced epidemiology surveillance, improved methods of identifying and tracking pathogens as well as the emergence of pathogens with low infective dose has also contributed immensely to an increase in fresh produce related foodborne

## Chapter 2

outbreaks being reported (Tauxe, *et al.*, 1997; Lynch *et al.*, 2009; Ijabadeniyi, 2010).

Reported foodborne outbreaks due to the consumption of fresh produce will thus vary between developed and developing countries (Ijabadeniyi, 2010). Developed countries such as Europe and USA may have higher reported cases of foodborne outbreaks due to enhanced epidemiology surveillance that are in place (Lynch *et al.*, 2009).

As mentioned before the epidemiology of foodborne disease is changing (Tauxe, 1997; Johnston *et al.*, 2006; Taeye, 2010). New pathogens have emerged and in recent times it is easy for them to be spread worldwide. A wide array of new food vehicles of transmission have also been implicated in recent years. In the past foods of animal origin were implicated in foodborne outbreaks, across the world. Only in recent years foods, such as fruits and vegetables, previously thought of as safe were considered as hazardous (Tauxe, 1997; WHO, 2006; Lynch *et al.*, 2009). Fresh produce poses a food safety risk because they are mostly consumed raw or are only minimally processed (Abadias *et al.*, 2008; Bourquin, 2009). It was discovered that contamination of fruits and vegetables typically occur early in the production process, rather than just before consumption (Tauxe, 1997; Ackerman, 2002).

In the past there was no relationship between specific pathogenic microorganisms being present on a specific food product, but due to an immense amount of research a link between certain pathogens and food combinations have emerged. These food-pathogen pairs may shed more light on the mechanisms and routes involved that takes place during the contamination process (Johnston *et al.*, 2006; Lynch *et al.*, 2009).

Fruits and vegetables can become contaminated during various stages in the production process namely while still growing in the fields, during harvest, while being handled, during processing and distribution as well as during consumption (Brackett, 1999; EC, 2002; Johnston *et al.*, 2006; Panigrahy *et al.*, 2011).

A produce-associated foodborne outbreak is commonly defined as the occurrence of two or more reported cases of the same illness in which the same uncooked fruit, vegetable, salad or juice was implicated in an epidemiologic investigation (Sivapalasingam *et al.*, 2004). After Sivapalasingam *et al.* (2004) analysed the Foodborne Outbreak Surveillance System data in the United States for 1973 through 1997, it was found that 190 produce-associated outbreaks were reported between these years. During these 190 outbreaks, 16 058 illnesses were reported, 598 hospitalisations occurred and eight people died. Fresh produce most frequently implicated in foodborne outbreaks included salad, lettuce, juice, melons, sprouts and berries. In 103 of the 190 produce-associated outbreaks, the pathogen responsible for illness was identified, 62 of which were caused by bacterial pathogens (Sivapalasingam *et al.*, 2004).

In the USA, outbreaks linked to fresh produce increased from <1% of all reported foodborne outbreaks with known food vehicle in the 1970s to 6% in the 1990s. The median size of fresh produce related foodborne outbreaks increased from <1% to 12% in the USA (Lynch *et al.*,

2009). Each year in the USA 76 million people suffer from foodborne disease, 325 000 of them are hospitalized and 5,000 die (Ackerman, 2002; Taeye, 2010). Fresh produce accounted for 4% of all foodborne outbreaks reported between 2001 and 2005, in Australia (Lynch *et al.*, 2009). Even though foodborne illness is a common occurrence in South Africa, finding literature reporting foodborne outbreaks related to consumption of contaminated fresh produce is uncommon. This can be attributed to a lack of acceptable surveillance systems, the lack of an established data basis for the documentation of foodborne outbreaks as well as misinformed consumers (Taeye, 2010; Niehaus *et al.*, 2011; Huisamen, 2012).

To date the world's largest reported fresh produce-associated outbreak occurred in 1996. More than 6000 cases of *E. coli* O157:H7 infection were reported in Japan and resulted in four deaths. Raw radish sprouts that had been prepared in central kitchens appeared to have transmitted the pathogen. In the past sprout-related disease outbreaks have also been reported in the United Kingdom, Finland, Denmark, Sweden and Canada (Buck *et al.*, 2003).

Also in 1996 raspberries, contaminated with *Cyclospora*, were imported into the United States and caused a large epidemic. Contaminated surface water used to spray the berries with fungicides before harvest was later implicated as the possible cause of the outbreak (Tauxe, 1997).

In 2006 an *E. coli* O157:H7 outbreak, due to the consumption of fresh spinach, affected 26 states in the United States and was responsible for approximately 200 cases of illness, including some of Hemolytic Uremic Syndrome (HUS) and resulted in three deaths (Abadias *et al.*, 2008).

In December 2008, 216 people presented to a local hospital in KwaZulu-Natal with symptoms of gastroenteritis. After microbial investigations were performed, it was found that *Salmonella* species was the cause. The patients contracted it after consuming a meal at a local primary school and presented with symptoms within a ten day period. The meal consisted of beef stew, chicken, rice, beetroot salad, coleslaw, kidney bean salad, pumpkin, chakalaka, fruit juice, tomatoes and pineapple. A sample of the food was tested to determine a specific food vehicle, but since all of the food was stored in one container, the specific source responsible could not be determined (Niehaus *et al.*, 2011).

In the beginning of 2011 an outbreak of *E. coli* O104:H4 initially occurred in Northern Germany but also led to some outbreaks in France. Most of the more than 4 000 victims that fell ill came from Germany. More than 50 people died and approximately 1 000 cases of HUS were reported. In the end fenugreek seeds were implicated as the cause of the outbreak. To date this was probably the most devastating case of produce-associated outbreaks (Griffith, 2011).

By the end of March 2012 the Democratic Republic of Congo (DRC) already experienced approximately 8 000 cases of cholera this year alone. In these three months 120 deaths had been recorded. The Eastern DRC was the province most affected by these outbreaks. Cholera is an acute intestinal infection caused when individuals that come into contact with or consumes contaminated food and water. The DRC has not had water and sanitation systems that function

**Table 2** Foodborne outbreaks associated with fresh and minimally processed fruits and vegetables (Tauxe, 1997; Beuchat, 2002; Buck *et al.*, 2003; Tournas, 2005; Johnston *et al.*, 2006; Abadias *et al.*, 2008; Lynch *et al.*, 2009; Griffith, 2011 & Anon., 2012b)

Year	Country	Pathogen	Fruit or vegetable source
1990	Central America	<i>Salmonella chester</i>	Cantaloupe
1990	United States	<i>Salmonella javania</i>	Tomatoes
1990	United States	Hepatitis A	Strawberries
1991	USA / Central America	<i>Salmonella poona</i>	Cantaloupe
1992	USA	<i>Giardia lamblia</i>	Raw vegetables
1993	USA	<i>E. coli</i> O157:H7	Apple cider
1994	Central America	<i>Shigella flexneri</i>	Scallions
1995	USA	<i>E. coli</i> O157:H7	Leaf lettuce
1996	Japan	<i>E. coli</i> O157:H7	Radish sprouts
1996	United States	<i>E. coli</i> O157:H7	Leaf lettuce
1996	United States	<i>Cyclospora</i>	Raspberries
1997	Peru	<i>Cryptosporidium parvum</i>	Raw vegetables
1997	Central America	<i>Cyclospora</i>	Raspberries
1997	USA	<i>Salmonella infantis</i>	Sprouts
1998	USA	<i>Shigella sonnei</i>	Parsley
1998 / 1999	USA	<i>Salmonella typhi</i>	Mamey
2000	Australia / China	<i>Salmonella</i>	Bean sprouts
2003	USA	Hepatitis A	Green onions
2005	Denmark	<i>Cryptosporidium hominis</i>	Carrots / red peppers
2006	New Jersey	<i>E. coli</i> O157:H7	Green onions
2006	North America (California)	<i>E. coli</i> O157:H7	Spinach
2007	Europe	<i>Salmonella</i>	Alfalfa sprouts
2007	Australia / Denmark	<i>Shigella sonnei</i>	Raw baby corn
2008	North America	<i>Salmonella</i>	Peppers / tomatoes
2008	United States	<i>Salmonella enterica</i>	Raw peppers / tomatoes
2011	Northern Germany	<i>E. coli</i> O104:H4	Fenugreek seeds
2011	Oregon	<i>E. coli</i> O157H7	Strawberries

properly in terms of sewage, draining and access to clean water, in many years. Until the aforementioned is not properly implemented, the death toll will most likely continue to rise in the DRC (DeCapua, 2012).

In December 2013 one of the biggest *Cyclospora* outbreaks occurred due to affected salads and cilantro at two different restaurants (CDC, 2013). Two outbreaks occurred, one in Iowa and Nebraska and the other is Texas. In total 631 illnesses were reported.

## **MICROORGANISMS OF CONCERN ASSOCIATED WITH FRESH PRODUCE**

The transmission of foodborne illness on foods of animal origin is well established, but awareness only in recent years started to grow that fresh or minimally processed fruits and vegetables can also be sources of pathogenic related foodborne illnesses (Steele & Odumeru, 2004).

There are four main groups of waterborne pathogenic microorganisms responsible for foodborne illnesses, namely bacteria, protozoa, viruses and helminths (ANZECC, 2000; Steele & Odumeru, 2004; GHD, 2005; WHO, 2006). The amount of pathogenic microorganisms that are present in surface water is primarily affected by the amount of precipitation, the season of the year as well as the socioeconomic status of the community (Nasser, 2005). *Campylobacter* spp., *Clostridium botulinum*, *Clostridium perfringens*, enterotoxigenic *Bacillus cereus*, *Escherichia coli* O157:H7, *Listeria monocytogenes*, *Salmonella* spp., *Shigella* spp., enterotoxigenic *Staphylococcus aureus*, *Vibrio cholerae* and *Yersinia enterocolitica* are all pathogenic bacteria of concern to human health. Pathogenic protozoa include *Cyclospora cayetanensis*, *Cryptosporidium parvum*, *Giardia lamblia* and *Entamoeba histolytica*. Hepatitis A, enteroviruses, echoviruses, rotaviruses and Norwalk-like viruses are all examples of pathogenic viruses. An example of a helminth pathogen that can be transmitted by food is the nematode (roundworm) *Ascaris lumbricoides* (Droste, 1997; ANZECC, 2000; Steele & Odumeru, 2004; GHD, 2005; Sela & Fallik, 2009).

The survival and or growth of pathogenic microorganisms are affected by intrinsic and extrinsic factors as well as processing factors. These factors include nutrient composition, pH, presence of scales and fibres, redox potential, temperature and gaseous atmosphere. Mechanical shredding, cutting and slicing of the produce are only a few of the processing factors responsible for opening up the plant's surface to microbial attack (Beuchat, 2002; EC, 2002; Sela & Fallik, 2009).

Bacteria of faecal origin give rise to the greatest concern as it is very difficult to control or prevent entry into the river systems (Oliver *et al.*, 2005; Ackermann, 2010). Although some pathogenic microorganisms are commonly found in the environment, the presence of some pathogens is indicative of recent human or animal faecal contamination (ANZECC, 2000; Steele & Odumeru, 2004; GHD, 2005; Sela & Fallik, 2009; McCaffrey, 2011; Elqert, 2012). An assumption commonly made in microbial water quality risk assessment models is that human faecal matter poses a much greater risk than faecal matter of animal origin (Chaidez *et al.*, 2005; Santo-Domingo & Ashbolt, 2010). This means that microbial contamination due to faecal matter

contamination is more manageable since human activities are more controlled than that of animals. These are mainly pathogenic bacteria and therefore should fruits or vegetables become contaminated with them, it will pose a health risk to the consumers (Ackermann, 2010; McCaffrey, 2011; Elqert, 2012). Once river water has been contaminated, little can be done to improve the quality of the water. Therefore if possible, contaminated water should not be used to irrigate fresh produce (Ackermann, 2010).

Water can be a vector for many microorganisms, including pathogenic strains such as *Escherichia coli*, *Vibrio cholerae*, *Cryptosporidium* and *Giardia* which are most often associated with waterborne diseases (Leclerc *et al.*, 2002; Coetzer, 2006; Wilkes *et al.*, 2009). Irrigation water is also frequently contaminated with the plant pathogen, *Phytophthora*, which is able to cause fruit rot (Yamak *et al.*, 2002; Hausbeck *et al.*, 2012). It is also important to take aerobic colony count (ACC) and total coliforms into account when assessing the acceptability of water for irrigation (WHO, 2006).

#### *Aerobic colony count*

Aerobic colony count (ACC), sometimes referred to as the total viable count, colony count, or plate count is commonly defined as the total number of bacteria able to grow in an oxygenated or aerobic environment. The ACC test is applied to indicate the microbial quality, not safety, of food (Anon., 2011). The test is used to estimate the total numbers of viable individual microorganisms present in a set volume. The counts may include bacteria, yeasts and mould species (Cheshire Scientific, 2012).

The significance of ACC results varies according to the type of food product being analysed and according to the processing it has received (Anon., 2011; Cheshire Scientific, 2012). It is of great importance to know the composition of the food tested and whether it is raw or cooked. Without this knowledge, it is extremely difficult to interpret ACC results. It is also important to test different samples at approximately corresponding shelf-life times, since foods sampled close to their expiry date will likely have ACC results approaching the upper limit (Anon., 2011).

Fruits and vegetables which are consumed raw are expected to have high numbers of bacterial organisms present from the environment in which they are grown and as a result will have high aerobic colony counts (Abadias *et al.*, 2008; Sela & Fallik, 2009). Except for bacteria from the field, pathogens resulting from the produce being past its shelf-life, poor sanitation practices, use of non-hygienic packaging materials as well as post-processing contamination such as poor food handling or hygiene practices are all factors that contribute to high ACC results (Sela & Fallik, 2009; Anon., 2011).

It is also of great importance to do multiple tests on each sample and keeping the results for comparison, since comparing a series of ACC results is of greater value than assessing a single sample (Anon., 2011).

## Chapter 2

### Coliforms

It is extremely time consuming, technically demanding and costly, depending on the methodology used, to test water for individual pathogens. As a result it is more practical to test for species of bacteria, referred to as indicator organisms that indicate that undesirable microbial pathogens are present in a sample (Jamieson *et al.*, 2002; Harwood *et al.*, 2005). An indicator organism is defined as a microorganism or group of microorganisms that indicate whether food or water has been exposed to conditions that is capable of increasing pathogen contamination (James, 2006).

For over a century coliform bacteria has been used to indicate the general bacterial quality of water and the possible presence of human pathogens in the tested water (Tate & Arnold, 1990; Jamieson *et al.*, 2002; Bitton, 2005). Coliforms are naturally present in the intestinal tract of humans and warm-blooded animals and millions are excreted in faecal matter. Apart from humans and animals, coliform bacteria are also naturally present in soil and decaying organic matter (Jamieson *et al.*, 2002; Bitton, 2005). Coliforms are relatively easy to detect and quantify in a given sample, most probably because they survive longer in water than most bacterial pathogens. If a water sample tests positive for the presence of coliforms, the chance of it being contaminated with faecal matter is great. In the case of a positive test result, further tests need to be performed to establish if pathogens are present. If a water sample does not contain any coliforms it is relatively safe to assume that no pathogenic organisms are present (Jamieson *et al.*, 2002).

Coliforms are further divided into total coliforms and faecal coliforms (Tate & Arnold, 1990; Jamieson *et al.*, 2002; Bitton, 2005). Total coliforms refer to the entire group of coliform bacteria and belong to the family *enterobacteriaceae* which include aerobic as well as facultative anaerobic, gram-negative, non-sporeforming and rod-shaped bacteria such as *Escherichia coli*, *Enterobacter*, *Klebsiella* and *Citrobacter* (Bitton, 2005; Wilkes *et al.*, 2009). These coliforms are excreted in large amounts in animal and human faeces, but are also naturally present in the environment (Bitton, 2005; Anon., 2011). In water treatment, total coliforms are one of the best indicators to determine the efficacy of disinfectants and are used worldwide as an indicator of faecal pollution (Bitton, 2005; Park *et al.*, 2006). Elevated counts of total coliforms on the surface of fruits and vegetables may be as a result of inadequate processing, extended shelf-life and post-processing contamination (Anon., 2011).

Total coliforms have no definite origin, whereas faecal coliforms are only present in the intestines of warm-blooded animals (Jamieson *et al.*, 2002; Bitton, 2005). Faecal coliforms, also referred to as thermotolerant coliforms, comprise of bacteria such as *Escherichia coli* and *Klebsiella pneumoniae* (Bitton, 2005). They provide stronger evidence of the possible presence of faecal contamination than do total coliforms (Tate & Arnold, 1990; Bitton, 2005).

### Bacterial pathogens

#### *Escherichia coli*

Human enteric pathogens such as *E. coli* O157:H7, *Salmonella* species, *Cryptosporidium* species and enteric viruses have been found in environmental waters due to faecal pollution from various

sources (Masters *et al.*, 2011). Since it is time consuming, difficult and costly to test water for individual pathogens, an indicator organism is tested for instead (Jamieson *et al.*, 2002; Masters *et al.*, 2011).

*E. coli* is a widely studied genus of bacteria and is part of the natural intestinal flora of humans and other warm blooded mammals, optimum growth occurs at elevated temperatures (Bitton, 2005; Johnston *et al.*, 2006; Sela & Fallik, 2009; Masters *et al.*, 2011). As a result they are almost exclusively of faecal origin and their presence is a definitive indicator of a food or water source being contaminated with faecal matter (Anon., 2011; Masters *et al.*, 2011). In rare instances *E. coli* can be present in nature without any faecal contamination (Masters *et al.*, 2011).

The presence of *E. coli* in or on the surface of fruits and vegetables is usually indicative of faecal contamination. Agricultural runoff and sewer overflows may also contribute to high *E. coli* counts in environmental waters (Masters *et al.*, 2011). It is thus an indicator for the presence of enteric pathogens (Anon., 2011; Masters *et al.*, 2011).

Enterotoxigenic *E. coli* is a common cause of travellers' diarrhoea, an illness some people contract after visiting developing countries. It is believed that raw vegetables are the most common cause of travellers' diarrhoea. During a conference held in Mexico City in 1974 it was found that enterotoxigenic *E. coli* was the most common cause of illness after 59 of 121 people fell ill after consuming salads containing raw vegetables, at the conference (Harris *et al.*, 2003).

Enterohemorrhagic *E. coli* O157:H7 is a well-known food and waterborne pathogen and together with *Salmonella* is recognised as the most common bacterial entero-foodborne pathogens associated with fruits and vegetables (Johnston *et al.*, 2006; Abong'o *et al.*, 2007). Its vehicles of transmission include contaminated foods, person to person, contact with infected animals or their manure as well as the consumption of fruits and vegetables irrigated with *E. coli* O157:H7 contaminated water (Abong'o *et al.*, 2007; CADE, 2011). Contamination of fresh produce may also occur during cultivation, harvesting, packaging and transportation to consumers. It has a very low infective dose and symptoms include abdominal cramps, nausea, vomiting, chills and can escalate to bloody diarrhoea and haemolytic uremic syndrome (HUS) if gastroenteritis was part of the initial symptoms (Harris *et al.*, 2003; CADE, 2011). Haemolytic uremic syndrome can eventually lead to kidney failure. Escalating symptoms are most common in individuals with a compromised immune system, especially young children under the age of five and in the elderly (Johnston *et al.*, 2006; CADE, 2011). Outbreaks of enterohemorrhagic O157:H7 illness has been reported for raw produce such as lettuce, salad mixes, mixed vegetables, cilantro, coriander and celery (Tauxe, *et al.*, 1997; Johnston *et al.*, 2006; Lynch *et al.*, 2009; Ijabadeniyi, 2010).

*E. coli* O157:H7 grows extremely well in several types of fruits and vegetables, especially when stored at temperatures above 12°C (Harris *et al.*, 2003). Modified atmosphere packaging of fresh produce has little or no effect on the survival and growth of *E. coli* O157:H7. It has a low infective dose, can develop acid resistance and has the ability to form biofilms on fruits and

vegetables making it difficult to sanitize fresh produce after contamination (Beuchat, 2002; Harris *et al.*, 2003; Ijabadeniyi, 2010).

### *Vibrio cholerae*

Faeces of infected individuals are a source of *Vibrio cholerae* cells (IAEA, 2001). When viable cells are ingested, cholera infection occurs. The mechanisms of transmission are similar to the mechanisms of other enteric infections. The O1 and O139 serogroups are the causes of epidemic cholera (EC, 2002; Bitton, 2005). Contaminated water and consumption of a wide variety of food are the main vehicles responsible for the initiation of cholera epidemics. According to literature vegetables, meat and seafood are some of the main food groups responsible for cholera epidemics (IAEA, 2001; Mena, 2006; Ijabadeniyi, 2010; CADE, 2011).

Since a lot more consumers started to consume fresh and uncooked fruits and vegetables, cholera epidemics have grown (IAEA, 2001). Green leafy vegetables, mixed vegetables as well as others that grow close to the ground, that are irrigated with contaminated irrigation water, such as wastewater, may more frequently present as cholera vehicles than seafood or meat (IAEA, 2001; Mena, 2006; FDA, 2013). In the 1970's Israel had a cholera epidemic which was later found to be caused by vegetables that were irrigated with sewage water and as a result contained *V. cholerae*. Irradiation of fresh produce, after contamination, could be used as a safe method for decontamination (IAEA, 2001).

*Vibrio cholerae* is predominantly a waterborne pathogen and has a very high infective dose (EC, 2002; Mena, 2006). Poor sanitation and hygiene may also contribute to *V. cholerae* contamination of fresh produce (Mena, 2006). Profuse watery diarrhoea is one of cholera's main symptoms and is mainly due to the effects of a heat labile enterotoxin elaborated by the organism in the intestine of humans. Other symptoms include nausea, vomiting and leg cramps. The onset of symptoms is quite rapid and can lead to dehydration and even death only a few hours after being infected, if left untreated. The duration of the illness is usually between three and seven days (EC, 2002; CADE, 2011).

## **Protozoan pathogens**

### *Cryptosporidium*

*Cryptosporidium parvum* is a protozoan pathogen often implicated in fresh produce-related as well as several waterborne outbreaks (EC, 2002; Johnston *et al.*, 2006; Sela & Fallik, 2009). Contamination is often due to contaminated irrigation water or food handlers. *Cryptosporidium parvum* causes the illness cryptosporidiosis (EC, 2002; Bitton; 2005 Mena, 2006; CADE, 2011; Duhain *et al.*, 2011). Most cryptosporidiosis outbreaks are waterborne, but the infection is just as easily contracted by consuming food contaminated with *Cryptosporidium* species. In the past more *C. parvum* related foodborne outbreaks was due to fruit than contaminated vegetables. Outbreaks in the past have mainly been associated with raspberries, basil, mesclun lettuce and snow peas

(Johnston *et al.*, 2006; Mena, 2006; CADE, 2011; Duhain *et al.*, 2011). As of yet no correlation has ever been found between the presence of *Cryptosporidium* oocysts, the infective stage of the organism, and the presence of faecal coliforms or *E. coli* (Harris *et al.*, 2003).

It is an obligate unicellular parasite capable of surviving extreme environments, in the form of an oocyte, even chemical disinfectants such as chlorine treatment used as a common form of disinfection in water, though it is susceptible to ultraviolet light and drying (EC, 2002; Harris *et al.*, 2003; Kartaginer, 2009; Sela & Fallik, 2009; Duhain *et al.*, 2011).

There are different forms of *C. parvum* available, but currently it is believed that the form responsible for infecting humans is the same species responsible for causing disease in young calves. The form that infects birds and mice are not capable of infecting humans (Harris *et al.*, 2003; Bitton, 2005).

It causes an acute self-limiting, watery diarrhoeal illness in immuno-competent individuals and a chronic, debilitating disease in immuno-suppressed individuals (Sela & Fallik, 2009). Other symptoms include abdominal cramping, headache, nausea, vomiting as well as a low-grade fever (CADE, 2011). Some healthy individuals stay asymptomatic, but are still able to pass the pathogen on to other individuals. The infectious dose of *C. parvum* is less than 10 organisms, but as little as one organism can initiate an infection. Oocysts are shed in the infected individual or animal's faeces. Theoretically *C. parvum* oocysts can occur on any food or in water which was contaminated by coming into contact with an infected individual (Harris *et al.*, 2003; CADE, 2011).

Patients with a compromised immune system are extremely sensitive to this pathogen, especially AIDS patients and cancer patients while receiving chemotherapy (Sela & Fallik, 2009; CADE, 2011).

### *Giardia*

*Giardia lamblia* is responsible for causing the illness giardiasis (Bitton, 2005; Sela & Fallik, 2009; CADE, 2011). *Giardia* can be found in soil, food, water or on surfaces contaminated with faeces from infected animals or humans (CADE, 2011). It is the most identifiable waterborne agent in the United States (Johnston *et al.*, 2006). The most common route of infection is through faecal-oral transmission. Fruits salads, iceberg lettuce and raw sliced vegetables such as tomatoes and onions have been implicated as the most common causes of foodborne giardiasis (Johnston *et al.*, 2006; Sela & Fallik, 2009).

Organisms of *G. lamblia* isolated from cats, dogs, beavers and bears appear to be identical to those responsible for causing human giardiasis (Harris *et al.*, 2003). Signs of human giardiasis may last for up to one or even two weeks, but in some chronic cases individuals can experience symptoms for up to a year. Chronic cases are extremely difficult to treat even in individuals with a strong immune system. Severities of symptoms differ for different individuals, even when infected with the same strain at the same time (Harris *et al.*, 2003; Bitton, 2005; CADE, 2011).

Infective dose is very low. Ingestion of one or more cysts may cause disease (Harris *et al.*, 2003). The consumption of contaminated water is usually one of the main causes for contracting giardiasis. *G. lamblia* thrive under cool and moist conditions and are resistant to disinfection with chemical agents such as chlorine (Bitton, 2005; Chaidez *et al.*, 2005).

*Giardia intestinalis* cysts are also responsible for causing giardiasis and are transmitted by individuals with dirty hands, contaminated drinking and irrigation water and food contaminated with faeces (EC, 2002; Bitton, 2005; Johnston *et al.*, 2006). These cysts survive well in most environmental conditions and are also resistant to chlorination (EC, 2002; Bitton, 2005). Symptoms of giardiasis include chronic diarrhoea, vomiting, malabsorption of vitamins and minerals, stomach cramps, loss of appetite as well as weight loss (EC, 2002; CADE, 2011).

## Water moulds

### *Phytophthora*

*Phytophthora* species are one of the most important groups of plant pathogens that are spread primarily through contaminated irrigation water (Yamak *et al.*, 2002; Hong en Moorman, 2005). Numerous fruit and vegetable crops are subject to *Phytophthora* contamination (Hong en Moorman, 2005). Even though most *Phytophthora* species have a wide host range, there are a few that are specialised on single-host plants (Ufer *et al.*, 2008).

Sporangia also known as spores may form on the surface of a host plant after the plant was infected by *Phytophthora* (Hausbeck *et al.*, 2012). Once the sporangia come into contact with water, they start dividing into swimming zoospores (Hausbeck *et al.*, 2012). Zoospores are not only transferred through flowing water, including irrigation water, but are also able to swim through moist soil (Kay *et al.*, 2011). Swimming zoospores are able to find plant roots by making use of chemical and electrical signals. Even after zoospores have stopped swimming, they are capable of surviving and causing infection for several days in a water source. As a result *Phytophthora* can spread to different fields via irrigation water and initiate epidemics if susceptible fruits and vegetable crops are adjacent or near a contaminated field. Contaminated surface runoff from infested fields can also flow into nearby creeks, rivers and ponds, thus infecting the entire water source (Hausbeck *et al.*, 2012).

*Phytophthora* species are part of the oomycete genus and comprise of over 100 species, most of which are known plant pathogens (Kay *et al.*, 2011). Morphologically and physiologically oomycetes resemble fungi, but in reality they are actually closer related to their phylogenetic cousins of diatoms and brown algae. They fall within the kingdom Stramenopiles and are well adapted in aquatic environments (Hong en Moorman, 2005). The most well-known and also most devastating species of *Phytophthora* is *P. infestans* which not only contributed to a large potato scarcity in Ireland, but also resulted in almost a million deaths due to starvation. The pathogen has a large impact on agriculture and is annually responsible for billions of dollars lost due to potato infestation (Kay *et al.*, 2011).

It is important to successfully manage water in the fields if *Phytophthora* contamination is to be prevented (Yamak *et al.*, 2002; Hausbeck *et al.*, 2012). Drip irrigation systems are recommended to reduce field wetness and the travel of zoospores (Hausbeck *et al.*, 2012). If overhead irrigation is the only available option, reduced watering must be implemented during fruit or vegetable growing time to reduce infection of crops without significantly affecting the yield. Another possible solution might be to heat irrigation water to between 10°C and 32°C since *Phytophthora* is unable to cause infection because zoospores are unable to survive between these temperatures (Hausbeck *et al.*, 2012).

### **Control of bacterial pathogens**

It is nearly impossible to control or prevent contamination of water sources and fields in production areas (Brackett, 1999). The only options farmers have in controlling bacterial contamination of their crops during production are by avoiding fields where animals recently grazed, using water free from pathogens for irrigation after planting. If these measures are not possible, on-farm treatments must be used to treat water if it is contaminated and it is of great importance that farmers realise what part they play in assuring the safety of fresh produce (Brackett, 1999). The number of informal settlements near fresh and natural water resources should be reduced and making sure that sewage treatment systems are fully operational will help to prevent the pollution of fresh water sources (Lötter, 2010). These two options are not currently feasible in South Africa's infrastructure (Lötter, 2010). Prevention of fresh produce contamination is especially important if a crop has a short expiry date and is consumed not long after harvesting (Brackett, 1999).

By the time a produce-associated outbreak has been identified, in most cases, harvesting is already finished, thus making it nearly impossible to find the field where initial contamination occurred (Lynch *et al.*, 2009). Food safety regulators' limited jurisdiction and a lack of well established procedures to follow in the case of an outbreak, further stands in the way of proper field work that can result in the implementation of proper practical control measures (Lynch *et al.*, 2009).

### **KEYS TO PREVENTION**

Fresh produce items that are not cooked before consumption should be viewed as 'ready to eat' (Lynch *et al.*, 2009; Ijabadeniyi, 2010). It is important for producers to realise that pathogen contamination cannot be washed off completely. Instead of trying to control pathogen contamination, contamination should rather be prevented from the start (Lötter, 2010). Hazard Analysis Critical Control Points (HACCP) is most often implemented to prevent contamination of fruits and vegetables because once contamination has occurred, it is extremely difficult to remove microbial hazards successfully (Lynch *et al.*, 2009; Ijabadeniyi, 2010).

The United States Food and Drug Administration (FDA) issued several guidance documents to deal with general problems associated with fresh fruit and vegetable production.

These documents were implemented to promote good agricultural practices (GAP) for fresh produce production and good manufacturing practices (GMP) for processing (Lynch *et al.*, 2009).

To improve the documentation and keep track and as a result prevent future produce-associated outbreaks, when an outbreak occurs investigations need to include all information so fresh produce can rapidly be traced back to the field where it was produced (Lynch *et al.*, 2009; Ijabadeniyi, 2010). Knowledge of the field location where produce was grown and what irrigation and harvesting techniques were used can, when put together, improve understanding of why outbreaks occurred and thus help to develop effective on-farm preventative measures of contamination. A possible long term solution is to treat contaminated water sources on-farm before using it to irrigate crops (Lynch *et al.*, 2009).

### **ON-FARM TREATMENT OPTIONS OF IRRIGATION WATER**

Good quality water for irrigation purposes is becoming harder and more expensive to obtain (Newman, 2004; Yiasoumi *et al.*, 2005). Disinfection of water is of great importance since it controls growth of microbiological pathogens in the irrigation system and reduces the risk of introducing disease to the farm through irrigation water (Yiasoumi *et al.*, 2005; Pehlivanoglu-Mantas *et al.*, 2006). There are several different disinfection techniques to choose from, but in most cases for disinfection to be successful, some systems require water that is free of colloidal material, organic matter and sediment, the water should be within a certain pH range and it should be low in iron and manganese. If all of the aforementioned requirements are met, before disinfection starts, the process itself will be a lot easier, cheaper and more effective (Yiasoumi *et al.*, 2005).

In the past, four mechanisms have been proposed to explain how disinfectants work. These mechanisms are as follows: 1) disinfectants damage the cell wall and 2) as a result, the cell's permeability change, 3) in response the colloidal nature of the protoplasm is altered and 4) inhibition of enzyme activity takes place. Thus, if the cell wall is damaged in any way, lysis takes place in the cell and eventually the cell dies (Tchobanoglous, 1979).

Disinfection treatments can generally be divided into three main technologies namely chemical (bromine, chlorine based, hydrogen peroxide, ozone), mechanical / physical (sand filtration, ultrafiltration) and alternative technologies better known as photochemical treatments (ultrasound, ultraviolet light) (Table 3) (Tebbutt, 1992; Acher *et al.*, 1996; Yiasoumi *et al.*, 2005; Momba *et al.*, 2008; Ali, 2010; Anon., 2014).

Several factors need to be taken into consideration before the right disinfectant method can be chosen for sanitising water for irrigation purposes (Tebbutt, 1992; Lazarova & Bahri, 2005).

## Chapter 2

**Table 3** Comparison of different on-farm irrigation water treatment options

	Bromine	Chlorination	Hydrogen Peroxide	Ozonation	Slow Bed Sand Filtration	Ultrafiltration	Ultrasound	Ultraviolet***
<b>Treatment</b>	Chemical	Chemical	Chemical	Chemical	Physical	Physical	Alternative	Alternative
<b>Capital expense</b>	medium <sup>1</sup>	low <sup>2</sup>	low <sup>3</sup>	high <sup>4</sup>	medium <sup>5</sup>	very high <sup>6</sup>	very high <sup>7</sup>	medium <sup>8</sup>
<b>Operating cost</b>	medium <sup>1</sup>	medium <sup>2</sup>	medium <sup>3</sup>	medium <sup>4</sup>	medium <sup>5</sup>	very high <sup>6</sup>	very high <sup>7</sup>	low <sup>8</sup>
<b>Power consumption</b>	medium	low*	low	very high	medium	high	very high	medium / high
<b>Safety</b>	medium	bad	bad	medium	good	good	good	good
<b>Maintenance involvement</b>	low	medium	medium	low	medium	very high	low	low
<b>Ease of installation</b>	good	good	good	bad	good	bad	good	good
<b>Reliability</b>	low	low	low	good	reasonable	very good	good	very good
<b>pH dependant</b>	yes	yes	yes	yes	no	no	no	no
<b>TSS dependant</b>	limited	limited	limited	limited	yes	no**	no	yes
<b>COD dependant</b>	yes	yes	yes	yes	yes	no**	no	yes
<b>Carbon footprint</b>	small	small	small	medium	large	large	medium	small
<b>Contact time</b>	10 - 30 minutes	30 - 90 minutes	15 - 30 minutes	10 - 20 minutes	hours	16 - 20 hours	15 - 20 minutes	seconds
<b>Disinfectant by-products</b>	yes	yes	yes	yes (less severe)	no	no (CIP chemicals)	no	no
<b>Acceptance</b>	bad	good	good	good	good	good	good	good

\*High power consumption during chlorine production.

\*\*Microbiological no, physical yes.

\*\*\*Very accurate predictions can be done when the UV transmission, target bacteria and flow rate are known.

<sup>1</sup>(Hugo & Malan, 2006)

<sup>2</sup>(Lazarova & Bahri, 2005)

<sup>3</sup>(Newman, 2004)

<sup>4</sup>(Freese et al., 2003)

<sup>5</sup>(Hugo & Malan, 2006)

<sup>6</sup>(Freese et al., 2003)

<sup>7</sup>(Mahamuni & Adewuyi, 2010)

<sup>8</sup>(Bolton & Cotton, 2008)

These considerations include: the sanitising capability of the disinfectant; the potential toxicity of the disinfectant at high levels and the effects it might have in water, soil and plants; if by-products are formed when the disinfectant reacts with water and the effects this may have on individuals that come into contact with it; if water quality parameters might influence the sanitising efficacy of the disinfectant, if the product is safe for use as well as the cost of the disinfectant. The cost of the disinfectant is made up of the capital and operating costs (Tebbutt, 1992; Lazarova & Bahri, 2005).

### **Chemical disinfection methods**

These methods are based on the oxidation potential of chemicals which are capable of damaging the cell walls of microorganisms through oxidation and eventually result in cell death (Acher et al., 1996; Yiasoumi *et al.*, 2005). The oxidation potential is not the only factor that has to be taken into consideration when deciding which disinfection agent to use, since water quality parameters also plays a significant role in the germicidal properties of disinfectants (Acher et al., 1996; Yiasoumi *et al.*, 2005). These parameters which may influence the efficacy of disinfectants include temperature, pH, conductivity, turbidity, chemical oxygen demand (COD) as well as total suspended solids (TSS). Depending on these parameters, the dose of disinfection governs the efficacy of the treatment. The dose of disinfection is a combination of the disinfectant concentration and the contact time (Freese *et al.*, 2003; GHD, 2005; Lazarova & Bahri, 2005; Yiasoumi *et al.*, 2005; WHO, 2004; WHO, 2006; Yang *et al.*, 2008; Ali, 2010).

Even though chemical disinfectants generally deliver good results, modern water analytical techniques have revealed that they release disinfection by-products (DBPs) into the water (Acher *et al.*, 1996; Yiasoumi *et al.*, 2005). Disinfection by-products are formed when disinfectants such as bromine, chlorine, hydrogen peroxide and ozone react with organic and inorganic matter in water (Tate & Arnold, 1990; Woo *et al.*, 2002; Westerhoff, 2006; Momba *et al.*, 2008). Researchers found that some DBPs such as trihalomethanes, di-/trichloroacetic acids, and 3-chloro-4-(dichloromethyl)-5-hydroxy-2(5H)-furanone are carcinogenic, mutagenic or even teratogenic in animal studies and as a result have raised public concern over the possible adverse health effects of DBPs on humans (Tate & Arnold, 1990; Woo *et al.*, 2002; Bitton, 2005).

Determining appropriate DBP regulations in water disinfection is a complex situation. Disinfectants are necessary to protect against waterborne pathogens, and thus DBPs are unavoidable. Source water quality and constituents vary widely throughout the world. Combined with the assortment of chemical disinfectants available, this means that DBPs differ from site to site in both occurrence and concentration (Tate & Arnold, 1990; Woo *et al.*, 2002; Westerhoff, 2006). Other disadvantages of chemical disinfectants are that their manufacturing, storage, transport and use pose a continuous threat to anyone who works with them as well as serious consequences for the environment (Acher *et al.*, 1996; Yiasoumi *et al.*, 2005).

## Chapter 2

### **Bromine**

#### *Background and practical application*

In 1825, C. Löwig, a German chemistry student discovered bromine after conducting tests on swamp water. When the word bromine is translated from the Greek word bromos, it means 'smell' and refers to the unpleasant odour of bromine (Kamlet, 1953).

In the past bromine disinfection has mainly been used to treat swimming pool and cooling tower water, although there are a few cases in literature where bromine was used for wastewater disinfection, but it is not recommended for the disinfection of drinking water since it imparts tastes and odours to most water. Bromochlorodimethylhydantoin, in the shape of a stick, was introduced in 1958 as a means for swimming pool disinfection. Since then, several bromine based disinfectants have been developed for household use and swimming pool treatment. In the 1990's some wastewater utilities in the USA started using bromine in combination with chlorine as disinfectant treatment (Tate & Arnold, 1990; Freese *et al.*, 2003).

#### *Mode of action*

A chemical such as bromine has excellent disinfecting properties since it is a strong oxidiser (Newman, 2004; Punyani *et al.*, 2006). Disinfection takes place because bromine is a strong enough oxidising agent to alter the chemical structure of unwanted pathogens as well as other organic material that might be present and as a result forms chemical by-products in the water (Newman, 2004).

The process entails transforming bromine into hypobromous acid (Droste, 1997; Yiasoumi, 2005; Yiasoumi *et al.*, 2005). This is best achieved when sodium bromide is added to sodium hypochlorite. Hypobromous acid is a very effective sanitising agent over a wide pH range. At a pH of 8.5, 60% of bromine is still in hypobromous acid form and able to successfully disinfect water (Yiasoumi, 2005; Yiasoumi *et al.*, 2005). Recycled water commonly used in horticulture contains fluctuating levels of ammonium and other nitrogen-based compounds. Both bromine and chlorine are capable of reacting with these compounds and as a result forms broamines and chloramines, respectively. Chloramines are poor biocides, while broamines show disinfection properties comparable to hypobromous acid (Yiasoumi, 2005; Momba *et al.*, 2008).

During the oxidation process, oxidising compounds are reduced and lose their activity. It is thus of great importance to maintain a high concentration of bromine, at all times during disinfection, in the water to ensure that complete disinfection takes place throughout the process (Newman, 2004). Investigations performed according to Freese *et al.* (2003) have shown that bromine disinfection of pathogens present in sewage is almost equal in efficacy to chlorine. In sewage with a pH above four, bromine disinfection was more efficient than chlorine disinfection (Droste, 1997).

## Chapter 2

### *Advantages*

Bromine dissolves three times faster than chlorine does in water, no dangerous gasses are required during the production of bromine, its activity is short since it does not bind strongly to water, as a result, the residual concentrations stay low and no additional substances are necessary to remove bromine after disinfection is completed (LENNTECH, 2011). Bromine has similar disinfection properties to chlorine, also a halogen and cost for disinfection is comparative with that of chlorine gas (Tebbutt, 1992; Yiasoumi, 2005). Cost of commercial treatment of irrigation water with bromine for the elimination of pathogens is economically justifiable (Hugo & Malan, 2006). Other advantages include bromine's long shelf life and the fact that it is an effective disinfectant of water over a wider pH range than chlorine (Korslin, 2012).

### *Disadvantages*

Disinfection of water with bromine is pH and COD dependable, requires a contact time of 10 to 30 minutes and to maintain sufficient disinfection, a lot of bromine needs be added to the water in comparison with chlorine (Freese *et al.*, 2003; LENNTECH, 2011). A high concentration of bromine is capable of killing most pathogenic organisms, but it is incapable of killing more resistant protozoan pathogens such as *Cryptosporidium* and *Giardia* (Freese *et al.*, 2003).

Bromine is also very reactive and corrodes materials such as metal when it comes into contact with it, for example the pipes and system used for disinfection (LENNTECH, 2011). During bromine disinfection bromamines and hypobromous acid react with organic matter present in the water and forms carcinogenic disinfection by-products, such as tribromomethanes, that can be harmful to humans as well as the environment and also imparts taste and odours into waters which may affect the taste of fresh produce (Freese *et al.*, 2003; Westerhoff, 2006; LENNTECH, 2011). Bromine should be transported, stored and used with care since exposure can lead to eye and mucous membrane irritation (LENNTECH, 2011).

### *Conclusion*

Even though bromine is cost effective and can be used to kill most microorganisms, it is unable to kill protozoan pathogens such as *Cryptosporidium* and *Giardia*. It also produces disinfectant by-products such as tribromomethanes during treatment which may be harmful when consumed. Bromine is also a very reactive disinfectant capable of corroding metal and imparts tastes and odours in treated water. Thus bromine will not be an effective method for disinfection of water used for irrigation purposes on fresh produce.

## **Chlorine**

### *Background and practical application*

Chlorine disinfection has been practiced for over a century, it was first discovered in Sweden in 1744 (Tchobanoglous, 1979; Lazarova & Bahri, 2005; Momba *et al.*, 2008). Back then people

believed that the odours in water were responsible for illness. As early as 1835, chlorine was used to remove odours from water, but the sanitising qualities of chlorine in water were not discovered until 1890. After this discovery chlorination began in Great Britain and expanded to the United States and Canada in the early 1900's (Lazarova & Bahri, 2005).

In modern society chlorination is the most popular sanitising method and is used to treat drinking water, water for agriculture as well as recreational water all over the world (Cheremisinoff, 2002; Lazarova & Bahri, 2005; Yiasoumi *et al.*, 2005; Momba *et al.*, 2008). Chlorine disinfection is effective against most bacteria, but an extremely high dose is needed to kill viruses and most protozoa such as *Cryptosporidium* and *Giardia* are resistant to chlorine (Cheremisinoff, 2002; Lazarova & Bahri, 2005). Chlorine for water disinfection purposes is available in gas, liquid and powder forms. Sodium hypochlorite, a liquid form of chlorine, is used most often and has been approved by the United States Food and Drug Administration for the disinfection of water (Lazarova & Bahri, 2005).

The effectiveness of water disinfection with chlorine is dependent upon water quality parameters such as organic material present in the water, the contact time, pH and temperature (Lazarova & Bahri, 2005; Yiasoumi *et al.*, 2005; Momba *et al.*, 2008). Typical chlorine doses used to disinfect municipal wastewater in the United States are approximately 5 - 20 mg.L<sup>-1</sup> of chlorine and require a contact time of at least 30 - 90 minutes to insure complete disinfection of the water and to comply with regulatory limits for bacterial indicator organisms. Higher doses and or contact times are required if low quality wastewater is used for irrigation purposes or if the water has to comply with drinking or recreational water standards (Lazarova & Bahri, 2005; Gadgil, 2008).

#### *Mode of action*

Chlorine is a chemical with strong oxidising properties and causes irreparable damage to bacterial cells, but the mode of action may differ for viruses (Newman, 2004; Bitton, 2005). The process that takes place is similar to bromine disinfection (Newman, 2004; Bitton, 2005; Yiasoumi, 2005).

Chlorine gas and water react to form hypochlorous acid (HOCl) and hydrochloric acid (HCl). In turn, the HOCl dissociates into a hypochlorite (OCl<sup>-</sup>) and a hydrogen ion (H<sup>+</sup>). The reactions proceed as follow: (1)  $\text{Cl}_2 + \text{H}_2\text{O} \rightleftharpoons \text{HOCl} + \text{HCl}$ , (2)  $\text{HOCl} \rightarrow \text{H}^+ + \text{OCl}^-$  (WHO, 2004). The reactions are reversible and pH dependent. The OCl<sup>-</sup> and HOCl species are commonly referred to as free chlorine, which is extremely reactive with numerous components of the bacterial cell (WHO, 2004; Bitton, 2005). HOCl can produce oxidation, hydrolysis and deamination reactions with a variety of chemical substrates, and produces physiological lesions that may affect several cellular processes (WHO, 2004).

The mode of action for bacterial cells can be divided into three phases (Bitton, 2005). Firstly, free chlorine damages the cell membrane, leading to the loss of cell permeability and other vital functions within the cell, leading to DNA and RNA leakage and eventually cell death. Secondly, chlorine also harms nucleic acids and enzymes in the bacterial cell. Lastly exposure to

chlorine may result in sulfhydryl groups of the cell being oxidised by hypochlorous acid and as a result can disrupt transport, inhibit cell respiration and prevents cells from maintaining an adequate amount of adenylate energy to remain viable (Bitton, 2005).

### *Advantages*

Chlorine is widely used for disinfection of drinking and irrigation water since it is readily available in gas, liquid and powder form and is capable of destroying most bacteria and viruses when available in high doses (Cheremisinoff, 2002; Lazarova & Bahri, 2005). It is relatively cheap to treat water with chlorine, partially because of the small doses, two to three mg.L<sup>-1</sup>, needed to achieve a 3-log reduction (Tebbutt, 1992; Cheremisinoff, 2002; GHD, 2005; Lazarova & Bahri, 2005; WHO, 2004; Yiasoumi *et al.*, 2005; Momba *et al.*, 2008). The chlorine doses necessary in wash water to disinfect fresh produce after harvest is usually a lot higher and can be as much as 50 – 200 mg/L<sup>-1</sup> (FAO & WHO, 2008a). The capital cost is low when compared to an ultraviolet (UV) unit and its yearly operating costs include power as well as chemicals (Cheremisinoff, 2002; GHD, 2005; Lazarova & Bahri, 2005; Momba *et al.*, 2008). Chlorine is easy to apply to water since it is highly soluble, 7 000 mg.L<sup>-1</sup>, and releases free chlorine into the water, to insure that disinfection of the water is sufficient (Lazarova & Bahri, 2005). Chlorine is also capable of reducing unpleasant odour and taste compounds that might be present in water (Momba *et al.*, 2008).

### *Disadvantages*

Chlorination has several disadvantages such as a long contact time and high doses, depending on the quality of the water to be sanitised (Tebbutt, 1992; Cheremisinoff, 2002; Lazarova & Bahri, 2005; Yiasoumi *et al.*, 2005). Chlorine disinfection is most effective between a pH range of 6.5 - 9.5, but efficacy decreases as the pH of water increases (Momba *et al.*, 2008). It is important to follow manufacturer's guidelines on proper handling and storage of chlorine because the gas is poisonous and can cause taste and odour problems in water, particularly when phenols are present in the water (Lazarova & Bahri, 2005; Yiasoumi *et al.*, 2005). For safety purposes chlorine can be stored in the form of hypochlorite since it is less toxic than chlorine gas, while for transport sodium sulfite or bisulfite can be added to neutralise the chlorine (Yiasoumi *et al.*, 2005). In existing irrigation systems, backflow equipment preventing chlorine from entering in the source water is of great importance since chlorine can have a negative influence on aquatic systems (Tebbutt, 1992; Cheremisinoff, 2002; Lazarova & Bahri, 2005). Since chlorine is a strong oxidising agent it will rapidly react when it comes into contact with reducing agents and unsaturated organic compounds and forms carcinogenic by-products such as chloroform as a result (Newman, 2004). Another disadvantage of chlorine treatment is that protozoa such as *Cryptosporidium* and *Giardia* are resistant to this type of treatment at a dose of two to three mg.L<sup>-1</sup> (WHO, 2004; Lazarova & Bahri, 2005).

### *Conclusion*

Even though chlorination is relatively inexpensive, requires low doses for general disinfection and provides residual protection against microbial growth in the distribution system, it is not a suitable option for irrigation water disinfection since it requires a relatively long contact time, it is toxic to humans as well as the environment and it releases carcinogenic by-products such as trihalomethanes into the disinfected water. Chlorine treatment is also not capable of killing protozoan microorganisms such as *Cryptosporidium* and *Giardia*. Another factor making chlorine unacceptable for disinfection is the taste and odour changes it may cause in the water. These changes may affect the taste of fruits and vegetables that are consumed raw by consumers.

### ***Hydrogen peroxide***

#### *Background and practical application*

Hydrogen peroxide was discovered in 1818 by Louis Jacque Thenard. It was originally used to bleach straw hats in the early twentieth century. Pure hydrogen peroxide was produced from electrolysis from 1920 to 1950. In modern times hydrogen is used to produce hydrogen peroxide by way of self-oxidation methods (LENNTECH, 2011).

Hydrogen peroxide consists of two hydrogens and two oxygens and is available in trace amounts of rain and snow (Newman, 2004; LENNTECH, 2011). It is an extremely versatile disinfectant and can be used in air, water, wastewater as well as soils (LENNTECH, 2011). In general it is mostly used to remove impurities such as off-odours, tastes and pathogens from wastewater (McDonnell & Russell, 1999; Gil & Selma, 2006; LENNTECH, 2011). Except for disinfection it has several other properties and is commonly used to bleach paper, teeth, hair, in the production of washing powder and as a disinfectant in food production (Newman, 2004). Hydrogen peroxide and the formulation hydrogen dioxide, known in industry as ZeroTol, is capable of killing bacteria, viruses, fungus, algae, yeasts as well as their spores on contact and is frequently used as a disinfectant for irrigation water, equipment and other surfaces (McDonnell & Russel, 1999; Newman, 2004).

#### *Mode of action*

In nature hydrogen peroxide is produced when rain combines with ozone in the atmosphere (Newman, 2004; LENNTECH, 2011). Hydrogen peroxide reacts very fast when it comes into contact with water and fragments into an oxygen and water molecule without forming any by-products (McDonnell & Russell, 1999; LENNTECH, 2011). As a result the amount of oxygen present in the water increases.

Hydrogen peroxide acts as an oxidant by producing hydroxyl free radicals ( $\bullet\text{OH}$ ) which attack essential cell components of microorganisms, including lipids, proteins, and DNA. Exposed sulfhydryl groups and double bonds are particularly targeted by hydrogen peroxide (McDonnell & Russell, 1999; LENNTECH, 2011). Peroxides such as hydrogen peroxide ( $\text{H}_2\text{O}_2$ ), perborate,

peroxiphosphate and persulphate are good disinfectants and oxidisers capable of destroying most microorganisms that might be present in irrigation water (LENNTECH, 2011). Microorganisms, present in the water are destroyed by free oxygen radicals, and only water remains (LENNTECH, 2011). Certain proteins are destroyed during hydrogen peroxide oxidation (Marris, 1995; Kim *et al.*, 2007; LENNTECH, 2011).

### *Advantages*

It is a strong oxidiser and unlike other chemical disinfectants, hydrogen peroxide is completely water soluble, it does not form any carcinogenic by-product residues when it comes into contact with water and it has good sanitising properties (Gil & Selma, 2006; LENNTECH, 2011). Hydrogen peroxide is capable of killing bacteria, viruses, fungus, algae, yeasts as well as their spores on contact and is frequently used as a disinfectant for irrigation equipment and other surfaces (McDonnell & Russell, 1999; Newman, 2004; Gil & Selma, 2006). Ronen *et al.* (2002) found that treatment of wastewater with 50 mg.L<sup>-1</sup> hydrogen peroxide is enough to reduce faecal coliforms to less than 1 cfu.100 mL<sup>-1</sup>.

### *Disadvantages*

Hydrogen peroxide is a weak oxidising agent when compared to ozone (Runia, 1995). It reacts rapidly with various substances and breaks down certain proteins it comes into contact with (Kim *et al.*, 2007; LENNTECH, 2011). High concentrations are necessary for disinfection and use can become quite costly if it is used on a continuous scale (Newman, 2004). As a safety measure it gets diluted during transport (Kim *et al.*, 2007; LENNTECH, 2011). Exposure can take place through inhalation, consumption of food as well as through skin or eye contact and can irritate the lungs, skin, eyes and mucous membranes (LENNTECH, 2011). The efficacy of hydrogen peroxide as a disinfectant depends on several factors namely concentration, pH, catalysers, contact time and temperature (Ronen *et al.*, 2002; Newman, 2004; Kim *et al.*, 2007; LENNTECH, 2011).

Hydrogen peroxide is generally recognized as safe (GRAS) for some food applications but has not yet been approved as an anti-microbial wash for produce (Sapers, 2001; Gil & Selma, 2006). It produces no residue since it is rapidly decomposed by catalase, an enzyme found throughout the plant kingdom, to water and oxygen. Sapers (2001) found that hydrogen peroxide can impose injuries to some commodities, causing browning of apple skin at temperatures greater than 60 °C and bleaching of anthocyanins in mechanically damaged berries. Even though hydrogen peroxide is capable of destroying most microbial pathogens, Kim *et al.* (2007) found hydrogen peroxide significantly decreased the phenolic and Vitamin C content in fresh cut tomatoes during storage at 4 °C.

### *Conclusion*

Hydrogen peroxide is capable of killing bacteria, viruses, fungus, algae, yeasts as well as their spores on contact and is frequently used as a disinfectant for wastewater, irrigation equipment and other surfaces. Large concentrations are needed to destroy all of the aforementioned microorganisms, making it a very expensive disinfection method. Treatment of wastewater with 50 mg.L<sup>-1</sup> hydrogen peroxide is enough to reduce faecal coliforms to less than 1 cfu.100 mL<sup>-1</sup>, but it is incapable of destroying protozoan pathogens such as *Giardia* and *Cryptosporidium*. It is also a dangerous chemical and can have serious consequences if individuals come into contact with it. Thus hydrogen peroxide will not be a suitable choice for irrigation water disinfection.

## **Ozone**

### *Background and practical application*

Ozone is an extremely strong oxidising agent and was initially used mostly to remove taste, colour and odours from water (Bitton, 2005). Ozone was first applied as a potable water disinfectant in 1893 at Oudshoorn, Netherlands (Haas, 1990). In 1906, the city of Nice (France), had the first facilities to utilise ozone in a water treatment plant. In the early 1970's in the United States ozone treatment became a feasible option for wastewater disinfection as an alternative for using chlorine (Tchobanoglous, 1979; Haas, 1990; Lazarova & Bahri, 2005). In recent years the Middle East, South Africa, France and Spain have constructed their own wastewater treatment facilities (Lazarova & Bahri, 2005). Ozone is also effective in the treatment of drinking water and is used as an alternative to chlorine, in bottled water purification systems (Gil & Selma, 2006). Ozone is also utilised as a primary disinfectant for pathogenic microorganism inactivation and for the oxidation of taste and odour-causing compounds, colour, refractory organic materials, iron and manganese (Glaze, 1990; Bitton, 2005).

### *Mode of action*

If the standard oxidation potential was the only factor controlling the effectiveness of water and wastewater disinfection, ozone would be far more effective than all of the other chemical disinfectants. Since the disinfectant properties are dependent on several water quality factors, namely oxidisable matter, pH, suspended solids and temperature, this is not necessarily the case (Gottschalk, 2000; Freese *et al.*, 2003).

The main commercial source for producing ozone is an ozonator (Hugo & Malan, 2006; NS, 2012). Ozone consists of three oxygen molecules and is an extremely effective antioxidant which decomposes in to oxygen after a few hours (Hugo & Malan, 2006).

Two mechanisms of ozone disinfection are commonly available, these include a method during which compounds are directly oxidised by ozone molecules as well as a reaction involving ozone decomposition products namely a hydroxyl radical. The second method involving hydroxyl radicals is the most common mode of ozone disinfection during water treatment (Gottschalk *et al.*, 2000; Freese *et al.*, 2003; WHO, 2004; Lazarova & Bahri, 2005).

Ozone is a strong oxidiser, in aqueous media, it produces free radicals that inactivate microorganisms either by direct reaction with molecular ozone or by indirect reaction, with the radical species formed, when ozone decomposes (Gottschalk *et al.*, 2000; Newman, 2004; WHO, 2004; Bitton, 2005). Ozone inactivates bacterial cells by affecting their permeability, enzymatic activity as well as guanine and thymine bases composition in DNA. Bacterial spore DNA appear unaffected by ozone treatment, most damage is done to the spore's inner membrane. Most viruses are deactivated due to ozone damage to their nucleic acid core and protein coat, but not all viruses are affected equally (Bitton, 2005).

During ozone disinfection an organism's cell wall is either partially or completely destroyed, resulting in lysis. Ozone is also responsible for destroying chromosomes, nitrogen-carbon bonds that connects sugar and bases, DNA hydrogen bonds and phosphate sugar bonds. When these bonds are broken; aldehydes, ketones or carbonyl compounds are formed. The aforementioned changes are responsible for depolymerisation and cellular constituent leakage as well irreversible enzyme inhibition (Freese *et al.*, 2003; WHO, 2004; Lazarova & Bahri, 2005).

#### *Advantages*

Ozone is a powerful disinfectant, capable of killing bacteria, viruses and protozoan parasites such as *Cryptosporidium* and *Giardia* (Gottschalk *et al.*, 2000; Xu *et al.*, 2002; WHO, 2004; Lazarova & Bahri, 2005; Momba *et al.*, 2008). Ozone is effective in treating drinking water as well as all kinds of effluents to moderate standards for the use of unrestricted irrigation. For good quality effluent doses as low as 3 - 5 mg.L<sup>-1</sup> is efficient in destroying most microorganisms, whereas doses of around 15 mg.L<sup>-1</sup> is required for poor quality wastewater (Lazarova & Bahri, 2005). This makes ozone one of the most effective disinfectants, since it is capable of successfully treating wastewater and destroying protozoan parasites that might be present in the water. Ozone also decomposes spontaneously into oxygen after a few hours of disinfection, thus producing no harmful by-products as is the case for chlorine (WHO, 2004; Lazarova & Bahri, 2005; Yiasoumi, 2005; Gil & Selma, 2006; Selma *et al.*, 2008).

#### *Disadvantages*

Ozone is quite expensive and because of its instability, has to be produced on-site (Freese *et al.*, 2003; WHO, 2004; Yiasoumi, 2005; Momba *et al.*, 2008). Since the outcome of the hydroxyl radical reaction cannot be predicted, ozonation of wastewater is usually confined to effluents which may contain organic matter or other hydroxyl scavengers (Freese *et al.*, 2003; Selma *et al.*, 2008). Another concern with ozone is the many disinfection by-products, especially non-halogenated, that form during treatment even though some of them are not toxic (aldehydes, ketones or carbonyl compounds) (Freese *et al.*, 2003; WHO, 2004; Lazarova & Bahri, 2005). It still raises a health concern because some ozone by-products are mutagenic or carcinogenic (Momba *et al.*, 2008).

The efficacy of ozone disinfection is also dependant on whether suspended solids or oxidisable matter is present in the water as well as on the pH and temperature of the water (Gottschalk, 2000; Freese *et al.*, 2003). For best ozone stability, the pH of the water has to be 4. Other disadvantages include that water has to be batch-treated in holding tanks or in pipes if ozone is used, since it takes such a long time to achieve the correct redox value (Hugo & Malan, 2006). The oxygen demand of the drainage water and the composition and nature of the oxidation compounds also influences ozone disinfection efficacy (Gottschalk, 2000; Hugo & Malan, 2006).

### *Conclusion*

Ozone is a powerful disinfectant that can be used to treat drinking water as well as effluents for irrigation purposes. In good quality water, low doses are needed to destroy bacteria, viruses and protozoan parasites such as *Cryptosporidium* and *Giardia*. This makes ozone one of the most effective disinfectants, since it is capable of successfully treating wastewater and destroying protozoan parasites that might be present in the water. Some of ozone's biggest disadvantages are its high cost and instability. As a result of its instability, ozone has to be prepared on-site, thus increasing the chance of individuals being exposed to it. Ozone disinfection is also affected by various factors such as pH and temperature and can form disinfection by-products. Thus when taking all of these factors in to account, it can be concluded that ozone will not be the most efficient disinfection method to treat irrigation water.

## **Mechanical / Physical disinfection methods**

These disinfection methods are based on the principal of mechanical retention of microorganisms from water by filtration through sand or synthetic membranes and are commonly used for the treatment of municipal water and wastewater (Acher *et al.*, 1996; Yiasoumi *et al.*, 2005; LENNTECH, 2011). These disinfection methods are useful in wastewater treatment for purification and recycling (Kesari *et al.*, 2011). To increase efficacy, these methods can be used in combination with other disinfection methods (Acher *et al.*, 1996; Yiasoumi *et al.*, 2005; LENNTECH, 2011).

### ***Slow bed sand filtration***

#### *Background and practical application*

Slow bed sand filters were initially developed in Europe for improving the quality of drinking water, drawn from questionable raw water sources such as lakes and reservoirs (Cleasby, 1990; GHD, 2005). The first slow sand filter was developed and built by John Gibb in 1804 to treat water discharged from his bleachery (Huisman & Wood, 1974). The first ever municipal filtration plant was built during the early 1800's in Scotland. Almost three decades later, in 1829, the first ever slow sand filter was built to treat London's water supply (Huisman & Wood, 1974; Cleasby, 1990; Droste, 1997). At that time the existence of pathogenic bacteria was unknown and slow sand

filters were mainly utilised to reduce turbidity and suspended solids in water (Huisman & Wood, 1974). The use of slow sand filters only expanded after several cholera outbreaks occurred and John Snow realized that deaths due to waterborne diseases decreased dramatically when water supplies were filtered (Huisman & Wood, 1974; Cleasby, 1990). Even after this realisation, the rest of the world was sceptical to implement slow bed sand filters since they found it difficult to associate clear water with freedom of disease (Cleasby, 1990). Slow sand filters are mostly utilised by communities of smaller than 10 000 people because capital and operating costs are a lot cheaper than for rapid sand filters. The first sand filter in the USA was installed in 1872 in Lawrence Massachusetts to remove *Salmonella typhi* from water (Bitton, 2005).

The slow bed sand filtration method has also been used to treat domestic wastewater for more than 150 years. Nowadays this process is referred to as intermittent sand filtration (Tchobanoglous, 1979; Cleasby, 1990). In slow bed sand filters the bed operates as a biological contact bed and is capable of removing several bacteria. The biological process to remove microorganisms is dependent on temperature (GHD, 2005).

#### *Mode of action*

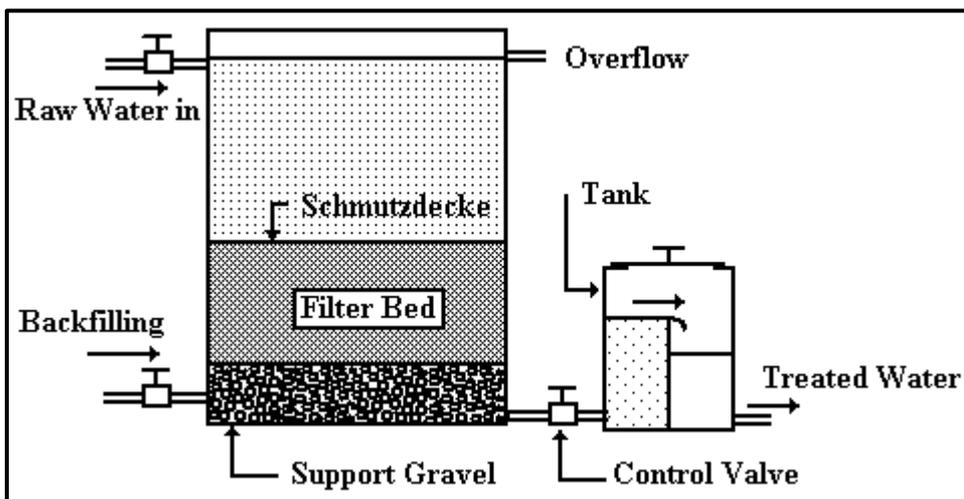
A properly constructed slow bed sand filter typically consists of a tank, a bed of fine filter sand, a gravel layer to support the sand, a proper draining system to collect the filtered water and a flow regulator to control and ensure an even filtration rate (Fig. 1) (GHD, 2005). Most slow bed sand filters contain a 60 to 120 centimetre layer of sand which are supported by a 30 to 50 centimetre gravel layer. The particle size of the sand can range between 0.15 and 0.35 millimetres and the flow rate is usually between 0.04 and 0.40 metres/hour (Huisman & Wood, 1974; Bitton, 2005).

Biological growth within the sand filter consists of a vast variety of microorganisms including algae, bacteria, protozoa, viruses and many more (Huisman & Wood, 1974; Bitton, 2005). Even though the slow bed sand filtration method is capable of removing all of the aforementioned microorganisms, it is not effective in removing plant parasitic nematodes from irrigation water because of the sand bed membrane's large pore size (Hugo & Malan, 2006).

During the normal operation of a slow bed sand filter a biologically active layer builds up. This build-up layer is referred to as the *schmutzdecke* (Huisman & Wood, 1974; Bitton, 2005; Bauer *et al.*, 2011). The layer is made up of filtered particulate and biological growth. This problem can be easily resolved by removing or scraping the top layer of sand. The time between scrapings can vary from one week to several months depending on the turbidity of the water being filtered. After scraping, the top layer is replaced by clean sand in a process known as resanding. For several days after the scraping the quality of the filtered water might be of lesser quality, but quickly improves during the ripening period (Huisman & Wood, 1974; Bitton, 2005).

### Advantages

Slow bed sand filters are relatively inexpensive and are easily built and maintained (Hugo & Malan, 2006; Langenbach *et al.*, 2009). It does not require chemicals or energy to achieve disinfection and needs a smaller space for water treatment compared to other natural technologies for pathogen removal (Langenbach *et al.*, 2009). It is capable of preventing waterborne diseases by removing algae, bacteria, protozoa such as *Giardia* and *Cryptosporidium* (Hijnen *et al.*, 2007), viruses as well as several *Phytophthora* species from irrigation and drinking water. The success of this treatment method is unaffected by the water's pH (Huisman & Wood, 1974; Runia, 1995; Bitton, 2005). The slow bed sand filtration method is also effective in disinfecting wastewater (Tchobanoglous, 1979; Cleasby, 1990).



**Figure 1** Schematic representation of a slow bed sand filter (Anon., 2012e).

### Disadvantages

The slow bed sand filtration method is a rather time consuming process and because of build-up, the sand has to be replaced every few weeks depending on the turbidity of the water that is filtered (Huisman & Wood, 1974; Droste, 1997; Bitton, 2005). A combination of several filter systems is usually necessary to optimally remove pathogens and organic matter from the water (Hugo & Malan, 2006). Even though slow bed sand filters are capable of removing most pathogenic microorganisms from the water, it is not proficient in successfully removing plant parasitic nematodes from irrigation water (Bitton, 2005; Hugo & Malan, 2011). Another disadvantage of this disinfection method is that for several days after resanding, the quality of the filtered water might be reduced (Huisman & Wood, 1974; Bitton, 2005).

### Conclusion

Slow bed sand filters are relatively inexpensive and safe to use when compared to other water treatment methods such as chlorination or bromination, are easily built and do not require chemicals or energy to achieve disinfection. It is capable of preventing waterborne diseases by

removing algae, bacteria, protozoa such as *Giardia* and *Cryptosporidium*, viruses as well as several *Phytophthora* species from irrigation and drinking water. Slow bed sand filters is a rather time consuming treatment. One of its biggest disadvantages is that the sludge build-up has to be treated with additional disinfection methods before it can be discarded. Another disadvantage of this disinfection method is that for several days after resanding, the quality of the filtered water might be of lesser value. Slow bed sand filtration will thus not be an effective method for irrigation water disinfection if it is used as the sole treatment method.

### **Ultrafiltration**

#### *Background and practical application*

Ultrafiltration is a membrane filtration method that was developed in the 1930's. The first ever ultrafiltration manufacturing company was founded by Alan Michaels in 1962 (Conlon, 1990). In the past, membrane filtration processes were mostly used in the removal of salt from water, wine and juice filtration as well as industrial waste filtration (GHD, 2005; Vickers, 2005). It was not until the late 1980's that ultrafiltration was implemented to produce high quality drinking water (Vickers, 2005).

In recent years it is increasingly used for the removal of bacteria and other microorganisms, particulate material as well as natural organic material, which can be responsible for colour, taste and odour changes in the water (GHD, 2005; Konieczny *et al.*, 2009; Arnal *et al.*, 2010). Since not all pathogenic microorganisms are removed during ultrafiltration, an additional disinfection treatment is necessary. It is of great importance to remove organic material from irrigation water, since it can react with other disinfectants to form carcinogenic disinfection by-products (Freese *et al.*, 2003).

In industry ultrafiltration is widely used for a wide variety of applications not always involving water. Ultrafiltration is often used to recover paint from primers applied by wet electrode-position processes in auto and appliance factories, to recover proteins in cheese whey for other dairy applications, to separate biologically active particles and fractions from fluids, to reduce water pollution by concentrating organisms from the water in the retentate and to filter cells and cell fractions from liquid media (Cheremisinoff, 2002; LENNTECH, 2011).

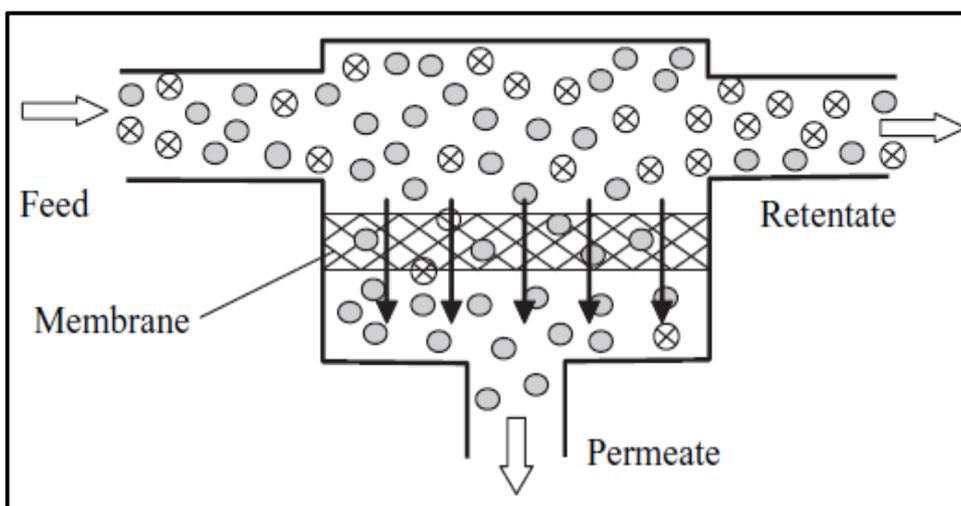
#### *Mode of action*

Ultrafiltration is a process commonly described as a method of separating particles from a solution by applying pressure to pass them through a physical barrier, for instance a semipermeable membrane (Fig. 2) (Conlon, 1990; GHD, 2005; Jacangelo & Noack, 2005). The pressure separates the solution into a permeate and a retentate. The permeate is usually pure water and the retentate is a concentrated solution, separated from the original solution, that must be disposed of or treated by other disinfection methods. Pressure-driven membrane processes use the dissimilarity in pressure between the permeate and the feed emulsion side as the driving force to

transport water through the membrane (Van der Bruggen *et al.*, 2003; Jacangelo & Noack, 2005). Any particles that might be present in the water are retained by the membrane based on their size and shape, and in the case of reverse osmosis, charge. The separation efficacy is expressed in terms of the rejection of a particle (Van der Bruggen *et al.*, 2003; Jacangelo & Noack, 2005).

Ultrafiltration membranes have small pores ranging between 2 to 100 nanometres and as a result, the permeability is considerably lower than other membranes such as microfiltration membranes which have bigger pores (Van der Bruggen *et al.*, 2003; GHD, 2005; Jacangelo & Noack, 2005). Since ultrafiltration membranes has smaller pores than microfiltration membranes, a larger pressure is necessary to separate the retentate from the permeate. Ultrafiltration is typically used to remove large natural organic material from water (Van der Bruggen *et al.*, 2003).

In ultrafiltration, the molecular weight cut-off concept is mostly used to determine whether a particle will be retained by the membrane or be able to move through. Rejection of particles through the membrane increases with the molecular weight of the particles present in solution. Approximately 90 percent of the time components that are larger than the molecular weight cut-off criteria are successfully retained by the membrane (Conlon, 1990; Van der Bruggen *et al.*, 2003). Shape, size and flexibility of the particles are also critical factors in determining whether particles will be retained by the membrane or not. Usually some low molecular weight materials and water are passed through the membrane when hydrostatic pressure is applied. Unlike in other filtration methods, no build-up of retained materials occur on the membrane filter since the rejected particles are usually much bigger than the pores in the membrane (Conlon, 1990; Cheremisinoff, 2002; Jacangelo & Noack, 2005).



**Figure 2** Schematic representation of an ultrafiltration membrane (Arnal *et al.*, 2010).

### Advantages

These days a wide variety of synthetic polymers can be used as ultrafiltration membranes (Cheremisinoff, 2002; Konieczny *et al.*, 2009). Many of these membranes can be handled dry,

have greater organic solvent resistance and are less sensitive than reversed osmosis membranes to pH and temperature. Other advantages of ultrafiltration is its ability to reduce the strength of waste present in a solution and being able to recover valuable by-products such as proteins at the same time (Cheremisinoff, 2002; Jacangelo & Noack, 2005).

Ultrafiltration is not only capable of removing large organic molecules from irrigation water it can also remove most bacteria, viruses as well as protozoan cysts such as *Giardia* and *Cryptosporidium*, as these organisms are generally larger than the membrane pore size (GHD, 2005; Speth & Reiss, 2005; Konieczny *et al.*, 2009). A log-reduction of up to 4.5 can be achieved for *Giardia* and *Cryptosporidium* (Speth & Reiss, 2005). This method is also capable of reducing the turbidity of water and as a result ultrafiltration is often used as a pre-treatment method to remove molecules with high molecular weight from water. Such molecules include organic components which are able to react with other disinfectants. Another advantage of ultrafiltration is that no disinfection by-products are formed by this method (Van der Bruggen *et al.*, 2003; Konieczny *et al.*, 2009).

#### *Disadvantages*

One of the biggest disadvantages of ultrafiltration is its high capital and operating cost (Freese *et al.*, 2003; GHD, 2005). Its operating time of 16 to 20 hours per cycle to remove particles from water also contributes negatively to the use of this method for disinfection purposes (Cheremisinoff, 2002). Ultrafiltration is not capable of removing all pathogenic microorganisms from irrigation water since some viruses are a lot smaller than the membrane pore size and are able to pass through (Conlon, 1990; GHD, 2005). To get rid of pathogenic organisms that were able to pass through the membrane and to treat the concentrated retentate, containing bacteria, protozoa and so forth, an additional disinfectant method has to be applied, increasing the cost immensely (Van der Bruggen *et al.*, 2003; GHD, 2005). Membrane lifetimes are usually two years or more when treating clean water sources but can decrease dramatically when ultrafiltration membranes are used to filter dirty samples such as wastewater (Cheremisinoff, 2002).

#### *Conclusion*

An advantage of ultrafiltration include its ability to reduce the strength of waste present in a solution and at the same time being able to recover valuable by-products such as proteins in the process. Ultrafiltration can also remove most bacteria, viruses as well as protozoan cysts such as *Giardia* and *Cryptosporidium*, as these organisms are generally larger than the membrane pore size. This method is also capable of reducing the turbidity of water and as a result ultrafiltration is often used as a pre-treatment method to remove molecules with high molecular weight from water. Another advantage of ultrafiltration is that no disinfection by-products are formed by this method. Ultrafiltration's biggest disadvantages are its high cost and extremely long operating time. Even though these systems are adjustable to reduce the operating time needed for water purification,

this will proportionately increase the total cost of the system. Another disadvantage of ultrafiltration is its inability to remove particles that are smaller than the membrane pores. This poses a problem since pathogens which are smaller than ultrafiltration membrane's pore sizes, cannot be removed from the water without an additional disinfection method being applied to the water. Thus ultrafiltration of irrigation water will not be an acceptable treatment method, since unsuccessful treatment of water can have a negative influence on consumers.

### **Alternative / Photochemical disinfection methods**

Since chemical disinfectants have so many disadvantages not only to humans and animals, but also against the environment, environmental and public agencies were adamant that chemical disinfectant methods be replaced with more ecologically friendly treatment options. As a result photochemical disinfection methods were developed, having the advantage of no disinfectant by-products forming (Acher *et al.*, 1996; Yiasoumi *et al.*, 2005).

### ***Ultrasound***

#### *Background and practical application*

The use of ultrasound to inactivate microorganisms was reported in the late 1920's, but at the time its limited lethal effect on spoilage microbes prohibited it from being used as a sterilisation method (Cameron *et al.*, 2009). Ultrasound was investigated as a potential method for microbial inactivation in the 1960s after it was discovered that the sound waves emitted by the anti-submarine warfare boats, killed fish (Piyasena *et al.*, 2003). Not long after, it was found that ultrasonic energy is a promising method in the treatment of industrial and domestic wastewater (Gonze *et al.*, 1999). In the past, ultrasound was used in the production of cavitation, degassing of water and to accelerate chemical reactions. Since then ultrasound has been used in various applications such as disinfection of water by causing the microorganism cells to disrupt, crystallisation, polymerisation, cleaning, flow measurements as well as wastewater treatment to name just a few (Mahamuni & Adewuyi, 2010).

In recent years ultrasound has extensively been used for wastewater treatment, especially as part of an advanced oxidation process (AOP). Wastewater treatment of various pollutants as well as pathogenic microorganisms such as bacteria was successfully performed with ultrasound (Mahamuni & Adewuyi, 2010).

#### *Mode of action*

Ultrasound commonly refers to pressure waves, generated by mechanical vibrations, with a frequency of 20 kHz or more (Piyasena *et al.*, 2003; Cameron *et al.*, 2009). Ultrasound equipment generally uses frequencies between 20 kHz to 10 MHz. Higher frequencies between 20 to 100 kHz are known as power ultrasound and have the ability to cause cavitation. Cavitation has the

ability to inactivate microbiological organisms (Chu *et al.*, 2001; Thiem *et al.*, 2001; Gil & Selma, 2006; Cameron *et al.*, 2009; Kesari *et al.*, 2011).

Killing of microbes due to ultrasound treatment mainly occurs because of thinning cell membranes, localised heating in certain areas as well the production of free radicals when the ultrasound waves break down the water molecules (Gonze *et al.*, 1999; Piyasena *et al.*, 2003; Cameron *et al.*, 2009; Mahamuni & Adewuyi, 2010; Kesari *et al.*, 2011). Regions of interchanging compression and expansion are created during the sonication process due to longitudinal waves that are created when a sonic wave comes into contact with a liquid medium. Cavitation takes place and gas bubbles are created due to the regions of pressure change that occur in the medium. The surface area of the bubbles, which were formed in the medium, increases during the expansion cycle and as a result increases gas diffusion, causing the bubbles to expand (Gonze *et al.*, 1999; Piyasena *et al.*, 2003; Kesari *et al.*, 2011).

At the point where the ultrasonic energy is incapable of retaining the vapour phase in the bubbles, rapid condensation starts occurring. Shock waves are created since the condensed molecules collide violently when they come into contact with each other. Regions of high temperatures of up to 5 500 °C and pressure up to 50 000 kPa are created due to the shock waves in the liquid (Piyasena *et al.*, 2003). The main bactericidal effect in ultrasound is caused due to the pressure changes that occur because of the bubbles that are imploding. The areas with extremely hot temperatures are also capable of killing some bacteria, but since these regions are localized they do not affect a large enough area to kill all of the microorganisms that might be present in the irrigation water (Thiem *et al.*, 2001; Piyasena *et al.*, 2003; Cameron *et al.*, 2009; Kesari *et al.*, 2011).

### *Advantages*

Ultrasound is effective in treating almost all types of wastewater and is also capable of removing pathogenic microorganisms, such as bacteria as well as protozoa such as *Cryptosporidium* and *Giardia*, from irrigation water (Sangave & Pandit, 2004; Mahamuni & Adewuyi, 2010). Ultrasound treatment is also effective in reducing algae and fungi such as *Phytophthora*, it can reduce biofilm formation, reduce water turbidity as well as iron and sulphur that might cause damage to the irrigation system (Oyib, 2009). A study on wastewater has shown that 20 kHz ultrasound unit, operated at 700 W.L<sup>-1</sup> is capable of causing four log-reductions of faecal coliforms within six minutes. The same unit is also capable of destroying 90% *Cryptosporidium* oocysts within one and a half minutes (Bitton, 2005). Heat and extreme pH changes, in combination with ultrasound, can successfully be used to further decrease microbial counts in irrigation water without the production of any disinfection by-products (Chu *et al.*, 2001; Piyasena *et al.*, 2003; Bitton, 2005).

## Chapter 2

### *Disadvantages*

Ultrasound treatment of irrigation water has an extremely high capital and operating expenditure (Johnston *et al.*, 2006). Even though extremely high temperatures are reached in this process, the disinfection is mainly localised and as a result ultrasound on its own does not always eliminate all microorganisms that might be present in the irrigation water (Piyasena *et al.*, 2003; Bitton, 2005). Better disinfection and a lowering in cost can be expected when ultrasound is used in combination with other disinfection methods such as chlorine, but this can lead to the formation of unwanted carcinogenic by-products (Johnston *et al.*, 2006).

Furthermore the effectiveness of ultrasound treatment to kill pathogenic microorganisms is dependent on various factors. Most microorganisms, especially spores, are fairly resistant to ultrasound treatment thus extended periods of ultrasonication is necessary to kill these microorganisms. Other factors that influence ultrasound treatment efficacy are the amplitude of the ultrasonic waves, the exposure or contact time needed to eliminate the microorganisms, the amount or volume of water that needs to be treated as well as the composition of the water (Gonze *et al.*, 2003; Piyasena *et al.*, 2003; Kesari *et al.*, 2011).

The amount of time needed to eliminate microorganisms in the irrigation water is in turn dependant on the type of microorganisms present in the water, the water's temperature, the amount of light, the amount of nutrients that are present in the water, the depth and size of the water source that needs to be treated, the total suspended and dissolved solids present in the water as well as the turbidity of the water (Oyib, 2009; Kesari *et al.*, 2011).

### *Conclusion*

Ultrasound is effective in treating almost all types of wastewater and is also capable of removing pathogenic microorganisms, such as bacteria as well as protozoa such as *Cryptosporidium* and *Giardia*, from irrigation water. Ultrasound treatment is also effective in reducing algae and fungi such as *Phytophthora* from irrigation water without the formation of any disinfectant by-products. A disadvantage of using ultrasound treatment is that a very long time is required for disinfection to occur, increasing the cost of this already very expensive treatment method even further. Ultrasound efficacy is dependent on various water quality parameters and is not always effective in killing all microorganisms and as a result it is mostly just used as a pre-treatment for other disinfection methods. When taking all advantages and disadvantages of ultrasound treatment into consideration, it is clear that this method will not be the most effective method for treating irrigation water.

## **Ultraviolet irradiation**

### *Background and practical application*

Ultraviolet (UV) light was discovered by a pharmacist from Poland in 1801, by demonstrating that silver chloride was successfully disintegrated by the invisible rays beyond the violet rays. In 1903 it

was discovered that bacteria were most sensitive to inactivation at a wavelength of approximately 250 nanometres. From 1904 to 1905 it was discovered that UV light from arc lamps were a lot more powerful in the deactivation of microorganisms than sunlight was (Bolton & Cotton, 2008). It was found that the order of efficacy of UV wavelengths for inactivation was UVC > UVB > UVA (WHO, 2004; Bitton, 2005; Bolton & Cotton, 2008; Anon., 2012c). In 1910 in Marseille, France, UV irradiation was used for the first time to disinfect drinking water after the mercury vapour lamp and quartz tube was developed and the germicidal effect of UV irradiation was established (Hijnen *et al.*, 2006). It was not until 1916 that UV irradiation was being used to disinfect drinking water in the United States (NDWC, 2005).

General application was hindered for many years because of relatively high costs, unreliable equipment, maintenance problems and the arrival of chlorination as a possible treatment for the disinfection of water. Not only did chlorine appear a lot cheaper than UV, it was also thought to be more reliable and it was possible to measure the potential disinfectant residual of chlorine which was not possible at the time for UV disinfection (Hijnen *et al.*, 2006).

Over the years UV irradiation became a more acceptable method for water disinfection due to the increased information that came to light on the production of carcinogenic oxidation by-products during chemical treatment of water, using chlorine, ozone and other chemical disinfectants (NDWC, 2005; Hijnen *et al.*, 2006). As a result, UV costs declined with the development and use of UV methods that are capable of disinfecting drinking water, wastewater and other water sources for irrigation purposes (NDWC, 2005). The breakthrough of the applicability of UV irradiation as a primary disinfection was in 1998 when it was discovered that UV irradiation was an extremely effective disinfectant against the protozoa, *Cryptosporidium* and later also against *Giardia* (Chang *et al.*, 1985; GHD, 2005; Hijnen *et al.*, 2006; Bolton & Cotton, 2008). Since then UV irradiation has been widely applied in the disinfection of water to control incidental or deliberate microbial contamination of surface and groundwater sources (Hijnen *et al.*, 2006).

Ultraviolet irradiation has several applications in the food and beverage industry, in industrial areas, municipal drinking water and wastewater companies, in hotels, hospitals, care homes, sport centres, in public swimming pools as well as in horticulture for the disinfection of irrigation water for plant cultivation purposes (Anon., 2012c; Anon., 2012d).

### *Mode of action*

Ultraviolet light is the part of the electromagnetic spectrum that is located between visible light and X-rays. The spectral range of UV light lies between 100 and 400 nanometres (nm) and can be divided into four main areas. These include UVA (long-wave, 315 - 400 nm), UVB (medium-wave, 280 - 315 nm), UVC (short-wave, 200 - 280 nm) and vacuum UV (100 - 200 nm) (Bitton, 2005; Anon., 2012c). Germicidal activity takes place due to irradiation at wavelengths between 200 to 300 nm (Das, 2001; Zimmer *et al.*, 2003; Bolton & Cotton, 2008). This region is referred to as the germicidal region since UV light in this region is capable of killing algae, bacteria, fungi, moulds,

nematode eggs, protozoa, viruses as well as yeasts (Chang *et al.*, 1985; Das, 2001; GHD, 2005; Hijnen *et al.*, 2006; Bolton & Cotton, 2008). According to literature the most destructive wavelength for these organisms is at 260 nm (Das, 2001; Bitton, 2005; Hijnen *et al.*, 2006; Bolton & Cotton, 2008).

Ultraviolet inactivation of pathogenic microorganisms is based on the amount of damage done to the nucleic acids namely DNA and RNA of the specific microorganism (Hijnen *et al.*, 2006). The formation of pyrimidine dimers, other photo-products of nucleic acids as well as nucleic acid lesions are responsible for the inhibition of replication and transcription and as a result prevent a microorganism from multiplying after it came into contact with UV light (Hijnen *et al.*, 2006). DNA absorption peaks around 260 nm and decreases at lower or higher wavelengths (Das, 2001; Zimmer *et al.*, 2003; Bitton, 2005; Hijnen *et al.*, 2006; Bolton & Cotton, 2008). Absorbance increases again when wavelengths decrease below 230 nm (Hijnen *et al.*, 2006).

There are mainly two types of UV disinfection methods available namely a flow-through open channel system mainly utilised for wastewater disinfection and an in-pipe closed channel system mainly utilised for drinking water disinfection (Acher *et al.*, 1996; Lazarova & Bahri, 2005; Anon., 2012a). These days most wastewater treatment plants use in-pipe closed systems to treat discharged effluents. As most treated effluents are re-used for irrigation, it is transported in pipes after being treated, to the point of use (Buijs, 2012).

When an open channel system is used, UV modules which are stainless steel frames that manifest the low intensity, low-pressure UV lamps are immersed in the water that flows through the channel (Acher *et al.*, 1996). Low-pressure UV lamps have a peak monochromatic emission at a wavelength of 253.7 nm (Bitton, 2005). The number and size of UV modules needed is dependent on the flow rate of the water to be disinfected, the water quality as well as the disinfection requirements (Acher *et al.*, 1996). Sophisticated controls as well as carefully designed inlet conditions are of great importance to ensure that the lamps stay submerged under water and flow is evenly distributed to prevent short circuiting and loss of disinfection performance (Anon., 2012a). Most open channel systems require that lamps be manually cleaned by an operator, thus each lamp has to be removed by hand (Anon., 2012a). In open channel UV units the gravitationally fed water flows almost laminar to the UV lamps due to the low velocity of the fluid (Buijs, 2012; Anon., 2012a). As a result microorganisms pass through the area with the lowest UV intensity without receiving sufficient UV light exposure (Buijs, 2012).

The in-pipe closed systems utilises high intensity medium pressure lamps in a closed area which is installed in the effluent header pipe just before discharge (Acher *et al.*, 1996; Anon., 2012a). Medium-pressure UV lamps have a peak polychromatic emission at wavelengths ranging from 185 to 400 nm (Bitton, 2005; Anon., 2012c). In-pipe closed channel systems have different requirements for inlet design than open channel systems (Anon., 2012a). Water flows in a linear flow at relatively high flow rates, and flow is always evenly distributed inside the chamber, resulting in optimum disinfection performance (Anon., 2012a). Since the flow rate is very high, the

irradiation time is relatively short, thus high intensity UV lamps are necessary to insure the minimum UV dose required is applied (Acher *et al.*, 1996). The use of high intensity lamps enables the treatment of wastewater effluents in a relatively small area (Anon., 2012a). Closed channel systems have an automatic cleaning system that cleans everything that might have been deposited on the quartz lamp sleeves capable of reducing the UV light intensity transmitted into the water (Anon., 2012a). The high output from these lamps allows for the use of fewer lamps than in an open channel system to achieve the same amount of disinfection, significantly enhancing reliability and at the same time reducing maintenance costs. Another advantage of closed channel systems is that less head-loss occurs in comparison with open channel designs (Acher *et al.*, 1996; Zimmer & Slawson, 2002; Anon., 2012a).

Taking into consideration all of the advantages of closed channel systems in comparison with open channel systems, the use of monochromatic emitting low-pressure mercury UV lamps in water disinfection has mainly been replaced by polychromatic emitting medium-pressure mercury UV lamps that has a much broader spectrum to efficiently kill all pathogenic microorganisms (Zimmer & Slawson, 2002).

Exposure of microorganisms to UV irradiation results in damage to the nucleic acids as well as other components of the cell (Hijnen *et al.*, 2006). Even after UV irradiation some microorganisms are capable of retaining certain metabolic functions such as enzyme activity (Hijnen *et al.*, 2006). Since most microorganisms are exposed to UV irradiation from sunlight on a daily basis, many microorganisms have over time developed mechanisms to compensate for the damage done to them by UV irradiation. Nucleotide excision repair, also referred to as dark repair, and photo-reactivation are the two main pathways available to repair UV damaged DNA or RNA (Zimmer & Slawson, 2002; Hijnen *et al.*, 2006).

According to Zimmer & Slawson (2002), certain microorganisms are capable of repairing damage done to cells following exposure to low-pressure UV irradiation. Zimmer & Slawson (2002) compared the efficacy of low-pressure UV lamps with medium-pressure UV lamps. It was found that *E. coli* underwent photo-repair after exposure to low-pressure UV lamps (doses of 5, 8 and 10 mJ.cm<sup>-2</sup>), but no repair was evident after it was exposed to medium-pressure UV lamps (doses of 3, 5, 8 and 10 mJ.cm<sup>-2</sup>) at the same or even lower doses (Zimmer & Slawson, 2002). Zimmer *et al.* (2003) also found no evidence of repair to *Cryptosporidium parvum* following low doses, 1 and 3 mJ.cm<sup>-2</sup>, of both low- and medium-pressure UV lamps.

In general, microorganisms' resistance to UV follows the same pattern as with chemical disinfectants which are as follows: protozoan cysts > bacterial spores > viruses > vegetative bacteria (Bitton 2005; Lazarova & Bahri, 2005). Thus, since Zimmer *et al.* (2003) found that UV is effective against a protozoan organism, it can be assumed that medium-pressure UV will be effective in killing all pathogenic microorganisms without any repair to cells after exposure.

Based on UV disinfection and repair mechanisms, a UV dose of 30 mJ.cm<sup>-2</sup> will be sufficient to produce reclaimed water virtually free from pathogens and is an adequate method of disinfection of secondary effluent for agricultural irrigation purposes (Yoon *et al.*, 2007).

### *Advantages*

Chemical disinfection with chlorine is not effective against *Cryptosporidium* and *Giardia* protozoan microorganisms (Lazarova & Bahri, 2005; Hijnen *et al.*, 2006). Even though ozone is effective against these protozoan pathogens, it is not a viable treatment option since ozone is very unstable and it is almost impossible to predict how it would react with organic matter that might be present in the water (Freese *et al.*, 2003; Selma *et al.*, 2008). These days UV irradiation is regarded as a disinfection method that is extremely effective against all pathogens such as algae, bacteria, fungi, moulds, nematodes eggs, protozoa, viruses, yeasts as well as water moulds such as *Phytophthora* that could be transmitted through water (Maya *et al.*, 2003; Das, 2001; Bitton, 2005; Yiasoumi *et al.*, 2005; Hijnen *et al.*, 2006; Bolton & Cotton, 2008).

Disinfection of water and wastewater with UV irradiation for all kinds of purposes has many advantages (Bitton, 2005). These advantages include no production of carcinogenic, mutagenic or toxic by-products (Bitton, 2005; NDWC, 2005; Guo *et al.*, 2009; Buijs, 2012). Ultraviolet irradiation prevents the occurrence of taste and odour problems that can occur on-site or in the final water after treatment (Bitton, 2005; NDWC, 2005). No volatile toxic chemicals are needed for treatment (Bitton, 2005; NDWC, 2005; Bolton & Cotton, 2008), UV equipment and the water contact chamber requires a minimal amount of space and can usually be retrofitted into existing water treatment plants (Bitton, 2005; NDWC, 2005; Bolton & Cotton, 2008). Ultraviolet apparatus is relatively inexpensive with low capital and operating costs compared to other treatment methods that are effective in killing protozoan organisms, UV equipment is easy to operate and water treated with UV requires only a few seconds contact time to be properly disinfected and it does not affect the treated water's quality in anyway (NDWC, 2005; Bolton & Cotton, 2008).

### *Disadvantages*

One of UV's disadvantages is that it has no disinfectant residual in treated water and as a result certain susceptible microorganisms can become viable again if it was treated by low-pressure UV lamps in an open channel system. This problem can be overcome by treating water with medium-pressure UV lamps in an in-pipe closed system (Zimmer & Slawson, 2002; Zimmer *et al.*, 2003; Bitton, 2005; NDWC, 2005; Bolton & Cotton, 2008). Other disadvantages of UV are that it is not always possible to accurately measure the UV dose, so operators have to rely on secondary measurements such as sensor readings, UV transmittance as well as water flow rates (Bitton, 2005; NDWC, 2005; Bolton & Cotton, 2008). Disinfection reliability also decreases in high turbidity effluents and as a result water for irrigational purposes mostly has to be pre-treated by sand- or ultrafiltration before it can be treated with UV (Acher, 1993; Bitton, 2005; Lazarova & Bahri, 2005).

Since UV lamps contain mercury, breakage of lamps can in certain cases result in a mercury hazard (Bolton & Cotton, 2008). The amount of mercury contained in these UV lamps are minute and if it breaks are usually contained within the quartz sleeve and might only come into contact with the water due to negligence (Van Kamp, H. 2014, Winelands UV Technology, Stellenbosch, South Africa, personal communication, 20 January). Another disadvantage of UV lamps are that in case of power outages water can be under disinfected (Bolton & Cotton, 2008).

### *Conclusion*

Ultraviolet irradiation is regarded as a disinfection method that is extremely effective against all pathogens such as algae, bacteria, fungi, moulds, nematodes eggs, protozoa, viruses, yeasts as well as water moulds such as *Phytophthora* that could be transmitted through water. Ultraviolet disinfection of wastewater effluents is an economically competitive alternative to other chemical and physical methods of irrigation water treatment and has a contact time of only a few seconds. Even though UV irradiation experiences reduced disinfection performance in water with high levels of suspended solids, turbidity and organic matter, these problems can easily be resolved by pre-treating the water with sand- or ultrafiltration methods. Medium-pressure UV light is capable of killing bacteria, viruses, protozoa as well as water moulds, without the possibility of reactivation occurring. Another factor making UV treatment such a viable option for irrigation water disinfection is that it does not produce any carcinogenic, mutagenic or toxic by-products or change the chemical characteristics of the water being treated. Ultraviolet irradiation also prevents the occurrence of taste and odour problems that can occur on-site or in the final water after treatment.

### **CONCLUDING REMARKS**

South Africa is a semi-arid, water scarce country facing an undeniable national water crisis, not only in terms of availability, but also in terms of the quality of its fresh water sources. Fresh produce production is an important component of Western Cape agriculture as well as the economic viability of the country. As a result of the varying rainfall patterns many farmers are forced to use water, from rivers, to irrigate their crops. These rivers are often contaminated with high microbial loads and are thus of questionable quality for irrigation. A long term solution for these farmers would be to apply on-farm treatments of the water they use for irrigation. There are a wide range of disinfectants available in treating water used for irrigational purposes.

Bromine is a cost effective treatment option that can be used to kill most microorganisms, but it is incapable of killing protozoan pathogens. Another major disadvantage of treating water with bromine, is the formation of harmful disinfectant by-products. As a result bromine will be an ineffective method to treat irrigation water.

Chlorination is also a relatively inexpensive method, that requires low doses for disinfection and provides residual protection against microbial growth, but it is not a suitable option for irrigation

water disinfection since it is incapable of killing protozoan microorganisms, requires a relatively long contact time and it releases carcinogenic by-products into the disinfected water.

Hydrogen peroxide formulations are capable of killing most microorganisms, but it is not a suitable choice for irrigation water disinfection since it produces disinfection by-products and large concentrations are needed for disinfection making it a very expensive disinfection method. It is also a dangerous chemical, damaging all proteins it comes into contact with and can have serious consequences if individuals come into contact with it.

Ozone disinfection is capable of killing bacteria, viruses and protozoan parasites, but it is not a suitable choice for irrigation water disinfection since this method is quite expensive and produces carcinogenic or mutagenic disinfectant by-products. Ozone is also very unstable and it is almost impossible to predict how ozone might react with organic matter that might be present in the irrigation water.

Slow bed sand filtration is a relatively inexpensive method, with low maintenance and capable of removing most microorganisms, including protozoan microorganisms, from water but it is not an effective method for irrigation water disinfection since it is a time consuming process affected by the water's turbidity. It can successfully be used as a pre-treatment for other disinfection methods.

Ultrafiltration is capable of removing organic materials from water to decrease the formation of carcinogenic disinfectant by-products, but it is not an effective disinfection method for irrigation water treatment since all particles including microorganisms such as some viruses that are smaller than the membrane pores are capable of permeating through the membrane. Another disadvantage of using ultrafiltration is that all of the pathogenic microorganisms that are successfully removed from water, builds up and forms a retentate. Additional disinfection methods are necessary to destroy the microorganisms in the retentate. As a result, ultrafiltration is quite a costly method which requires a long contact time for proper disinfection.

Even though ultrasound treatment is capable of destroying most pathogenic microorganisms, it takes a long time (hours) for disinfection to occur, increasing the cost of this very expensive treatment method even further. Ultrasound is thus not an effective method for the disinfection of irrigation water, but it can be applied as a pre-treatment option for other disinfection methods.

Ultraviolet irradiation is regarded as a disinfection method that is extremely effective against all pathogens such as algae, bacteria, fungi, moulds, nematodes eggs, protozoa such as *Giardia* and *Cryptosporidium*, viruses, yeasts as well as water moulds such as *Phytophthora* that could be transmitted through water. Medium-pressure UV light is capable of killing all of the aforementioned organisms, without the possibility of reactivation occurring. Ultraviolet disinfection of wastewater effluents is an economically competitive alternative to other chemical and physical methods of irrigation water treatment since it has a very short contact time of only a few seconds. Even though UV irradiation experiences reduced disinfection performance in water with high levels of

suspended solids, turbidity and organic matter, these problems can easily be resolved by pre-treating the water with sand- or ultrafiltration methods. Another factor making UV treatment such a viable option for irrigation water disinfection is that it does not produce any carcinogenic, mutagenic or toxic by-products or change the chemical characteristics of the water being treated. As a result it can be concluded that UV will be the most effective method for irrigation water treatment when used together with a suitable pre-treatment method such as sand filtration.

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## CHAPTER 3

### SCOPING STUDY ON DIFFERENT ON-FARM TREATMENT OPTIONS TO REDUCE THE HIGH MICROBIAL CONTAMINANT LOADS OF IRRIGATION WATER TO REDUCE THE RELATED FOOD SAFETY RISK

#### SUMMARY

A baseline study was performed of the water at Limberlost Farms, located just outside Stellenbosch. The farm irrigates fresh produce with water obtained from the Eerste River. The study was done over a five month period, at six preselected sampling points, to determine the microbial and chemical parameters of the water so a baseline could be established to compare the results to when an ultraviolet (UV) apparatus is installed. Aerobic colony count (ACC), total coliforms (TC) and *Escherichia coli* (*E. coli*) were tested for during the microbial study, while the chemical analysis consisted of temperature, pH, conductivity, chemical oxygen demand (COD), alkalinity and total soluble solids (TSS) determinations. The UV unit was installed and functioning by the end of February 2013. The UV treatment study was performed over a five month timeline, at eight different sampling points. The same microbial tests were performed during the UV treatment study, while turbidity and percentage ultraviolet transmittance (% UVT) were additionally tested for during chemical analysis.

During the baseline study ACC, TC and *E. coli* counts as high as 9 600 cfu.mL<sup>-1</sup>, 13 799 MPN.100 mL<sup>-1</sup> and 2 098 MPN.100 mL<sup>-1</sup> were detected from the river, respectively. While performing the UV treatment study ACC, TC and *E. coli* counts as high as 142 000 cfu.mL<sup>-1</sup>, 241 960 MPN.100 mL<sup>-1</sup> and 6 867 MPN.100 mL<sup>-1</sup> were detected in the river, respectively. As a result it was concluded that the Eerste River was unsuitable for irrigation of fresh produce that is consumed raw. The higher counts in the river, during the UV treatment study might be attributed to the increase in rainfall that occurred in the sampling months (March to July 2013). The counts as measured at the point of irrigation are of greater importance, as the counts present in the river might still decrease to below the guideline levels after passing through sand filters and the addition of hydrogen peroxide (the farm's current mode of treatment) or after passing through the UV in the UV treatment study.

At the point of irrigation the ACC, TC and *E. coli* counts during the baseline study were as high as 8 800 cfu.mL<sup>-1</sup>, 24 196 MPN.100 mL<sup>-1</sup> and 85 MPN.100 mL<sup>-1</sup>, respectively. After hydrogen peroxide addition log-reductions between 0.0 and 2.0 were found, but the log reduction was never found to be constant. The counts at the point of irrigation remained more or less constant compared to the river due to contamination that occurred at the sand filters, making the water unsuitable for irrigation of fresh produce. During the UV treatment study ACC, TC and *E. coli* counts were as high as 35 000 cfu.mL<sup>-1</sup>, 10 462 MPN.100 mL<sup>-1</sup>

and 63 MPN.100 mL<sup>-1</sup> at the point of irrigation, respectively. Log-reductions in the range of 0.0 to 1.5 were achieved, but the results were inconsistent. After treatment with chlorine and re-sanding of the sand filters, the counts decreased to below South African Water Quality (DWAf, 1996b & DWAf 1996d) and Canadian (Monaghan & Hutchison, 2010) guideline limits, making the water safe for irrigational use in terms of the microbial parameters applied, but this was not necessarily due to the effect of UV. It is of importance to find a treatment that would bring the counts in the water to below the limits required for safe irrigation.

## INTRODUCTION

According to numerous studies performed in the last decade it was found that the water quality of many South African rivers has been declining dramatically due to an increase in pollution levels (Paulse *et al.*, 2009; Ackermann, 2010; Ijabadeniyi, 2010; Lötter, 2010; Kikine, 2011; Gemmell & Schmidt, 2012; Huisamen, 2012). Several factors are known to contribute to the condition of South Africa's rivers. These include pollution with improperly treated human, industrial and municipal wastes from improperly functioning or damaged sewage treatment plants, storm water overflows and agricultural effluent run-off (Schultz-Fademrecht *et al.*, 2008; Lötter, 2010). Informal settlements are another major source of source water contamination in South Africa, since they are mostly located upstream from areas of a river used for irrigation, thus all the waste and effluents produced upstream end up in the natural water sources and contribute to crop contamination (Provincial Development Council, 2005; Lötter, 2010).

Many South African farmers have to use water from nearby rivers for crop irrigation, since it is the most affordable and sometimes only source of water available to them. It is thus of utmost importance that the quality of the water used to irrigate crops is known, since pathogens can be carried over from water onto fresh produce (EC, 2002; Ijabadeniyi *et al.*, 2011). In the past few years consumers have started consuming more fruits and vegetables as they became increasingly aware of their health. As a result there has been an increase in produce-associated foodborne outbreaks (Buck *et al.*, 2003; Lynch *et al.*, 2009; Panigrahy *et al.*, 2011).

Once river water has been contaminated little can be done to improve the quality. Therefore if possible, contaminated water should not be used to irrigate fresh produce (Ackermann, 2010). Good quality water for irrigation purposes is becoming scarcer and more expensive (Newman, 2004; Yiasoumi *et al.*, 2005). Disinfection of water is of great importance since it can control growth of pathogens in irrigation systems and reduces the risk of introducing disease to the farm and crops through irrigation water (Yiasoumi *et al.*, 2005; Pehlivanoglu-Mantas *et al.*, 2006).

The objective of this study was firstly, to investigate the change in water quality (in terms of microbial and chemical parameters) over an entire irrigation system and, secondly to investigate the efficacy of a UV treatment system in the study irrigation system.

## **MATERIALS AND METHODS**

### **Site selection**

For this study an appropriate site was selected. As part of the site selection, certain aspects were taken into consideration so as to find the most appropriate site. These aspects included the irrigation water sources available on the farm, the type of contamination occurring (also referred to as the microbial loads present in the water), the type of farming, the type of vegetable or fruit crop being irrigated, the type of irrigation system used, the irrigation usage periods as well as the availability and access of the site over an extended time.

After visiting several potential sites, Limberlost Farms, was chosen (Figs. 3 & 4). The farm is situated approximately nine kilometres south-west of Stellenbosch on the Annandale road. Limberlost Farms is situated approximately eight kilometres downstream from where the Plankenburg and Jonkershoek Rivers merge to form the Eerste River (Fig. 3). Water samples were obtained from several preselected sampling points along the irrigation system on Limberlost Farms. Water drawn from the Eerste River is used, by Limberlost Farms, to irrigate strawberries, bell peppers and tomatoes. It also passes through a series of filters and dams and is dosed with chemicals before irrigation.

### **Sampling points**

Water is pumped from the Eerste River (Fig. 4), through sand filters (Conn 40 Manual sand filter 120 microns, South Africa) to a first holding dam (7 000 – 8 000 m<sup>3</sup> in size, lined with low-density polyethylene) at a flow rate of 90 m<sup>3</sup>.h<sup>-1</sup>. When the first holding dam is full, it overflows into a second holding dam. After the second holding dam (7 000 – 8 000 m<sup>3</sup>, lined with low-density polyethylene), the water passes through several sand filters (Conn 40 Manual sand filter 120 microns, South Africa) and hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) is added to the water as a disinfection measure. The farmer adds approximately 5 - 20 parts per million (ppm) H<sub>2</sub>O<sub>2</sub> to achieve an end concentration of at least 1 ppm at the point of irrigation. According to NETAFIM (2009), when H<sub>2</sub>O<sub>2</sub> is continuously injected at a low dosage into the irrigation cycle, the injected concentration (in the pump house at Sampling Point 4) should be between 10 - 50 ppm so a residual concentration (point of irrigation at Sampling Point 6) of at least 0.5 ppm could be reached for disinfection to be effective. Thereafter, the water is pumped to a holding tank (400 m<sup>3</sup>, to facilitate contact time for the H<sub>2</sub>O<sub>2</sub>). Water from the

holding tank is pumped, via a pump room, to the point of irrigation. An acid-mixture (sulphuric and phosphoric) dosing (concentration was pH dependant, the same amount was not always added) was regularly applied to the water leaving the holding tank to lower the pH to within the SAWQG for irrigation water of 6.5 to 8.5 (DWAF, 1996b; Gregory, 2001).

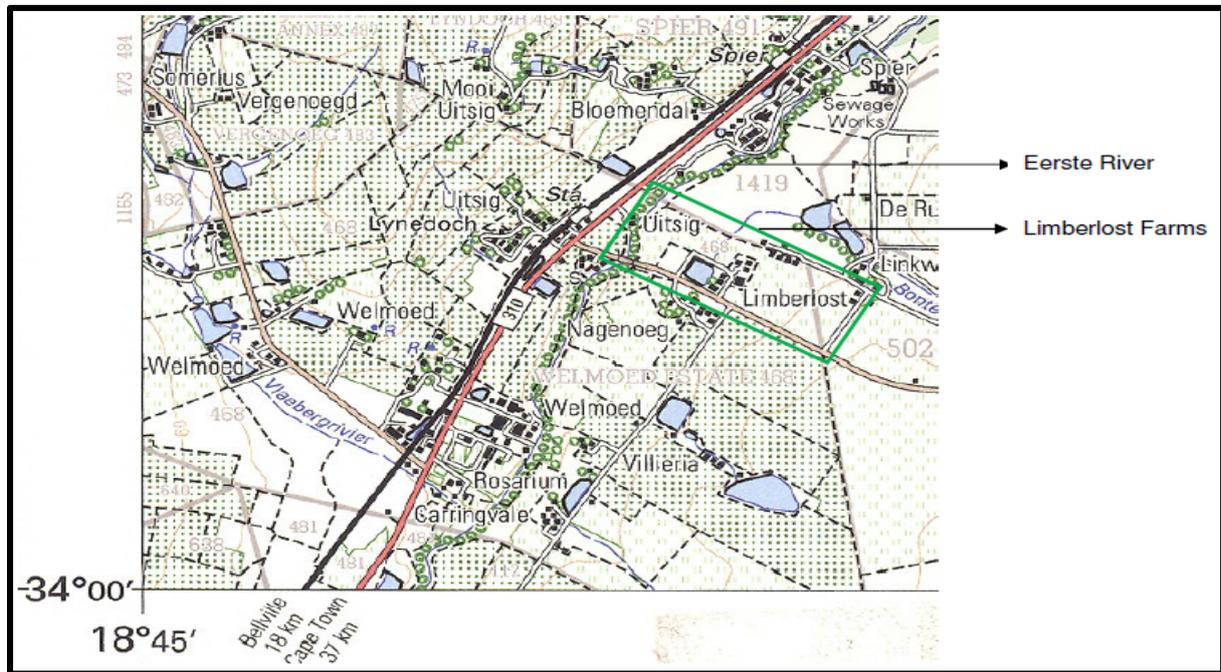
The study was divided into a baseline study (river water through on-farm irrigation system) and a UV treatment study (river water through irrigation system and UV system) (Fig. 5). The sampling points for the baseline river water study were as follows (in sampling order) (Fig. 5): 1) river and sand filters; 2) inlet into first holding dam; 3) overflow from first holding dam into second holding dam; 4) after second holding dam and sand filters ( $H_2O_2$  added); 5) in pump house after holding tank and 6) point of irrigation. The UV apparatus (Berson Inline<sup>+</sup> 100 WW, Lamp type: B810H berson Multiwave<sup>®</sup>) was installed in a closed off room after Sampling Point 4 and before Sampling Point 5. After the installation of the UV apparatus into the current irrigation system, at a point after the sand filters and before the pump room, an additional two sampling points were created. The sampling points for the river study after the installation of UV (also referred to as the UV treatment study) are referred to as follows (in sampling order): 1) river and sand filters; 2) before first holding dam; 3) overflow from first holding dam into second holding dam; 4) after second holding dam and sand filters; 5) before UV; 6) after UV; 7) in pump house after holding tank and; 8) point of irrigation. The flow rate of the water during the UV treatment study was set at 30  $m^3 \cdot h^{-1}$ .

Initially some microbiological tests were performed at different flow rates to determine whether it affects the success of the treatment. After initial testing it was decided to take samples at a flow rate of approximately 500  $L \cdot min^{-1}$ . Sampling took place over a three month period to gather information for the UV treatment study. Sampling was done from March till the end of July 2013 to determine the microbiological and chemical effects of UV on the water. For the purpose of this study, no  $H_2O_2$  was added to the water during the investigation on the efficacy of UV in reducing the microbial counts in the water, to below the recommended limits for irrigation water.

### Sampling frequency

Nine sample sets were collected over a five and three month period during the baseline (each set consisted of samples from Sampling Points 1 - 6) and UV treatment study (Sampling Points 1 - 8), respectively. Samples were collected every one to two weeks for the duration of the microbial and chemical baseline and UV treatment study performed from October 2012 up to February 2013 and March till May 2013, respectively. Samples were collected on a Tuesday morning usually between 08h30 and 09h30, after which samples

were transported back to the laboratory in cooler boxes for chemical and microbiological analyses.

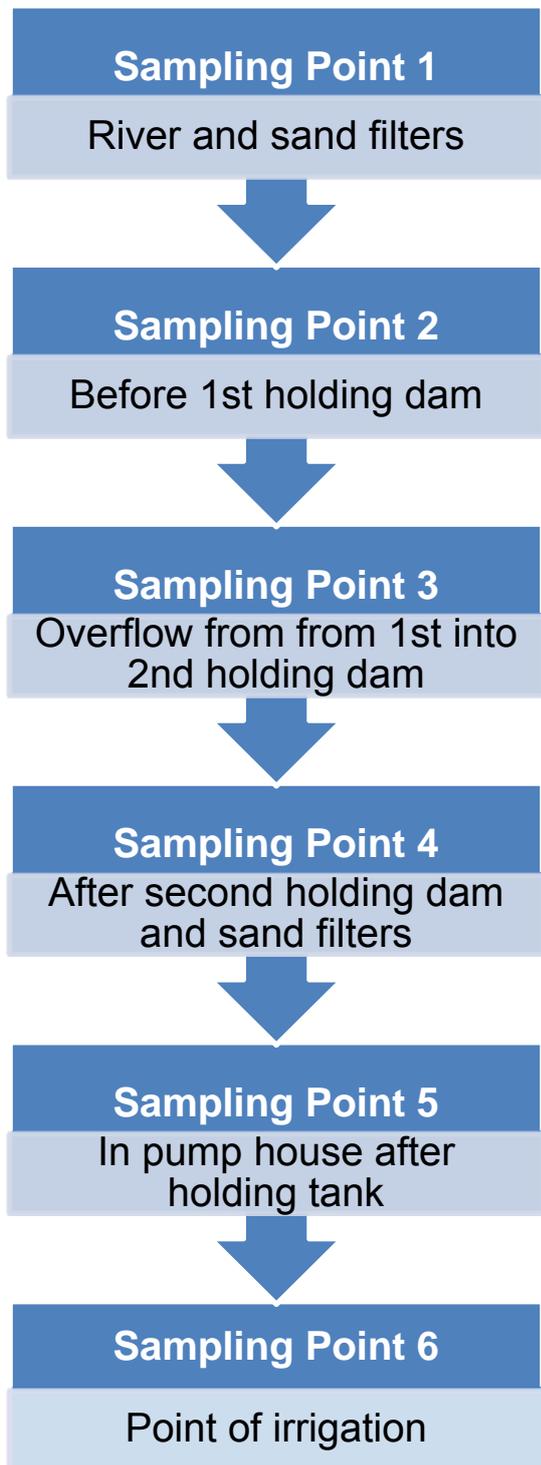


**Figure 3** A topographical map showing Limberlost Farm (Stellenbosch is to the north-east).

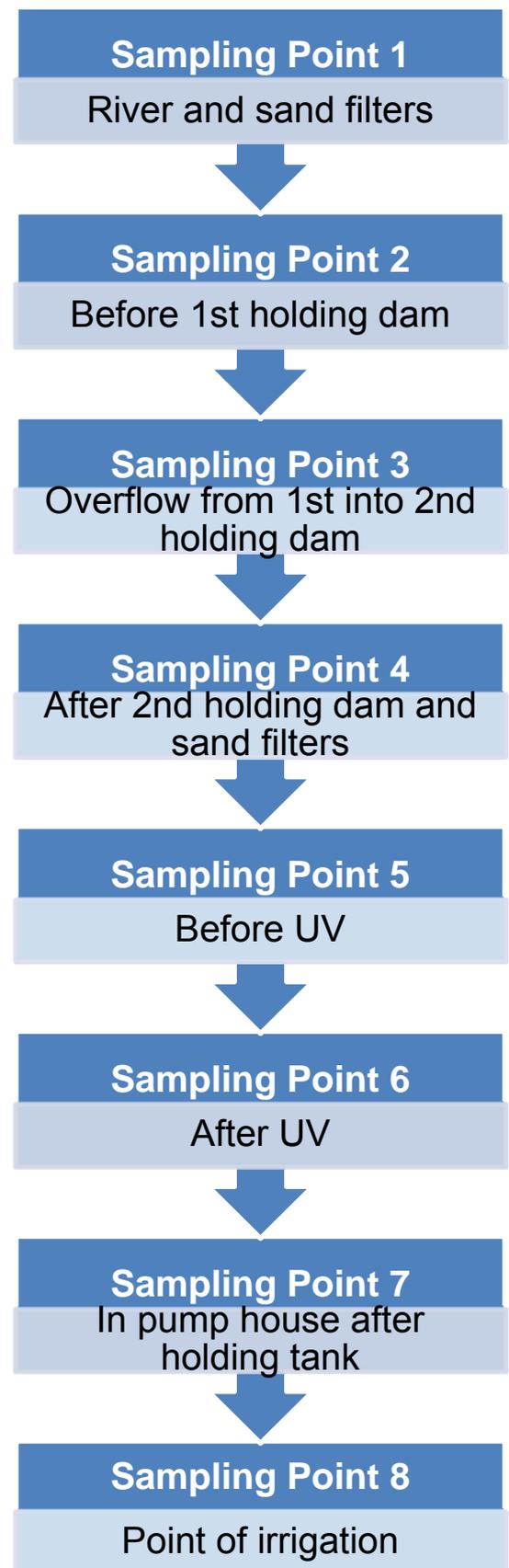


**Figure 4** Map of Limberlost Farms and layout of the farming operations and the Eerste River where samples were sampled.

a)



b)



**Figure 5** The sampling points for the baseline study (a) and UV treatment study (b).

### **Sampling method**

The sampling of river and irrigation water was carried out according to the SANS 5667-6 (2006) guidelines. Safety precautions were taken, to not only ensure the safety of the sampler, but also to improve the accuracy of the results achieved. Safety measures included wearing surgical gloves and protective waterproof footgear when sampling. Sterile, Schott bottles (1L) were used to collect the water at the different sampling points (six in the baseline study and eight in the UV treatment study). The Schott bottles were sterilised and marked beforehand at the laboratory and transported in cooler boxes containing ice bricks. Samples were transported back to the lab in the cooler boxes (as close to 4°C as possible) and analysed within six hours of sampling.

For the collection of the river samples, care was taken to not disturb any sediments and the sample was taken as far away from the river bank as possible. A sterile Schott bottle (1L) was opened under the water surface and submerged to a depth of approximately 30 cm (if permitted), pointing in the direction of the water flow. The bottle was filled to the top and the cap replaced before removing it from the water. If there was a noticeable difference in the flow of the river, appearance and any accompanying odours, these were also recorded. Collection of samples from Sampling Points 2 and 3 (inlet into first holding dam and overflow into second holding dam) was performed as follows: Sterile Schott bottles (1L) were opened while water was flowing over the bottles. The bottles were held facing the flow and caps were replaced once the bottles were full, while still being held in the water flow. Sampling at all the other Sampling Points (4 - 8) was done at taps. The caps of the Schott bottles were removed after opening the taps and only replaced once the bottles were full. Samples at the point of irrigation were taken from the drip irrigation system (October 2012 - February 2013) and from a sprinkler irrigation system (March - May 2013). All of the samples were placed upright in cooler boxes for transportation and were analysed within six hours after sampling.

### **Chemical and environmental parameters**

#### *Temperature and pH*

The temperature and pH of the water was measured simultaneously at each of the Sampling Points with the probe of a WTW pH320 digital pH-meter (Xylem Inc., Germany). The pH was determined according to Standard Methods (APHA, 1998).

#### *Conductivity*

The conductivity of the water was measured with a HI 8711 conductivity meter (Hanna Instruments, South Africa). The conductivity meter was calibrated once a month according

to the instruction manual (Hanna Instruments, South Africa) using 12880  $\mu\text{S}/\text{CM}$  @ 25°C Conductivity Calibration Solution (Hanna Instruments, South Africa). Once calibrated, the probe was placed into the sample. All air bubbles were removed before taking the reading. After calibration and between each sample the probe was cleaned with distilled water and dabbed dry with a piece of tissue paper. A reading was taken only once the display had stabilised. The units of measurement ( $\text{mS}\cdot\text{m}^{-1}$ ) were adjusted according to the instruction manual (Hanna Instruments, South Africa).

#### *Turbidity*

The turbidity of the water was measured with a Thermo Scientific ORION AQUAfast AQ3010 turbidity meter (Thermo Fisher Scientific Inc., United States). The meter was calibrated with Standard Calibration Solutions according to the instruction manual every time the standards no longer read within 10% of the nominal NTU value for the standard (Thermo Scientific ORION AQUAfast AQ3010 turbidity meter User Guide, United States). Once calibrated, samples were poured into glass vials up to the line and the cap replaced before being measured in the turbidity meter. The unit of measurement used is nephelometric turbidity units (NTU).

#### *Chemical oxygen demand*

A DR2000 spectrophotometer (Hach Co. Loveland, CO) and Standard Methods were used to colorimetrically determine chemical oxygen demand (COD) (APHA, 1998).

#### *Alkalinity*

Alkalinity was determined by means of a titration method as described according to Standard Methods (APHA, 1998). The unit of measurement used is  $\text{mg CaCO}_3\cdot\text{mL}^{-1}$ .

#### *Total suspended solids*

Total suspended solids (TSS) was determined according to Standards Methods (APHA, 1998).

#### *Ultraviolet transmittance (%UVT)*

Ultraviolet transmittance percentage was measured with a UVT meter (Berson, Netherlands). The meter was calibrated with de-ionised water to a reading of 100% UVT. There after the de-ionised water was removed from the cuvette. The cuvette was rinsed with distilled water after which the cuvette was filled with the sample. A cap was used to cover the sample cuvette to prevent any light from penetrating and influencing the results. The results were expressed in terms of percentage.

### *Anions and Cations*

Inductively coupled plasma atomic emission spectrometry (ICP-AES) was used to determine the cations (Iron and Manganese) (Anon., 2014). During this method, electrons of an atom absorb energy and as a result jump to higher energy levels. When these electrons return to normal states, they emit characteristic photons of energy. The types and concentrations of the elements present can be determined by isolating these photon wavelengths (Gong, 2008). Anions (Chlorine, Fluorine, Nitrate, Phosphate and Sulphate) were determined by ion chromatography (IC) analysis (Anon., 2014). The IC method includes all rapid liquid chromatography separations of ions in columns coupled on-line with detection and quantification in a flow-through detector (Eith *et al.*, 2007). The quantity of ammonia present in the water was analysed in the lab with Spectroquant® Ammonium cell test kits. A Spectroquant® Photometer NOVA60 (Merck) was used to determine the results.

### **Microbiological parameters**

#### *Aerobic colony count*

The aerobic colony count (ACC) technique was performed according to the methods described in SABS ISO 4833 (2007) and Standard Methods (APHA, 1998). The Schott bottles, containing the water samples (kept as close to 4°C in the cooler boxes), were shaken vigorously before 1 mL was withdrawn to prepare a dilution series of  $10^0$ - $10^{-6}$ , for each sample. The dilutions were done in McCartney's containing 9 mL sterile saline solution. A high dilution series was prepared since it was anticipated that the water might carry a high microbial load. Using a sterile pipette, 1 mL of each dilution was carried over into correspondingly marked Petri dishes. This was performed in duplicate for each of the water samples. Approximately 10 - 12 mL liquefied Plate Count Agar (PCA) (Merck) was aseptically added to each of the plates to create pour plates. After the addition of PCA, each of the Petri dishes was carefully moved in a figure eight motion to ensure that the samples were evenly distributed in the agar. Once the agar had fully set, the plates were inverted and incubated at 35°C for 48 hours. Only plates containing 30 to 300 colonies were counted. The total number of coli-forming units (cfu) per millilitre was determined by taking the dilution factor of each pour plate into consideration (APHA, 1998).

#### *Total coliforms and Escherichia coli*

The Schott bottles, kept at refrigerator temperatures in the cooler boxes, were shaken vigorously before 10 mL was used to aseptically prepare a dilution series of  $10^{-1}$  -  $10^{-5}$ , for each sample. The dilutions were done in 100 mL Schott bottles containing 90 mL sterile saline solution. Duplicates of each dilution to be tested were made in additional 100 mL

Schott bottles, originally containing 90 mL sterile saline solution to ensure an end-sample volume of 100 mL in each. Colilert-18 (IDEXX Laboratories, South Africa) nutrient-indicator also referred to as 4-methylumbelliferyl- $\beta$ -D-glucuronide (MUG) was added to each of the duplicates. After the MUG was completely dissolved, the samples were poured into Quanti-Tray's, after which they were sealed (Quanti-Tray<sup>®</sup> Sealer Model 2X) and incubated at 37°C for 18 hours. After incubation, total coliforms were determined by counting all the wells that turned yellow. *Escherichia coli* was determined by counting all the wells that fluoresced under UV light (Spectroline<sup>®</sup> Model CM-10 Fluorescence Analysis Cabinet) in a dark environment. After the positive counts were determined, the actual total coliforms and *E. coli* counts were established by reading the values of an IDEXX Quanti-Tray<sup>®</sup>/2000 most probable number (MPN) table. Both counts were presented as MPN.100mL<sup>-1</sup>.

## RESULTS AND DISCUSSION

### Baseline study

#### *Environmental and chemical results*

The averages of the environmental and chemical parameters obtained from samples taken at the various sampling points on the farm between October 2012 and February 2013, are summarised in Table 4. As expected the temperature of the river water increased as the ambient temperature increased (October to December 2012) and stayed relatively constant till the end of February 2013.

The river water temperature at Sampling Point 1 varied between 15.3°C in October 2012 and 19.8°C in February 2013, whereas the pH varied between 7.17 and 7.41 for the same time period. The Department of Water Affairs and Forestry (DWA, 1996b) specifies that there is a relationship between water temperature and the corresponding pH value (since pH can be influenced by water temperature). This might be a possible explanation for the slightly higher pH during months with a higher ambient temperature. According to the South African Water Quality Guidelines (SAWQG) water of an acceptable quality for irrigational purposes should have a pH value between 6.5 and 8.5 (DWA, 1996b). The pH values of the water at Sampling Point 1 always fell within the aforementioned guideline ranges, thus making the water “generally safe to use for irrigation and recreational purposes where chemical parameters are concerned” (DWA 1996a; DWA, 1996b). No connection was seen between pH and temperature and the other chemical parameters such as alkalinity, conductivity, COD and TSS. The alkalinity of the river water ranged between 25.0 and 125.0 mg CaCO<sub>3</sub>.L<sup>-1</sup>. According to Spellman (2008), a solution's alkalinity value should be above 80.0 mg CaCO<sub>3</sub>.L<sup>-1</sup>, for it to have an effective buffering capacity against the environment.

**Table 4** Chemical analysis of the water during the baseline study.

<b>Sampling date</b>	<b>Temperature (°C)</b>	<b>pH</b>	<b>Alkalinity (mg CaCO<sub>3</sub>.L<sup>-1</sup>)</b>	<b>Conductivity (mS.m<sup>-1</sup>)</b>	<b>COD (mg.L<sup>-1</sup>)</b>	<b>TSS (mg.L<sup>-1</sup>)</b>
<b>Sampling Point 1</b>						
Oct 2012	15.3	7.17	25.0	0.30	37	28
Nov 2012	17.4	7.38	100.0	0.37	23	13
Dec 2012	20.0	7.49	100.0	0.71	29	5
Jan 2013	19.9	7.53	117.0	0.71	21	4
Feb 2013	19.8	7.41	125.0	0.45	22	10
<b>Sampling Point 2</b>						
Oct 2012	15.9	7.07	62.5	0.29	12	37
Nov 2012	18.6	7.15	112.5	0.35	29	2
Dec 2012	21.5	7.32	143.8	0.51	23	2
Jan 2013	21.6	7.56	125.0	0.56	15	21
Feb 2013	21.6	7.37	149.8	0.53	20	33
<b>Sampling Point 3</b>						
Oct 2012	16.8	7.35	62.5	0.26	36	39
Nov 2012	20.0	7.00	112.5	0.34	14	6
Dec 2012	22.1	7.25	137.5	0.47	21	2
Jan 2013	22.1	7.41	125.0	0.51	22	9
Feb 2013	24.4	7.92	143.8	0.53	19	43
<b>Sampling Point 4</b>						
Oct 2012	17.9	7.03	100.0	0.26	14	21
Nov 2012	20.5	6.81	125.0	0.33	31	4
Dec 2012	23.1	6.80	137.5	0.46	28	3
Jan 2013	22.4	7.50	120.7	0.50	26	23
Feb 2013	23.3	8.08	143.8	0.53	19	19
<b>Sampling Point 5</b>						
Oct 2012	18.2	7.00	87.5	0.25	8	17
Nov 2012	20.8	6.98	187.5	0.32	19	2
Dec 2012	21.8	7.47	125.0	0.45	46	7
Jan 2013	21.8	7.42	116.7	0.49	25	17
Feb 2013	20.6	7.64	131.3	0.53	24	8
<b>Sampling Point 6</b>						
Oct 2012	19.6	4.39	0.00	1.12	25	13
Nov 2012	21.7	5.91	37.5	1.24	18	6
Dec 2012	25.0	6.98	100.0	0.76	22	11
Jan 2013	25.4	7.12	95.8	0.78	15	15
Feb 2013	24.7	7.27	125.0	0.66	14	17

The alkalinity of the water was only below 80.0 mg CaCO<sub>3</sub>.L<sup>-1</sup>, in October 2012. In November 2012 till February 2013 the alkalinity was above 80.0 mg CaCO<sub>3</sub>.L<sup>-1</sup>. Thus it can

be concluded that the water had a high buffering capacity and that it offers a great amount of resistance against the effect of environmental changes on the pH. Salinity is a measure of the dissolved salts that are present in water and is measured as electrical conductivity (McCaffrey, 2011). It is important that the salt content of water used to irrigate crops is not too high, since it might damage crops or in some cases even cause soil permeability problems (McCaffrey, 2011).

According to the SAWQG the Target Water Quality Range (TWQR) for electrical conductivity is  $40.00 \text{ mS.m}^{-1}$  (DWAF, 1996b). The conductivity of the water samples ranged between  $0.30$  and  $0.45 \text{ mS.m}^{-1}$  and thus never exceeded  $40.00 \text{ mS.m}^{-1}$  throughout all of the sampling months, thus indicating that the water contains low salt levels ensuring that salt sensitive crops can be grown without yield decreases (DWAF, 1996b). According to the SAWQG (DWAF, 1996d), the TWQR for COD in water used for agricultural irrigation is not available; therefore it was decided to use the DWAF guidelines for industrial use (DWAF, 1996c). According to DWAF (1996c), COD levels may not exceed  $30.0 \text{ mg.L}^{-1}$  in water used for industrial use. The COD levels ranged between  $21$  and  $37 \text{ mg.L}^{-1}$  during the sampling months. The COD levels for the sampling months November 2012 throughout February 2013 were all below  $30 \text{ mg.L}^{-1}$ . The COD levels which were below the limits were indicative of a low demand for oxygen from chemical pollution present in the water. As a result it can be concluded that the levels of chemical pollution in the water were low enough for the water to be considered acceptable for industrial use and indirectly, irrigational use (DWAF, 1996c). The COD levels of the sample taken at Sampling Point 1 in October 2012 were slightly higher than the limit, indicating that the water from that sample would not have been suitable for irrigation purposes (DWAF, 1996c). A limit of  $0.050 \text{ mg.L}^{-1}$  is recommended by Capra & Scicolone (2007) for TSS to prevent clogging of the irrigation system. The SAWQG of TSS for irrigational use is  $0 - 50 \text{ mg.L}^{-1}$  (DWAF, 1996b). This is also the limit referred to as the point after which uniform irrigation will be affected. The TSS values ranged between  $5$  and  $28 \text{ mg.L}^{-1}$ , thus never exceeding the SAWQG (DWAF, 1996b).

At Sampling Point 2, a small connection could again be seen between the temperature and pH values, in that the pH increased slightly with increasing temperatures. The water temperature ranged from  $15.9$  to  $21.6^\circ\text{C}$ , whereas the pH ranged from  $7.07$  to  $7.56$  for the same time period. The pH values all fell within the SAWQG thus making it safe for irrigational and recreational use (DWAF, 1996a; DWAF, 1996b). Again no relationship could be seen between temperature, pH and the other chemical parameters. The alkalinity of the water ranged between  $62.5$  and  $149.8 \text{ mg CaCO}_3.\text{L}^{-1}$ . The alkalinity of the water was only below the level recommended by Spellman (2008) of  $80 \text{ mg CaCO}_3.\text{L}^{-1}$  in October 2012, indicating that the water had a relatively high buffering capacity in the other sampling months. The conductivity of the water ranged between  $0.29$  and  $0.53 \text{ mS.m}^{-1}$  and never

exceeded the SAWQG of  $40.00 \text{ mS}\cdot\text{m}^{-1}$  thus making the water safe for irrigational use (DWAF, 1996b). The COD levels ranged between  $12$  and  $29 \text{ mg}\cdot\text{L}^{-1}$  and never exceeded the SAWQG for industrial use, thus making the water suitable for use (DWAF, 1996c). The TSS values ranged between  $2$  and  $37 \text{ mg}\cdot\text{L}^{-1}$  and never exceeded the SAWQG of  $50 \text{ mg}\cdot\text{L}^{-1}$ , thus making the water suitable for irrigational use (DWAF, 1996b; Capra & Scicolone, 2007).

At Sampling Point 3 the water temperature varied between  $15.9$  and  $21.6^\circ\text{C}$ , whereas the pH values varied from  $7.00$  to  $7.92$  for the same time period. The pH values were always within the SAWQG values, thus making it safe for irrigational and recreational use (DWAF, 1996a; DWAF, 1996b). The alkalinity of the water ranged between  $62.5$  and  $143.8 \text{ mg CaCO}_3\cdot\text{L}^{-1}$ , while the conductivity of the water ranged from  $0.26$  to  $0.53 \text{ mS}\cdot\text{m}^{-1}$ . Alkalinity was above  $80 \text{ mg CaCO}_3\cdot\text{L}^{-1}$  on all sampling occasions (except October 2012), indicating water of a good buffering capacity (Spellman, 2008). Conductivity of the water was also under the SAWQG values of  $40.00 \text{ mS}\cdot\text{m}^{-1}$ , making it suitable for irrigational use (DWAF, 1996b). The COD and TSS levels varied between  $14$  and  $36 \text{ mg}\cdot\text{L}^{-1}$  and  $2$  and  $43 \text{ mg}\cdot\text{L}^{-1}$ , respectively. The SAWQG for COD was only exceeded in October 2012, the rest of the samples fell below the guideline of  $30.0 \text{ mg}\cdot\text{L}^{-1}$ , making it safe for use (DWAF, 1996c). The TSS of the water was below the SAWQG of  $50 \text{ mg}\cdot\text{L}^{-1}$  on all sampling occasions, indicating the water is suitable for a drip irrigation system (DWAF, 1996b).

The water temperature at Sampling Point 4 varied between  $17.9$  and  $23.3^\circ\text{C}$ , while the pH values ranged between  $6.80$  and  $8.08$ . The pH values were always within the SAWQG, thus it was safe for recreational and irrigational use (DWAF, 1996a; DWAF 1996b). The alkalinity of the water varied from  $100.0$  to  $143.8 \text{ mg CaCO}_3\cdot\text{L}^{-1}$ , whereas the conductivity of the water ranged between  $0.26$  and  $0.53 \text{ mS}\cdot\text{m}^{-1}$ . Alkalinity was above  $80 \text{ mg CaCO}_3\cdot\text{L}^{-1}$  on all sampling occasions, indicating water of a good buffering capacity (Spellman, 2008). Conductivity of the water was also below the SAWQG values of  $40.00 \text{ mS}\cdot\text{m}^{-1}$ , making it suitable for irrigational use (DWAF, 1996b). The COD and TSS levels ranged between  $14$  to  $31 \text{ mg}\cdot\text{L}^{-1}$  and  $3$  and  $23 \text{ mg}\cdot\text{L}^{-1}$ , respectively. The SAWQG for COD was only exceeded in November 2012, the rest of the samples fell below the guideline of  $30.0 \text{ mg}\cdot\text{L}^{-1}$ , making it safe for use (DWAF, 1996c). The TSS of the water was below the SAWQG of  $50 \text{ mg}\cdot\text{L}^{-1}$  on all sampling occasions, indicating the water is suitable for a drip irrigation system (DWAF, 1996b).

At Sampling Point 5 the water temperature varied between  $18.2^\circ\text{C}$  and  $21.8^\circ\text{C}$ , while the pH values ranged from  $6.98$  to  $7.64$ . The pH values all fell within the SAWQG thus it is safe for irrigational and recreational use (DWAF, 1996a; DWAF, 1996b). The alkalinity of the water varied from  $87.5$  to  $187.5 \text{ mg CaCO}_3\cdot\text{L}^{-1}$  and was always above the recommended value of  $80 \text{ mg CaCO}_3\cdot\text{L}^{-1}$ , indicating that the water has good buffering capacity (Spellman, 2008). Conductivity of the water varied between  $0.25$  and  $0.53 \text{ mS}\cdot\text{m}^{-1}$ , thus it was always

below the SAWQG of  $40.00 \text{ mS}\cdot\text{m}^{-1}$ , indicating that the water contained low salt levels and were safe for irrigational use (DWAF, 1996b). The COD and TSS levels ranged from 8 and  $46 \text{ mg}\cdot\text{L}^{-1}$  and 2 to  $17 \text{ mg}\cdot\text{L}^{-1}$ , respectively. The SAWQG for COD was only exceeded in December 2012, the rest of the samples were below the guideline of  $30.0 \text{ mg}\cdot\text{L}^{-1}$ , making it safe for use (DWAF, 1996c). The TSS of the water always fell within the SAWQG of  $50 \text{ mg}\cdot\text{L}^{-1}$  on all sampling occasions, indicating the water is suitable for a drip irrigation system (DWAF, 1996b).

The water temperature varied from  $19.6$  to  $25.4^\circ\text{C}$  at Sampling Point 6, while the pH values varied between 4.39 and 7.27. The pH values did not meet the SAWQG guidelines in October or November 2012, thus it was not safe for irrigational and recreational use (DWAF, 1996a; DWAF, 1996b). The low pH values could be attributed to the addition of phosphoric and sulphuric acid in the pump house. These acids were added as a means to lower the pH values to within the SAWQG guidelines, but the dosing was not always correct, leading to pH values below the guidelines. The alkalinity of the water ranged between 0.0 and  $125.0 \text{ mg CaCO}_3\cdot\text{L}^{-1}$ . Alkalinity was below  $80 \text{ mg CaCO}_3\cdot\text{L}^{-1}$  in October and November 2012, during the rest of the sampling months the water had a good buffering capacity (Spellman, 2008). The alkalinity value of zero was caused by the extremely low pH of 4.39 during October 2012. The conductivity of the water varied between 0.66 and  $1.24 \text{ mS}\cdot\text{m}^{-1}$ , while the COD levels ranged between 14 and  $26 \text{ mg}\cdot\text{L}^{-1}$ . Conductivity of the water was always below the SAWQG values of  $40.00 \text{ mS}\cdot\text{m}^{-1}$ , making it suitable for irrigational use (DWAF, 1996b). The higher than usual conductivity level could possibly be attributed to the addition of the acids to the water just before the point of irrigation. The SAWQG for COD was never exceeded and all of the samples fell below the guideline of  $30.0 \text{ mg}\cdot\text{L}^{-1}$ , making it safe for use (DWAF, 1996c). The TSS values ranged between 6 and  $17 \text{ mg}\cdot\text{L}^{-1}$ . The TSS of the water was below the SAWQG of  $50 \text{ mg}\cdot\text{L}^{-1}$  on all sampling occasions, indicating the water is suitable for a drip irrigation system (DWAF, 1996b).

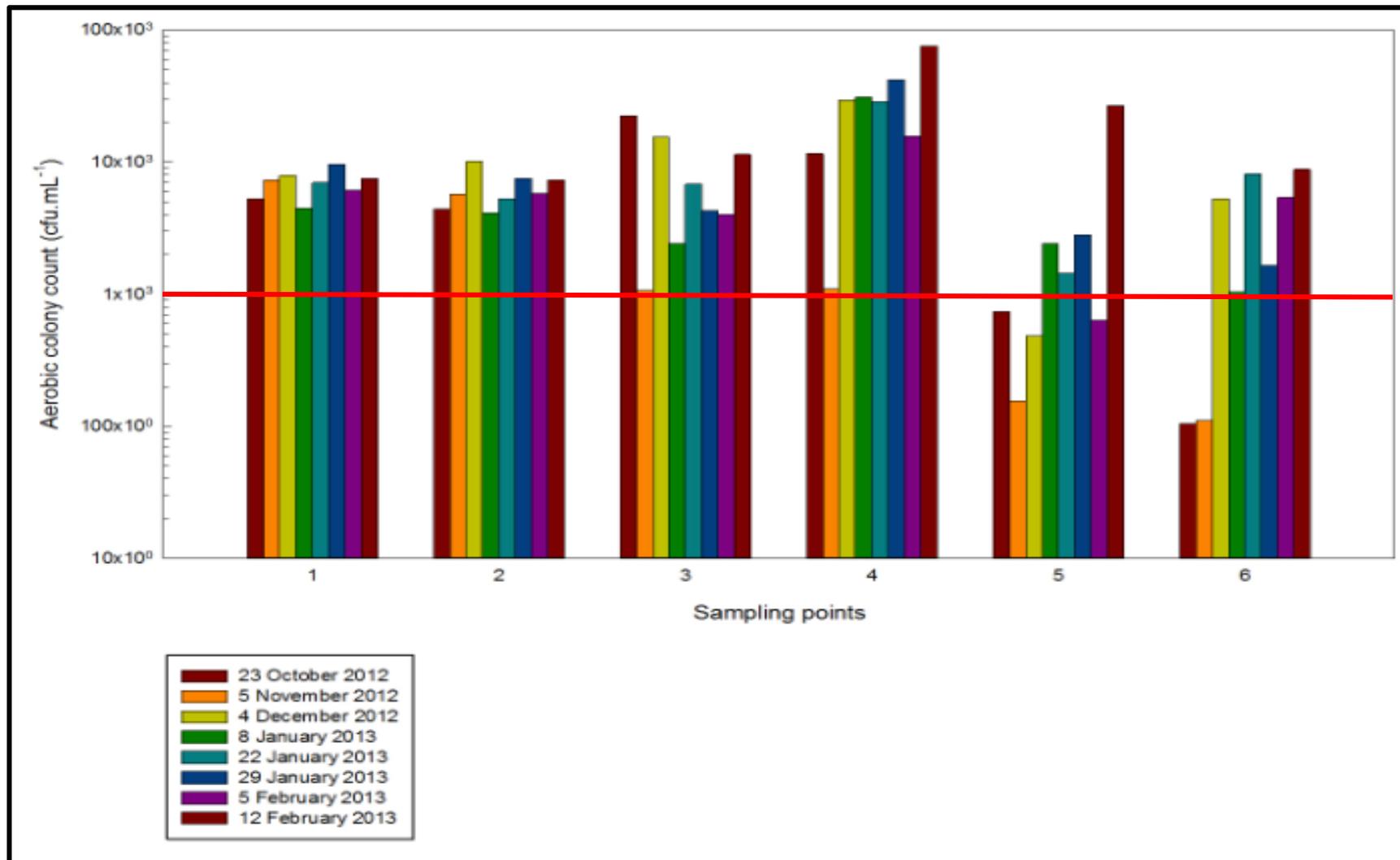
#### *Microbiological results*

All of the water samples taken from the various sampling points from October 2012 up to the end of February 2013 were subjected to certain microbiological tests. The results obtained for aerobic colony count (ACC), total coliforms (TC) and *Escherichia coli* (*E. coli*) are given in Figs. 6, 7 and 8, respectively.

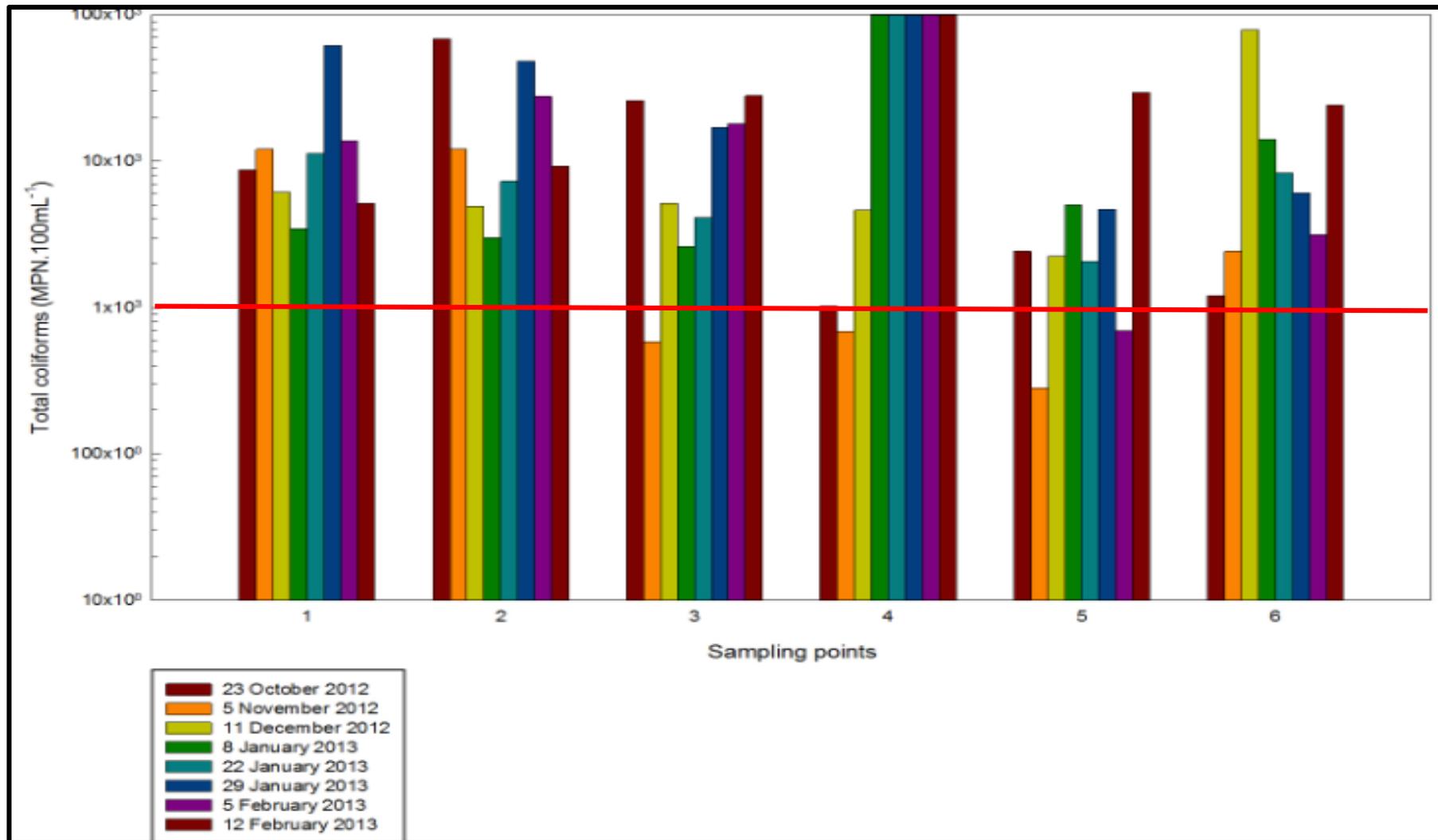
At Sampling Point 1 the ACC ranged between 4 500 and 9 600  $\text{cfu}\cdot\text{mL}^{-1}$  in the river water (Fig. 6). No SAWQG for ACC is available in either the Agricultural Water Use: Irrigation (DWAF, 1996b) or the Field Guide (DWAF, 1996d) guidelines, thus it was decided to use the SAWQG as set out for Domestic Use (DWAF, 1996a) as reference limit. According to SAWQG for Domestic Use an increased risk of disease transmission is

possible when counts are higher than 1 000 cfu.mL<sup>-1</sup> (DWAF, 1996a). Total coliforms varied between 3 469 and 61 600 MPN.100 mL<sup>-1</sup> (Fig. 7). No South African guideline could be found for TC present in irrigation water for the consumption of fresh produce eaten raw. Canadian guidelines, however, state that the TC count present in water used to irrigate crops that are consumed raw, should not exceed 1 000 cfu.100 mL<sup>-1</sup> since it is indicative of poor water quality and treatment (Monaghan & Hutchison, 2010). The counts were higher than the Canadian regulations during all of the sampling months which indicate that this water would not be considered as safe for the irrigation of crops which are consumed raw. In previous years, Kikine (2011) and Huisamen (2012) found coliform counts as high as 13 000 000 and 7 000 000 MPN.100mL<sup>-1</sup> in samples from the Eerste River, respectively. The *E. coli* counts at Sampling Point 1 ranged from 110 to 2 098 MPN.100 mL<sup>-1</sup> (Fig. 8). Both the World Health Organisation (WHO) and the South African DWAF have guidelines for the quality of irrigation water. According to the WHO (1989) irrigation water containing more than 1 000 faecal coliforms per 100 mL water is seen as a serious risk for the spread of disease. As *E. coli* is seen as an indicator of faecal contamination, some studies only report the count of *E. coli* present in the water. This applies to all water being used for the irrigation of crops, irrespective of its source. According to SAWQG (DWAF, 1996b) and WHO (1989), water used for irrigation of crops may not exceed 1 000 organisms.100 mL<sup>-1</sup>. The Canadian guidelines state that the *E. coli* count present in water used to irrigate crops that are consumed raw, should not exceed 100 cfu.100 mL<sup>-1</sup> since it is indicative of poor water quality and treatment (Monaghan & Hutchison, 2010). Canadian guidelines for *E. coli* are a lot stricter than the SAWQG for the irrigation of fresh crops which are consumed raw. It is important to test for *E. coli* since they are almost exclusively of faecal origin and their presence is a definitive indicator of a food or water source being contaminated with faecal matter (Anon., 2011; Masters *et al.*, 2011).

The *E. coli* limit for the SAWQG and WHO guideline was only exceeded in October 2012, making the water on those dates unsuitable for the irrigation of crops that is to be consumed raw (WHO, 1989; DWAF, 1996b). From November 2012 to February 2013 the SAWQG and WHO guideline was met, making the water from these sampling months suitable to use for the irrigation of fresh produce (WHO, 1989; DWAF, 1996b). Interestingly,



**Figure 6** Aerobic colony counts (ACC) at the six different sampling points obtained during the baseline study from October 2012 to February 2013.



**Figure 7** Total coliform (TC) counts at the six different sampling points obtained during the baseline study from October 2012 to February 2013.

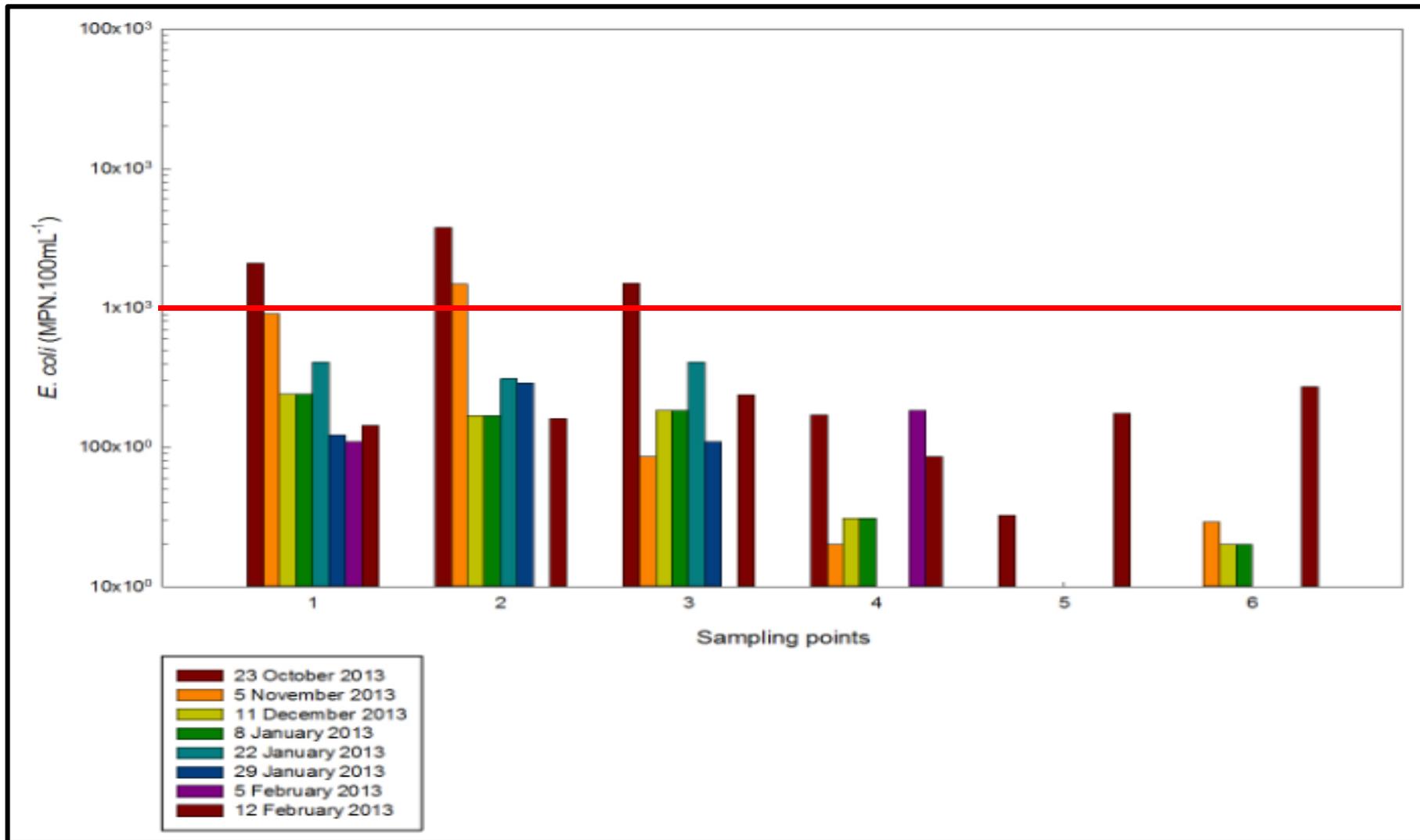


Figure 8 *Escherichia coli* counts at the six different sampling points obtained during the baseline study from October 2012 to February 2013.

the Canadian guidelines were only met in January and February 2013, which would have implied that the water sampled throughout all of the other sampling months were unsuitable to irrigate crops that are to be consumed raw (Monaghan & Hutchison, 2010). Although many of the river water samples exceeded the guidelines (WHO, 1989; DWAF 1996a; DWAF, 1996b; Monaghan & Hutchison, 2010) for irrigation of fresh produce, in many instances the levels were only slightly higher than the guidelines. This should be kept in mind, as water abstracted from rivers is usually not used directly for irrigation and often undergoes at least sand filtration before use.

The ACC at Sampling Point 2 ranged from 4 100 to 10 200 cfu.mL<sup>-1</sup> (Fig. 6). The ACC were very similar to those of the river water (Sampling Point 1). It was expected that the sand filters between the river and the inlet to the first holding dam would result in a slight reduction in the microbial load (Hijnen *et al.*, 2007). A possible reason for there being no decrease could be that the sand filters are used for extended periods without being re-sanded, leading to their inefficiency. An increased risk of disease transmission is possible due to ACC being higher than the SAWQG for domestic use in all of the water samples (DWAF, 1996a). The TC ranged between 3 005 and 68 670 MPN.100 mL<sup>-1</sup> (Fig. 7). Similar to the ACC, the range for TC stayed more or less the same as at Sampling Point 1. The fact that the TC levels have not been reduced by the sand filtration means that the loads are still above the 1 000 cfu.100 mL<sup>-1</sup> Canadian guideline (Monaghan & Hutchison, 2010). The *E. coli* counts varied between 10 and 3 790 MPN.100 mL<sup>-1</sup> (Fig. 8). The SAWQG and WHO guideline for irrigation water (1 000 *E. coli*.100 mL<sup>-1</sup>) were only exceeded in October and November 2012, thus the water could be regarded as suitable for irrigation of fresh produce in December 2012 till February 2013 in terms of the SAWQG and WHO guidelines for *E. coli* (WHO, 1989; DWAF, 1996b).

At Sampling Point 3 the ACC varied between 1 060 and 22 400 cfu.mL<sup>-1</sup> (Fig. 6). Clear fluctuations in the ACC are visible in Fig. 4 from Sampling Point 2 to Sampling Point 3. The ACC increased from Sampling Point 2 to Sampling Point 3 on 23 October 2012, 4 December 2012 and 12 February 2013 (Fig. 6). The ACC decreased from Sampling Point 2 to Sampling Point 3 on all the other sampling dates. The water was not safe for domestic use due to an increased risk of disease transmission in terms of ACC, since the SAWQG of 1 000 cfu.mL<sup>-1</sup> were exceeded on all of the sampling occasions (DWAF, 1996a). The TC ranged between 581 and 27 900 MPN.100 mL<sup>-1</sup> (Fig. 7). The TC levels at Sampling Point 3 were only once (November 2012) below the 1 000 cfu.100 mL<sup>-1</sup> guideline for safe irrigation water as set out in the Canadian guideline (Monaghan & Hutchison, 2010). Thus on all other sampling dates, the water would still be considered unsuited for irrigational use (Monaghan & Hutchison, 2010). The TC loads remained more or less the same from Sampling Point 2 to Sampling Point 3 on most of the sampling occasions (Fig. 6), with increases on some

occasions and decreases on others. The *E. coli* counts ranged between 10 and 1 505 MPN.100 mL<sup>-1</sup> (Fig. 8). The SAWQG and WHO guideline of 1 000 *E. coli*.100 mL<sup>-1</sup> were only exceeded in October 2012. Thus, at this stage of the irrigation cycle, the water can mostly be regarded as safe for irrigation of crops that are consumed raw (DWAF, 1996b). It can clearly be seen in Fig. 8 that the *E. coli* loads remained more or less constant from Sampling Point 2 to Sampling Point 3. The sample for Sampling Point 3 was taken at the overflow from the first holding dam to the second holding dam. The overflow channel was quite narrow and not very deep (2 to 15 centimetres depending on the flow rate). Since the dams are open to the environment, birds have access to them and as a result the overflow area was sometimes covered with bird faeces. This might be a possible explanation for the increased counts observed (on some of the sampling dates) between Sampling Point 2 and 3. Decreases might be attributed to higher flow at the overflow channel, thus less sediment and faeces might have been part of the sample, attributing to the lower loads on certain sampling occasions.

The ACC ranged between 1 090 and 76 000 cfu.mL<sup>-1</sup> at Sampling Point 4 (Fig. 6). It can clearly be seen in Fig. 6 that ACC remained more or less constant from Sampling Point 3 to Sampling Point 4 during October and November 2012, respectively. Thereafter, ACC at Sampling Point 4 were higher than the levels at Sampling Point 3. An overall log-reduction, of 0.5 to 1.0 was observed in ACC after passing through the sand filters. Although there is no guideline in the SAWQG for irrigation water, the guideline for domestic water of 1 000 cfu.100 mL<sup>-1</sup> was exceeded (DWAF, 1996a). The TC counts ranged from 683 to 461 100 MPN.100 mL<sup>-1</sup> (Fig. 7). Similar to the trend seen for ACC, it can clearly be seen in Figure 7 that TC loads decreased from Sampling Point 3 to Sampling Point 4 during October and November 2012, respectively, but from 8 January 2013 and onwards, the TC loads at Sampling Point 4 were higher than the levels at Sampling Point 3 (Fig. 7). The TC levels were only below 1 000 cfu.100 mL<sup>-1</sup> in October to December 2012 (i.e. below the Canadian guidelines for TC) (Monaghan & Hutchison, 2010), but exceeded these levels thereafter, displaying large increases after passing through the sand filters. Increases as high as 2.0 logs were seen on certain sampling dates. The *E. coli* counts ranged between 10 and 272 MPN.100 mL<sup>-1</sup>. In Fig. 8 it can clearly be seen that the *E. coli* loads mostly decreased from Sampling Point 3 to Sampling Point 4, except on 5 February 2013 when it increased. The SAWQG was met on all sampling occasions, thus the water was presumed as safe for the irrigation of fresh produce in terms of *E. coli* counts (DWAF, 1996b). It was expected that microbial loads would be reduced between Sampling Point 3 and Sampling Point 4, due to sand filtration that takes place (Hijnen *et al.*, 2007). This was, however, not the case for ACC and TC, which after initial decreases from Sampling Point 3 to Sampling Point 4, actually increased. The *E. coli* counts (except for one instance) decreased from Sampling

Point 3 to Sampling Point 4. A possible explanation for the increased ACC and TC loads, from December 2012 onwards could be ascribed to the fact that the sand filters are used for extended periods without being re-sanded or washed. As a result the filters might be clogged and not filtering properly. A possible explanation for this irregularity could be that a biofilm had formed throughout the sand, consisting mainly of ACC and TC, thus explaining the increase in counts at Sampling Point 4. The biofilm could thus be “trapping” the *E. coli*, which would possibly be “out competed” and thus die off, resulting in lower *E. coli* levels.

The on-farm irrigation system included a H<sub>2</sub>O<sub>2</sub> dosing step, which took place after the second holding dam and sand filters (Sampling Point 4) and the holding tank and pump house (Sampling Point 5). The function of the holding tank is to facilitate the necessary H<sub>2</sub>O<sub>2</sub> contact time. Water is pumped from the holding tank, via the pump house (Sampling Point 5) to the point of irrigation (Sampling Point 6). At Sampling Point 5 the ACC ranged from 155 to 26 800 cfu.mL<sup>-1</sup> (Fig. 6). It can be seen that in all sampling instances the ACC decreased from Sampling Point 4 to Sampling Point 5 (Fig. 6). The log-reductions, however, ranged from 0.5 to 1.5, but in most instances it was less than 1.0. The efficiency of the H<sub>2</sub>O<sub>2</sub> dosing thus varies and can be ascribed to insufficient contact time or ineffective dosing of H<sub>2</sub>O<sub>2</sub> (either the dosed amount varying or variations in the water flow rate). The TC counts varied between 282 and 29 500 MPN.100 mL<sup>-1</sup> (Fig. 7). In Figure 7 it can clearly be seen that the TC loads decreased from Sampling Point 4 to Sampling Point 5, except on 23 October 2012 when an increase occurred (Fig. 7). The log-reduction in TC after treatment with H<sub>2</sub>O<sub>2</sub> ranged from 0.5 to 2.0, but in most instances was also less than 1.0. *Escherichia coli* counts ranged between 10 and 175 MPN.100 mL<sup>-1</sup> (Fig. 8). In Figure 6 it can be seen that the counts decreased or remained constant from Sampling Point 4 to Sampling Point 5, except on 12 February 2013 when an increase in counts occurred. The addition of H<sub>2</sub>O<sub>2</sub> thus resulted in the *E. coli* counts decreasing by between 0.01 and 0.5 logs. Since the counts were so low (10 to 272 MPN.100 mL<sup>-1</sup>) after the sand filters (Sampling Point 4), it was expected that the addition of H<sub>2</sub>O<sub>2</sub> would be more effective in reducing the *E. coli* counts, but this was not the case. The *E. coli* levels were, however, below the SAWQG of 1 000 cfu.100 mL<sup>-1</sup>, making it safe to use for the irrigation of crops that are consumed raw (DWAF, 1996b). Log-reductions ranging from 0.5 to 1.5, 0.5 to 2.0 and 0.01 to 0.5 were achieved for ACC, TC and *E. coli*, respectively, during the baseline study. The average overall log-reduction achieved during the baseline study (H<sub>2</sub>O<sub>2</sub> dosing) was 0.65 to 1.13.

Sampling Point 6, the point of irrigation, was the most critical point in terms of meeting the guidelines for crops being irrigated that are to be consumed raw without any further hurdles implemented to reduce possible microbial loads. The ACC varied between 104 and 8 800 cfu.mL<sup>-1</sup> (Fig. 6). The SAWQG for domestic use was only met on the first two sampling dates (DWAF, 1996a). Total coliform counts ranged from 1 203 to 24 196

MPN.100 mL<sup>-1</sup> (Fig. 7). The counts were always above the Canadian guidelines, thus indicating an increased risk of infective disease transmission (Monaghan & Hutchison, 2010). During some sampling weeks there was an increase in ACC's and TC visible in the water from the pump house (Sampling Point 5) to the point of irrigation (Sampling Point 6). This could possibly be attributed to a dirty pipe system or the presence of a biofilm in the pipes from the pump house to the point of irrigation. As the irrigation system (from pump house to point of irrigation) has times of non-use, this could provide the opportunity for biofilm formation within the system. This is also more plausible, considering that the H<sub>2</sub>O<sub>2</sub> dosing was not very effective in reducing microbial loads even though the recorded results were already quite low. The *E. coli* counts ranged between 5.2 and 85 MPN.100 mL<sup>-1</sup> (Fig. 8). The SAWQG was met on all of the sampling dates, thus the water could be considered safe for irrigation of fresh crops in terms of *E. coli* counts (DWAF, 1996b).

### UV treatment study

#### *Environmental and chemical results*

The averages of the chemical parameters obtained from samples taken at the various sampling points on the farm, between March and May 2013 during the UV treatment study, are summarised in Table 5. During this part of the study the river water temperature decreased as the ambient temperatures decreased. This can be attributed to the change in seasons.

The river water temperature at Sampling Point 1 ranged between 19.3°C in March and 10.8°C in July 2013, with river water temperature decreasing as ambient temperature decreased. The pH varied between 6.01 and 7.16 for the same time period. As seen in the baseline study, there was a relationship between temperature and pH, with the temperatures decreasing from March to July 2013, while the corresponding pH values also decreased. According to the SAWQG (DWAF, 1996b) the water was not always considered of acceptable quality for irrigational purposes, as the pH during the month from May to July 2013 was below 6.5. During this study no visible link could be found between pH and temperature and the other chemical parameters. The alkalinity of the river water ranged between 87.5 and 154.2 mg CaCO<sub>3</sub>.L<sup>-1</sup>, well above the lower limit recommended by Spellman (2008), thus it could be concluded that the water always had an effective buffering capacity. The conductivity of the water varied from 0.34 and 0.86 mS.m<sup>-1</sup> and was always below the SAWQG value of 40.00 mS.m<sup>-1</sup>, making it suitable for irrigational use (DWAF, 1996b). According to the SAWQG (DWAF, 1996d) the TWQR for COD in water used for irrigational purposes is not available, but water used in industry should have a COD value below 30 mg.L<sup>-1</sup> (DWAF, 1996c). The COD levels ranged between 16 and 43 mg.L<sup>-1</sup> and

**Table 5** Chemical analysis of the water during the UV treatment study.

Sampling date	Temp. (°C)	pH	Alkalinity (mg CaCO <sub>3</sub> .L <sup>-1</sup> )	Conduct. (mS.m <sup>-1</sup> )	COD (mg.L <sup>-1</sup> )	TSS (mg.L <sup>-1</sup> )	Turbidity (NTU)
<b>Sampling Point 1</b>							
Mar 2013	19.3	7.16	154.2	0.56	17	9	4.44
Apr 2013	15.9	6.82	120.8	0.35	43	19	137.97
May 2013	13.4	6.18	116.7	0.38	32	11	12.60
Jun 2013	10.8	6.14	87.5	0.34	29	17	19.18
Jul 2013	11.7	6.01	100.0	0.86	16	12	9.92
<b>Sampling Point 2</b>							
Mar 2013	21.0	7.13	170.8	0.53	12	12	3.95
Apr 2013	17.4	6.91	104.2	0.42	22	25	5.48
May 2013	14.5	6.25	108.3	0.43	14	4	5.61
Jun 2013	12.1	6.36	100.0	0.34	22	4	17.16
Jul 2013	12.6	6.08	87.5	0.38	31	10	12.69
<b>Sampling Point 3</b>							
Mar 2013	21.6	7.21	158.3	0.50	23	14	3.69
Apr 2013	18.0	7.20	125.0	0.46	27	25	4.45
May 2013	14.5	6.43	120.8	0.44	15	12	4.17
Jun 2013	12.1	6.14	100.0	0.36	29	16	18.40
Jul 2013	12.7	6.70	112.5	0.39	36	11	7.85
<b>Sampling Point 4</b>							
Mar 2013	21.6	7.23	162.5	0.50	18	12	5.75
Apr 2013	17.8	6.96	116.7	0.45	21	21	4.88
May 2013	14.8	6.38	116.7	0.44	95	10	3.89
Jun 2013	13.2	6.28	112.5	0.38	25	4	6.21
Jul 2013	13.1	6.21	87.5	0.37	31	4	3.28
<b>Sampling Point 5+6</b>							
Mar 2013	22.2	7.25	150.0	0.49	20	6	3.79
Apr 2013	18.1	7.07	120.8	0.46	32	26	3.50
May 2013	14.9	6.44	108.3	0.44	25	7	3.09
Jun 2013	12.2	5.93	75.0	0.37	29	10	4.81
Jul 2013	11.6	6.08	87.5	0.41	41	11	2.75
<b>Sampling Point 7</b>							
Mar 2013	21.8	7.29	150.0	0.51	38	3	4.00
Apr 2013	18.5	7.11	108.3	0.47	35	25	3.72
May 2013	15.1	6.30	108.3	0.44	27	7	2.08
Jun 2013	13.5	5.90	75.0	0.35	38	11	4.21
Jul 2013	14.2	5.58	62.5	0.36	32	1	0.91
<b>Sampling Point 8</b>							
Mar 2013	24.2	7.14	145.8	0.50	26	5	4.40
Apr 2013	19.7	6.94	129.2	0.49	21	19	2.87
May 2013	15.5	6.30	100.0	0.46	19	1	1.94
Jun 2013	14.5	6.19	87.5	0.39	20	3	2.69
Jul 2013	13.1	6.53	100.0	1.20	25	1	1.76

were only below  $30 \text{ mg.L}^{-1}$  in March, June and July 2013 and thus would not be regarded as suitable in terms of industrial use (DWAF, 1996c). The TSS values varied between 9 and  $19 \text{ mg.L}^{-1}$ , thus never exceeding the SAWQG of  $50 \text{ mg.L}^{-1}$ , making it suitable to use for drip irrigation (DWAF, 1996b). The turbidity ranged from 4.44 and 137.97 NTU. Measuring turbidity gives an estimate of suspended solids in the water (McCaffrey, 2011; Elqert, 2012). Though high turbidity is often a sign of poor water quality and land management - crystal clear water does not always guarantee healthy water. Water with a very clear appearance can signify very acidic conditions or high levels of salinity (McCaffrey, 2011). According to the SAWQG Field Guide (DWAF, 1996d), there is no TWQR for turbidity for agricultural irrigation. The SAWQG for domestic use is set at 1.00 NTU (DWAF, 1996a) - a quality which was not met in any instances. During April 2013, when the turbidity of the river water was extremely high, the water was brown and had a murky appearance. According to Daphne *et al.* (2011) there is a positive correlation between TSS and turbidity taken from river water samples. After reviewing Table 2, a slight correlation was seen between TSS and turbidity within the first three sampling points, respectively, but this trend was not followed all the way through to Sampling Point 8.

A relationship was again evident between the temperature and pH values measured at Sampling Point 2. The water temperature ranged from  $12.1^{\circ}$  to  $21.0^{\circ}\text{C}$ , whereas the pH values varied between 6.08 and 7.13 for the same time period. The pH values only fell within the SAWQG (6.5 to 8.5) during March and April 2013, while the samples taken in May, June and July 2013 did not adhere to the SAWQG (DWAF, 1996b). Again no relationship could be found between temperature, pH and the other chemical parameters. The alkalinity of the water ranged between 87.5 and  $170.8 \text{ mg CaCO}_3.\text{L}^{-1}$  and was thus always above the  $80 \text{ mg CaCO}_3.\text{L}^{-1}$  recommended by Spellman (2008). The conductivity of the water varied between 0.34 and  $0.53 \text{ mS.m}^{-1}$  and never exceeded the SAWQG of  $40.00 \text{ mS.m}^{-1}$ , making the water suitable for irrigational use (DWAF, 1996b). The COD levels ranged between 12 and  $31 \text{ mg.L}^{-1}$ , thus the SAWQG of  $30 \text{ mg.L}^{-1}$  set for industrial use was only exceeded in July 2013 (DWAF, 1996c). The TSS values ranged from 4 to  $25 \text{ mg.L}^{-1}$  and never exceeded the set SAWQG of  $50 \text{ mg.L}^{-1}$ , making the water suitable for use in drip irrigation systems (DWAF, 1996b). The turbidity of the water ranged between 3.95 and 17.16 NTU, thus exceeding the SAWQG for domestic use during all of the sampling occasions (DWAF, 1996a).

The water temperature varied between  $12.1^{\circ}$  and  $21.6^{\circ}\text{C}$  at Sampling Point 3, while the pH values varied from 6.14 to 7.21 for the same time period. The pH values only fell within the SAWQG (6.5 to 8.5) for irrigation water during March, April and July 2013 (DWAF, 1996b). The alkalinity of the water varied from 100.0 to  $158.3 \text{ mg CaCO}_3.\text{L}^{-1}$ , whereas the conductivity ranged from 0.36 to  $0.50 \text{ mS.m}^{-1}$ . Alkalinity was above  $80 \text{ mg CaCO}_3.\text{L}^{-1}$  on all

sampling occasions, indicating water of a good buffering capacity (Spellman, 2008). Conductivity of the water was also under the SAWQG values of  $40.00 \text{ mS.m}^{-1}$ , making it suitable for irrigational use (DWAF, 1996b). The COD and TSS levels ranged between 15 and  $36 \text{ mg.L}^{-1}$  and 11 to  $25 \text{ mg.L}^{-1}$ , respectively. The SAWQG of  $30 \text{ mg.L}^{-1}$  COD set for industrial use was only exceeded in July 2013 (DWAF, 1996c), while the TSS of the water was below the SAWQG of  $50 \text{ mg.L}^{-1}$  on all sampling occasions, indicating the water to be suitable for a drip irrigation system (DWAF, 1996b). The turbidity varied from 3.69 to 18.40 NTU. Even though the values are relatively low, they were still above the SAWQG for domestic use (DWAF, 1996a).

The water temperature at Sampling Point 4 varied from  $13.1$  to  $21.6^\circ\text{C}$ , while the pH values ranged between 6.21 and 7.23. The pH values only fell within the SAWQG (6.5 to 8.5) during March and April 2013, making it unsuitable for irrigational use in May till July 2013 (DWAF, 1996b). The alkalinity of the water varied between 87.5 and  $162.5 \text{ mg CaCO}_3.\text{L}^{-1}$ , while the conductivity ranged from 0.37 to  $0.50 \text{ mS.m}^{-1}$ . Alkalinity was above  $80 \text{ mg CaCO}_3.\text{L}^{-1}$  during all of the sampling months, indicating water of a good buffering capacity (Spellman, 2008). Conductivity of the water was also under the SAWQG values of  $40.00 \text{ mS.m}^{-1}$ , making it suitable for irrigational use (DWAF, 1996b). The COD and TSS levels varied between 18 and  $95 \text{ mg.L}^{-1}$  and 4 to  $21 \text{ mg.L}^{-1}$ , respectively. The TSS of the water always fell within SAWQG of  $50 \text{ mg.L}^{-1}$  on all sampling occasions, indicating the water is suitable for a drip irrigation system (DWAF, 1996b). The turbidity of the water varied between 3.28 and 6.21 NTU. The values exceeded the SAWQG for domestic use, making it unsuitable for use (DWAF, 1996a).

At Sampling Point 5 and 6, where the UV apparatus was installed, the water temperature ranged from  $11.6^\circ$  to  $22.2^\circ\text{C}$ , whereas the pH values ranged from 5.93 to 7.25. The pH values only fell within the SAWQG during March and April 2013, making it unsuitable for irrigational use in May, June and July (DWAF, 1996b). The alkalinity of the water varied between 75.0 and  $150.0 \text{ mg CaCO}_3.\text{L}^{-1}$ . The water has relatively good buffering capacity since alkalinity was always above  $80.0 \text{ mg CaCO}_3.\text{L}^{-1}$  during most of the sampling months, except during June 2013 (Spellman, 2008). The conductivity of the water varied from 0.37 to  $0.49 \text{ mS.m}^{-1}$ , while the COD levels varied between 20 and  $41 \text{ mg.L}^{-1}$ . Conductivity of the water always was suitable for irrigational use since it always fell within the SAWQG values of  $40.00 \text{ mS.m}^{-1}$  (DWAF, 1996b). The SAWQG for COD was only exceeded in April and July 2013, the rest of the samples fell below the guideline of  $30.0 \text{ mg.L}^{-1}$ , making it safe for use (DWAF, 1996c). The TSS values varied from 6 to  $26 \text{ mg.L}^{-1}$ , whereas the turbidity values ranged between 2.75 and 4.81 NTU. The TSS of the water met the SAWQG on all sampling occasions, indicating the water is suitable for a drip irrigation system (DWAF, 1996b). The

turbidity counts exceeded the SAWQG for domestic use, making it unsuitable for use (DWAF, 1996a).

At Sampling Point 7 the water temperature ranged between 13.5° and 21.8°C, while the pH values ranged from 5.58 to 7.29. The pH values only fell within the SAWQG (6.5 to 8.5) during March and April 2013, making it unsuitable for irrigational use in May till June (DWAF, 1996b). The alkalinity of the water ranged from 62.5 to 150.0 mg CaCO<sub>3</sub>.L<sup>-1</sup>, thus the water had a relatively good buffering capacity from March till May 2013 since the values was always above 80.0 mg CaCO<sub>3</sub>.L<sup>-1</sup> during these sampling months (Spellman, 2008). The conductivity of the water varied between 0.35 and 0.51 mS.m<sup>-1</sup>, whereas the COD levels ranged between 27 and 38 mg.L<sup>-1</sup>. Conductivity of the water was below the SAWQG of 40.00 mS.m<sup>-1</sup> on all occasions, making it suitable for irrigational use (DWAF, 1996b). The SAWQG for COD was only met in May 2013, making the rest of the samples unsuitable for industrial use (DWAF, 1996c). The TSS values varied between 1 and 25 mg.L<sup>-1</sup>, while turbidity values ranging between 0.91 and 4.21 NTU was observed. The SAWQG for TSS was always met (DWAF, 1996b). Even though the turbidity loads are relatively low, it was still above the SAWQG for domestic use, making it unsuitable for use (DWAF, 1996a).

The water temperature varied between 13.1° and 24.2°C at Sampling Point 8, while the pH values ranged between 6.19 and 7.14. The SAWQG for pH was only met during March, April and July 2013, making it unsuitable for irrigational use in the May and June (DWAF, 1996b). The alkalinity of the water varied between 87.5 and 145.8 mg CaCO<sub>3</sub>.L<sup>-1</sup>, thus according to Spellman (2008) the water has relatively good buffering capacity since alkalinity was always above 80.0 mg.L<sup>-1</sup>. The conductivity of the water ranged from 0.39 to 1.20 mS.m<sup>-1</sup>, making it suitable for irrigational use since it always fell within the SAWQG of 40.00 mS.m<sup>-1</sup> (DWAF, 1996b). The COD and TSS levels varied between 19 to 26 mg.L<sup>-1</sup> and 1 to 19 mg.L<sup>-1</sup>, respectively. The SAWQG for COD of 30.0 mg.L<sup>-1</sup> was met during all of the sampling occasions, making it safe for use (DWAF, 1996c). The TSS is suitable for a drip irrigation system since the SAWQG was always met (DWAF, 1996b). Turbidity values varied from 1.76 to 4.40 NTU. The loads exceeded the SAWQG for domestic use, making it unsuitable for use (DWAF, 1996a).

#### *Anions and Cations*

Water used for irrigational purposes always contains some salt (Bauder *et al.*, 2013; Yiasoumi *et al.*, 2005). The salt present in the water usually comes from weathering of soil solution or groundwater, landscapes and stream banks during and following precipitation. Groundwater typically contains more salt than surface water. It has also been observed that irrigation water from arid and semi-arid regions usually contain more salt than humid and sub-humid areas.

As mentioned earlier salinity is a measure of the dissolved salts that are present in water and usually increase as water levels decrease (Bauder *et al.*, 2013; Yiasoumi *et al.*, 2005; McCaffrey, 2011). Salinity is measured as either total dissolved solids or as electrical conductivity and has an adverse effect on plant growth as well as soil properties.

According to Yiasoumi *et al.* (2005) and Hopkins *et al.* (2007) certain anions and cations must be tested for to characterize irrigation water. Cations include calcium, magnesium, sodium, potassium, iron and manganese. Anions to be tested include chloride, boron, carbonate, bicarbonate, sulphate and nitrate. In reality after analysis, the amount of anions and cations present in the water, must add up to the same amounts (Hopkins *et al.*, 2007; Bauder, *et al.*, 2013). If all tests are performed correctly, the sum of each one, respectively, should not differ by more than 20 percent (Hopkins *et al.*, 2007). Only certain anions and cations were tested for during this study, thus the calculation could not be made (Table 6).

**Table 6** Anions and cations present in the water samples.

Sampling date	Anions					Cations		
	F* (mg.L <sup>-1</sup> )	Cl <sup>-</sup> * (mg.L <sup>-1</sup> )	NO <sub>3</sub> <sup>-</sup> * (mg.L <sup>-1</sup> )	PO <sub>4</sub> <sup>2-</sup> * (mg.L <sup>-1</sup> )	SO <sub>4</sub> <sup>2-</sup> * (mg.L <sup>-1</sup> )	NH <sub>4</sub> <sup>-</sup> * (mg.L <sup>-1</sup> )	Fe* (mg.L <sup>-1</sup> )	Mn <sup>2+</sup> ** (mg.L <sup>-1</sup> )
<b>Sampling Point 1</b>								
March	0.3	78.6	24.4	nd	14.6	-	0.23	0.01
April	nd**	26.3	14.5	nd	8.4	-	0.67	0.01
May	nd	68.6	12.8	12.5	19.6	5.8	0.35	0.01
June	0.2	63.1	9.8	nd	16.4	2.3	0.34	0.02
<b>Sampling Point 5</b>								
March	nd	68.8	5	4.5	11.7	-	0.27	0.02
April	nd	68.4	57.5	11.6	15.5	-	0.48	0.04
May	nd	68.3	16.1	nd	20.6	5.9	0.25	0.01
June	0.3	58.9	28.7	3.2	18.4	2.5	0.37	0.01
<b>Sampling Point 8</b>								
March	0.1	75.1	14.6	13.4	11.7	-	0.36	0.04
April	0.6	73.6	55.6	16.1	17.9	-	0.28	0.05
May	nd	68.8	21	nd	20.5	5.3	0.12	0.01
June	0.3	62.1	33.8	nd	16.5	2.2	0.26	0.01

\*Fluorine, Chloride, Nitrate, Phosphate, Sulphate

\*Ammonium, Iron, Manganese \*\*nd = none detected

Water samples were collected from March till June 2013 from the river (Sampling Point 1), before UV (Sampling Point 5) and at the point of irrigation (Sampling Point 8) to test for certain anions and cations that might be present in the river water. This table gives an indication of the amount of anions and cations that are present at the river (Site 1), before UV (Site 5) and the point of irrigation (Site 8) (Table 6).

According to Yiasoumi *et al.* (2005) the suitable range for fluorine in water used for irrigation water of sensitive crops in soilless media such as strawberries is less than 1.0 mg.L<sup>-1</sup>. High levels of fluorine in irrigation water can disrupt the metabolic processes in plants and cause foliar lesions (Guatam & Bhardwaj, 2010). Even low concentrations of fluorine in irrigation water can adversely affect crop growth by causing physiological and biochemical changes to occur in plants. Fluorine concentrations at Sampling Point 1, Sampling Point 5 and Sampling Point 8 ranged between none detected to 0.3 mg.L<sup>-1</sup>, none detected to 0.3 mg.L<sup>-1</sup> and none detected to 0.6 mg.L<sup>-1</sup>, respectively, between March and June 2013. Fluorine was always below 1.0 mg.L<sup>-1</sup> at all of the sampling points and on all of the sampling occasions, thus the water could be used for irrigational purposes since it did not exceed the limits recommended by Yiasoumi *et al.* (2005).

The presence of chloride in irrigation water used in soilless media according to Yiasoumi *et al.* (2005) is suitable for most plants if the concentration is lower than 70 mg.L<sup>-1</sup>. Chloride is known to cause tip-burn in sensitive crops such as raspberries in concentrations higher than 200 mg.L<sup>-1</sup> and it is generally unsuitable for irrigation of crops if concentrations greater than 400 mg.L<sup>-1</sup> is present in the water. Hopkins *et al.* (2007) reports similar ranges for suitability of chloride present in water used for irrigational purposes, reporting a suitability range of 70 - 140 mg.L<sup>-1</sup>. The limit for sensitive crops is used as a guideline since this range closest relate to strawberries. High chloride levels in water may cause poor plant growth and death of sensitive plants, particularly if sprayed on leaves. Foliar damage (leaf burn, bronzing and leaf drop) and uptake through leaves with overhead irrigation can also occur if a high concentration of chloride is present in irrigation water (Yiasoumi *et al.*, 2005 & Hopkins *et al.*, 2007). The chloride concentrations at Sampling Point 1, Sampling Point 5 and Sampling Point 8 ranged between 26.3 and 78.6 mg.L<sup>-1</sup>, 58.9 and 68.8 mg.L<sup>-1</sup> and 62.1 and 75.1 mg.L<sup>-1</sup>, respectively, between March and June 2013. Chloride concentrations never exceeded the recommended limits, thus the water could be used to irrigate sensitive crops such as strawberries (Yiasoumi *et al.*, 2005 & Hopkins *et al.*, 2007).

The presence of nitrate in irrigation water used in soilless media according to Yiasoumi *et al.* (2005) is suitable for most plants if the concentration is lower than 10.0 mg.L<sup>-1</sup>. Nitrate concentrations higher than 25.0 mg.L<sup>-1</sup> is unsuitable for irrigation since precipitation of salts can occur causing blockages in the irrigation system. The nitrate concentrations at Sampling Point 1 ranged between 9.8 and 24.4 mg.L<sup>-1</sup>. Thus the water

was only suitable for plant irrigation in terms of nitrate concentrations, in June 2013. The nitrate concentrations at Sampling Point 5 ranged between 5.0 and 57.5 mg.L<sup>-1</sup>. Thus the water was only suitable for plant irrigation in terms of nitrate concentrations, in March 2013. In April and June 2013 the concentrations were higher than 25.0 mg.L<sup>-1</sup>, thus the water was unsuitable for irrigational purposes at this stage of the irrigation cycle. The nitrate concentrations at Sampling Point 8 ranged between 14.6 and 55.6 mg.L<sup>-1</sup>. Thus the water was unsuitable for plant irrigation in terms of nitrate concentrations, on all of the sampling occasions. In March and June 2013 the concentrations were higher than 25.0 mg.L<sup>-1</sup>, thus the water was unsuitable for irrigational purposes at this stage of the irrigation cycle since it might damage the plant and cause blockages to occur in the irrigation system. Sampling point 8 was the most critical point where the limits had to be met since this is the point of irrigation where the water comes into contact with the crop during irrigation.

The presence of phosphate in irrigation water used in soilless media according to Yiasoumi *et al.* (2005) is suitable for most plants if the concentration is lower than 1.0 mg.L<sup>-1</sup>. Phosphate concentrations at Sampling Point 1, Sampling Point 5 and Sampling Point 8 ranged between none detected to 12.5 mg.L<sup>-1</sup>, none detected to 11.6 mg.L<sup>-1</sup> and none detected to 16.1 mg.L<sup>-1</sup>, respectively, between March and June 2013. A sewage treatment plant is present upstream along the river from which the farm sources their water for irrigational purposes. If the treatment plant is not functioning properly, it might contribute to the high phosphate levels present in the water, since according to literature sewage contains natural nutrients such as phosphorus and ammonia (Jacob & Cordaro, 2000). Phosphate levels in untreated sewage can range between 6.0 and 20.0 mg.L<sup>-1</sup>. Phosphate was above 1.0 mg.L<sup>-1</sup> at all of the sampling points and on certain of the sampling occasions, thus the water could not always be used for irrigational purposes since it exceeded the limits set by Yiasoumi *et al.* (2005).

Even though sulphate is a major contributor to salinity, it rarely causes toxicity problems except at extremely high concentrations (Bauder *et al.*, 2013). The sulphate concentrations at Sampling Point 1, Sampling Point 5 and Sampling Point 8 ranged between 8.4 and 19.6 mg.L<sup>-1</sup>, 11.7 and 20.6 mg.L<sup>-1</sup> and 11.7 and 20.5 mg.L<sup>-1</sup>, respectively, between March and June 2013.

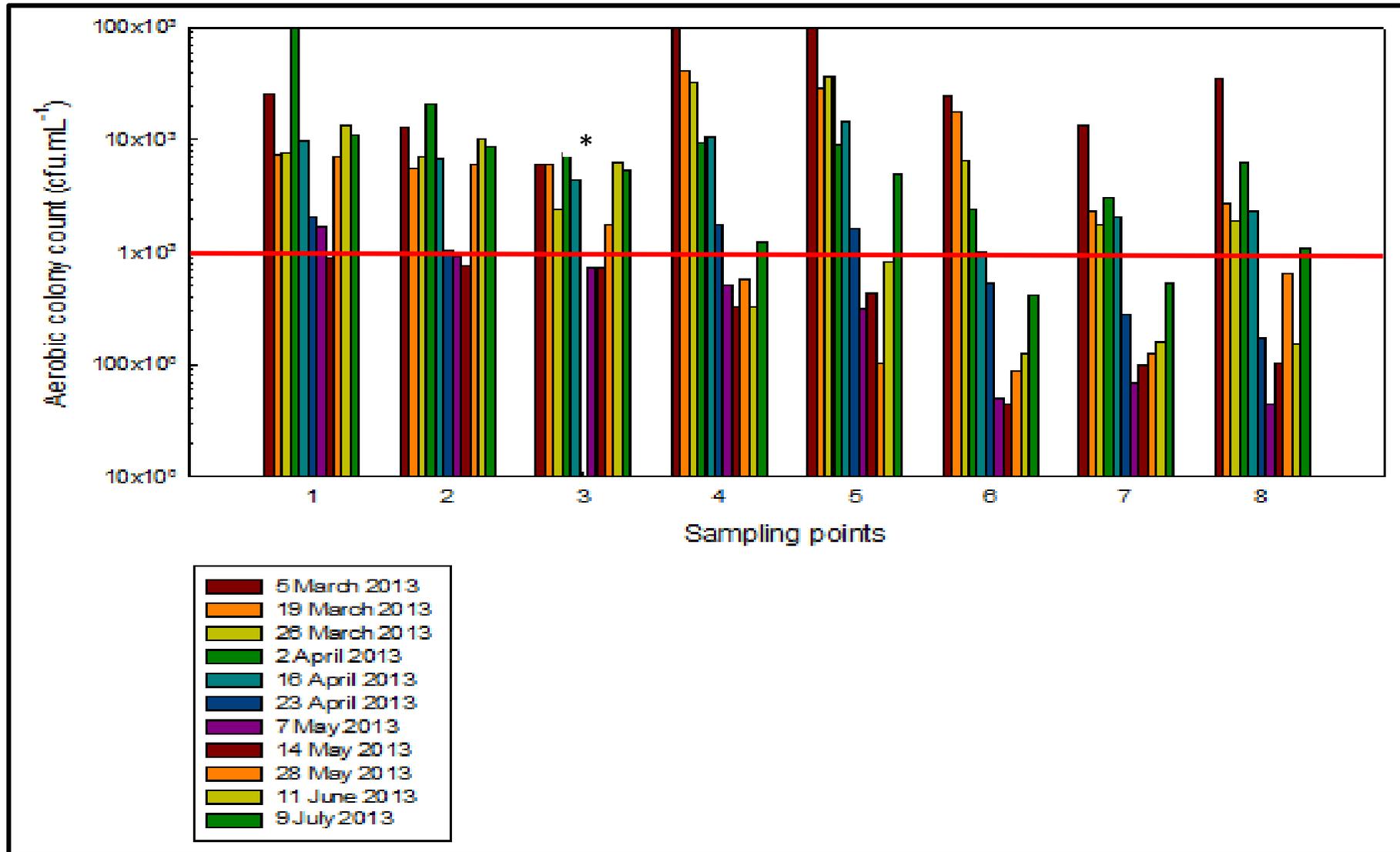
Ammonium levels in the river water were tested since according to literature, levels ranging between 12 and 50 mg.L<sup>-1</sup> can be detected in untreated sewage and as mentioned earlier a sewage treatment plant is present upstream from the source water (Sampling Point 1) (Jacob & Cordaro, 2000; Anon., 2013). No limit is available for the amount of ammonia allowed in irrigation water, since it is not usually tested for. Ammonium levels were only tested in May and June 2013. Ammonium concentrations at Sampling Point 1, Sampling Point 5 and Sampling Point 8 ranged between 2.3 and 5.8 mg.L<sup>-1</sup>, 2.5 and 5.9 mg.L<sup>-1</sup> and 2.2

and 5.3 mg.L<sup>-1</sup>, respectively. Since the levels were so low, it is not possible to speculate whether its presence in the river water was solely due to sewage contamination and thus an improperly functioning sewage treatment plant.

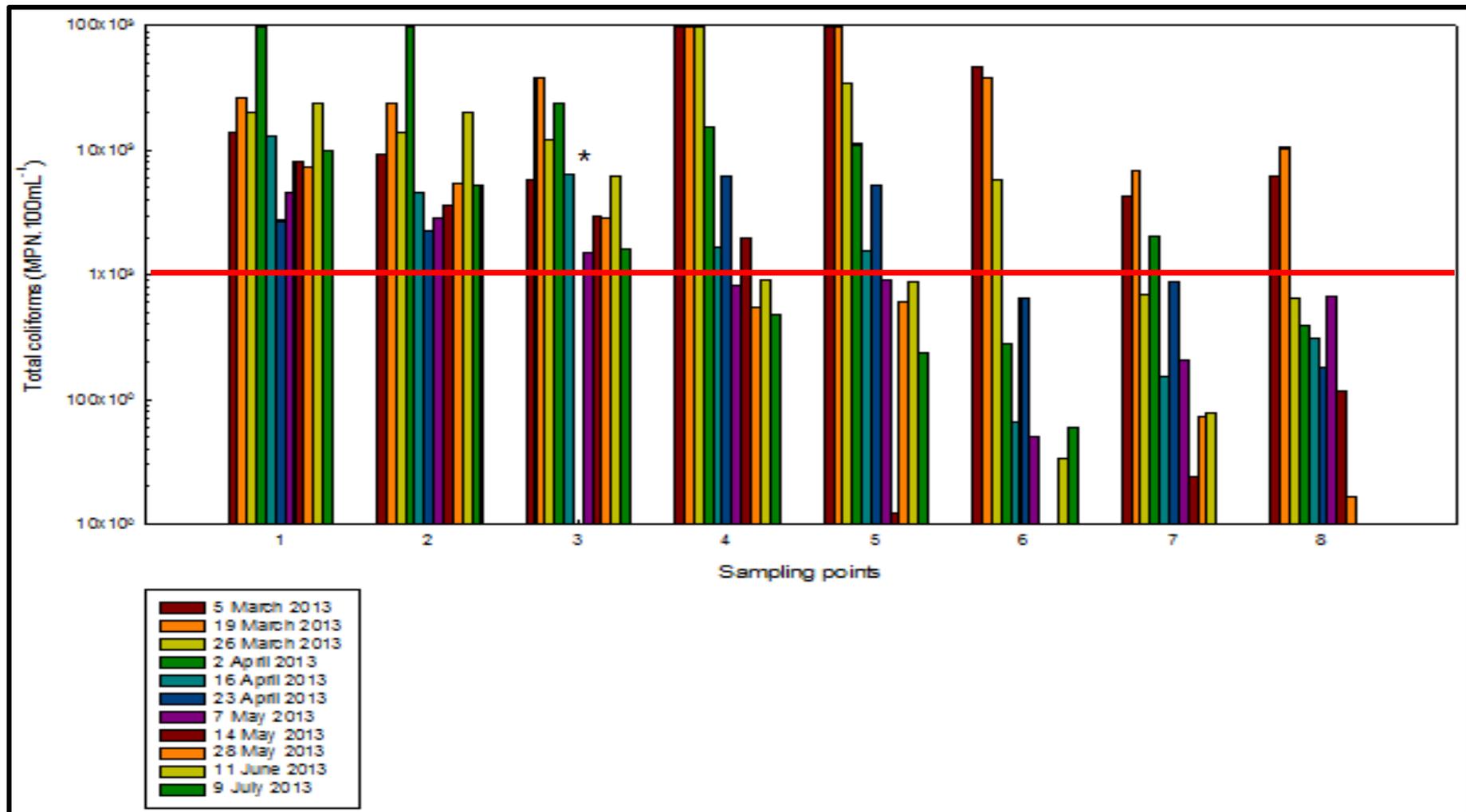
According to Hopkins *et al.* (2007) it is also beneficial to test for iron and manganese in river water, but it is not always necessary, depending on the water source. Even low concentrations of iron and manganese present in irrigation water can oxidize and form precipitates in the irrigation system capable of plugging drip emitters (Cahn, 2013). Iron concentrations at Sampling Point 1, Sampling Point 5 and Sampling Point 8 ranged between 0.23 and 0.67 mg.L<sup>-1</sup>, 0.25 and 0.48 mg.L<sup>-1</sup> and 0.12 and 0.36 mg.L<sup>-1</sup>, respectively. While manganese concentrations at the same sampling points ranged between 0.01 and 0.02 mg.L<sup>-1</sup>, 0.01 and 0.04 mg.L<sup>-1</sup> and 0.01 and 0.05 mg.L<sup>-1</sup>, respectively.

#### *Microbiological results*

The results for ACC on water samples from Sampling Point 1 - 8 are given in Figure 9. The ACC in the river water ranged between 900 and 142 000 cfu.mL<sup>-1</sup> at Sampling Point 1 (Fig. 9). Even though there are no guidelines for ACC in irrigation water in South Africa, it is worth noting that counts greater than 1 000 cfu.mL<sup>-1</sup> in domestic water, are considered to increase the risk of transmitting disease (DWA, 1996a). The TC counts ranged between 2 723 and 241 960 MPN.100 mL<sup>-1</sup> (Fig. 10). In the absence of South African guidelines for TC in irrigation water, the Canadian guideline of 1 000 cfu.100 mL<sup>-1</sup> can be used as a comparison (Monaghan & Hutchison, 2010). The counts were higher than the Canadian guidelines during all of the sampling months, indicating an increased safety risk when irrigating fresh crops that are consumed raw (Monaghan & Hutchison, 2010). The *E. coli* counts ranged from 195 to 6 867 MPN.100 mL<sup>-1</sup> (Fig. 11). The SAWQG and WHO *E. coli* limit of 1 000 organisms.100 mL<sup>-1</sup> was only exceeded on 2 April, 28 May and 11 June 2013 (WHO, 1989; DWA, 1996b). The SAWQG and WHO guideline for *E. coli* of less than 1 000 organisms.100 mL<sup>-1</sup> was met on all of the other sampling occasions (WHO, 1989; DWA, 1996b). Although many of the river water samples exceeded the guidelines for irrigation of fresh produce, in many instances the levels were only slightly higher than the guidelines. This should be kept in mind, as water abstracted from rivers is usually not used directly for irrigation and often undergoes at least sand filtration before use. The overall microbial counts for ACC, TC and *E. coli* were a lot higher at the river (Sampling Point 1) during the UV treatment study (900 to 142 000 cfu.mL<sup>-1</sup> for ACC, 2 723 to 241 960 MPN.100 mL<sup>-1</sup> for TC 195 to 6 867 MPN.100 mL<sup>-1</sup> for *E. coli*) than in the baseline study (4 500 to 9 600 cfu.mL<sup>-1</sup> for ACC, 3 468.5 to 61 600 MPN.100 mL<sup>-1</sup> for TC 110 to 2 098 MPN.100 mL<sup>-1</sup> for *E. coli*). The baseline study was conducted from October 2012 to February 2013, the summer months in the Southern Hemisphere, while the UV treatment study was done from March

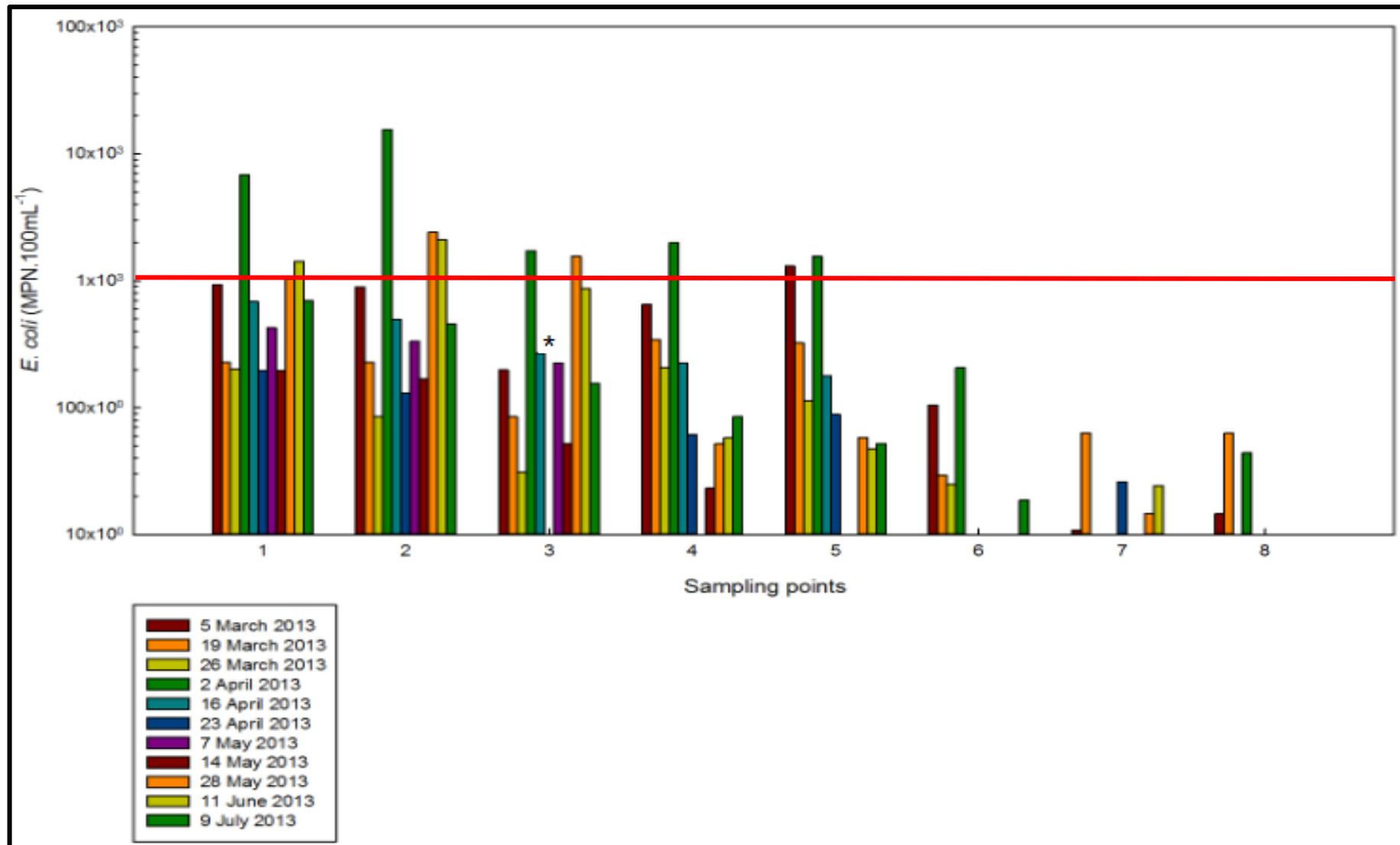


**Figure 9** Aerobic colony counts at the eight different sampling points as obtained during the UV treatment study, from March to May 2013. (\*no counts reported at Sampling Point 3 on 23 April - sample bottle broke during transport)



**Figure 10** Total coliforms at the eight different sampling points as obtained during the UV treatment study, from March to May 2013.

(\*no counts reported at Sampling Point 3 on 23 April - sample bottle broke during transport)



**Figure 11** *Escherichia coli* at the eight different sampling points as obtained during the UV treatment study, from March to May 2013. (\*no counts reported at Sampling Point 3 on 23 April - sample bottle broke during transport)

2013 to May 2013 (autumn and beginning of winter). According to Bruhn & Wolfson (2007) ultraviolet rays from the sun might kill bacteria on a warm and sunny day, leading to lower than expected counts. Heavy storms and rainfall have also been shown to contribute to higher microbial counts in rivers due to storm water overflows, pollution and runoff from pastures and wastewater treatment plant overflows (Kistemann *et al.*, 2002; Hill *et al.*, 2006). This might explain why counts are lower during the baseline study, which was conducted during the summer months (October 2012 to February 2013).

At Sampling Point 2 the ACC ranged between 760 and 21 300 cfu.mL<sup>-1</sup> (Fig. 9). From Figure 9 it is clear that the ACC were very similar to those of the river water (Sampling Point 1) except on 2 April 2013 where ACC were a lot lower at Sampling Point 2 (21 300 cfu.mL<sup>-1</sup>) than at Sampling Point 1 (142 000 cfu.mL<sup>-1</sup>). As mentioned in the baseline study, it was expected that the sand filters between the river and the inlet to the first holding dam would result in a slight reduction in the microbial load, but this was not the case (Hijnen *et al.*, 2007). The TC varied between 2 282 and 104 620 MPN.100mL<sup>-1</sup> (Fig. 10). Similar to the trend with ACC, the range for TC at Sampling Point 2 stayed more or less the same as at Sampling Point 1. The fact that the TC levels have not been reduced by the sand filtration means that the loads are still above the 1 000 cfu.100 mL<sup>-1</sup> Canadian guideline, indicating an increased risk of using such water to irrigate crops (Monaghan & Hutchison, 2010). The *E. coli* counts ranged from 85 to 15 531 MPN.100 mL<sup>-1</sup> (Fig. 11). The *E. coli* loads were very similar to those at the river (Sampling Point 1). The SAWQG and WHO guideline for irrigation water were only exceeded on 2 April, 28 May and 11 June 2013 (increase from Sampling Point 1 to Sampling Point 2), thus the water could be regarded as safe for irrigation of fresh produce on the rest of the sampling occasions, in terms of the guidelines for *E. coli* (WHO, 1989; DWAF, 1996b).

The ACC at Sampling Point 3 varied between 720 and 7 400 cfu.mL<sup>-1</sup> (Fig. 9). The ACC remained similar in loads from Sampling Point 2 to Sampling Point 3, with only a few visible decreases on 5 March, 26 March, 2 April, 8 January 2013 and on 28 May 2013. The TC varied from 1 515 to 38 730 MPN.100mL<sup>-1</sup> (Fig. 10). The TC levels at Sampling Point 3 were never below the 1 000 cfu.100 mL<sup>-1</sup> guideline for safe irrigation water as set out in the Canadian guideline (Monaghan & Hutchison, 2010). The *E. coli* counts varied between 31 and 1 725 MPN.100 mL<sup>-1</sup> (Fig. 11). The SAWQG and WHO guideline of 1 000 cfu.100 mL<sup>-1</sup> was only exceeded on 2 April and 28 May 2013, thus at this stage of the irrigation cycle, the water could mostly be regarded as safe for irrigation of crops that are consumed raw in terms of *E. coli* (WHO, 1989; DWAF, 1996b). It can clearly be seen in Figure 11 that the *E. coli* loads generally decreased from Sampling Point 2 to Sampling Point 3.

The ACC varied between 330 and 251 000 cfu.mL<sup>-1</sup> at Sampling Point 4 (Fig. 9). As part of the UV treatment study, no H<sub>2</sub>O<sub>2</sub> was added to the water. It can clearly be seen in Figure 9 that ACC increased (0.5 - 1.5 logs increase) from Sampling Point 3 to Sampling Point 4 during March

2013. The ACC at Sampling Point 4 remained constant similar to that of Sampling Point 3 on 2 April and thereafter the loads decreased slightly from Sampling Point 3 to Sampling Point 4 (less than 1.0 log decrease). The TC counts ranged between 488 and 547 500 MPN.100 mL<sup>-1</sup> (Fig. 10). The same trend was seen in the TC counts as with ACC, as it can clearly be seen in Fig. 8 that TC loads increased from Sampling Point 3 to Sampling Point 4 during March 2013, but decreased thereafter from Sampling Point 3 to Sampling Point 4. The Canadian guidelines were only met on 7 May, 28 May, 11 June and 9 July 2013, thus the water could be considered unsuitable for the irrigation of fresh crops which are consumed raw on the other sampling occasions (Monaghan & Hutchison, 2010). The rest of the time the Canadian guideline was exceeded after water passed through the sand filters, showing increases as high as 2.0 logs on certain sampling dates. *Escherichia coli* counts varied from 31 to 1 725 MPN.100 mL<sup>-1</sup> (Fig. 11). Unlike in the baseline study, in Fig. 11 it can clearly be seen that the *E. coli* loads increased from Sampling Point 3 to Sampling Point 4 during March, remained relatively constant during April and decreased during May, June and July. The SAWQG and WHO guideline was met on all sampling occasions except on 2 April 2013, thus the water was presumed as safe for the irrigation of fresh produce in terms of *E. coli* counts (WHO, 1989; DWAF, 1996b). It was expected that microbial loads would be reduced between Sampling Point 3 and Sampling Point 4, due to sand filtration that takes place (Hijnen *et al.*, 2007). A possible explanation for the initial increases in ACC, TC and *E. coli* loads could be due to the sand filters which are used for extended periods without being re-sanded. As a result the filters might be clogged and not filtering properly. As a result of the increase in counts from Sampling Point 3 to Sampling Point 4 during March, the farmer decided to take action by adding chlorine to the sand filters (Sampling Point 4) three/four days prior to the sampling day 7 May 2013. Chlorine was added as a rapid solution to prevent any further increases in counts at Sampling Point 4. It was suspected that an extensive clogging had taken place and that bacteria were being “washed out” of the sand filter. After the chlorine was added, it was left in the sand filters for an undetermined time where after the entire system was flushed (Zettler, L. 2013, Owner, Limberlost Farms, Stellenbosch, South Africa, personal communication, 7 May 2013). This explains why the counts stopped increasing from Sampling Point 3 to Sampling Point 4 after 7 May 2013. Even though the addition of chlorine had a positive effect on the counts, chlorine could not indefinitely be added to the system since it is known to cause harmful by-products during water treatment (Tate & Arnold, 1990; Woo *et al.*, 2002; Westerhoff, 2006; Momba *et al.*, 2008). The week prior to sampling day 14 May 2013, the sand filters were resanded (Zettler, L. 2013, Owner, Limberlost Farms, Stellenbosch, South Africa, personal communication, 14 May 2013). After the addition of chlorine and resanding, no further increases in counts from Sampling Point 3 to Sampling Point 4 were observed.

In the UV treatment study, Sampling Point 5 was added to monitor the microbial levels in the water directly before the installed UV system. The UV dose reading (mJ.cm<sup>-2</sup>) and % UVT was measured on each of the samplings days, since it may affect the efficacy of the UV apparatus in

lowering the microbial counts present in the water (Werschkun *et al.*, 2012). No H<sub>2</sub>O<sub>2</sub> dosing was added during the UV treatment study so the effectiveness of the UV to destroy microbial growth could be monitored. The ACC ranged between 103 and 273 000 cfu.mL<sup>-1</sup> at Sampling Point 5 (before UV treatment) (Fig.9). It can clearly be seen in Fig. 9 that the ACC remained constant from Sampling Point 4 to Sampling Point 5 during all of the sampling occasions except on 28 May 2013, when the loads decreased dramatically. This was to be expected since no hurdle was in place between Sampling Point 4 and Sampling Point 5. The high counts in March, which decreased thereafter can be explained by the chlorine addition and re-sanding as described above. The TC counts ranged from 12.1 to 579 400 MPN.100 mL<sup>-1</sup> (Fig. 10). The same trend (high counts in March followed by decreases) was seen in the TC counts as with ACC. It can clearly be seen in Figure 10 that TC loads remained relatively constant from Sampling Point 4 to Sampling Point 5. This was to be expected since no hurdle was in place between Sampling Point 4 and Sampling Point 5. The *E. coli* counts varied between 1 and 1 533.1 MPN.100 mL<sup>-1</sup> (Fig. 11). The *E. coli* loads remained more or less constant from Sampling Point 4 to Sampling Point 5 (Fig. 11). Once again this was to be expected since no hurdle was in place between Sampling Point 4 and Sampling Point 5.

After UV treatment at Sampling Point 6 the ACC varied from 44 to 25 000 cfu.mL<sup>-1</sup> (Fig. 9). It can clearly be seen in Fig. 9 that ACC decreased from Sampling Point 5 to Sampling Point 6 (after passing through UV) during all of the sampling occasions. These decreases in ACC represented ranged between 0.01 and 1.5 log-reductions. Even though the loads decreased after UV treatment, it was not always efficient in lowering the ACC to below SAWQG as set out for domestic use (DWAf, 1996a). The counts only fell within the SAWQG from 16 April 2013 and onwards. The TC ranged between 1 and 46 110 MPN.100 mL<sup>-1</sup> (Fig. 10). It can clearly be seen in Fig. 10 that TC loads decreased from Sampling Point 5 to Sampling Point 6 (after passing through UV) during all of the sampling occasions. These reductions in TC counts represented decreases ranging between 0.01 and 1.5 log-reductions. Even though the TC loads decreased after UV treatment, it was not always efficient in lowering the loads to below Canadian guidelines (Monaghan & Hutchison, 2010). The Canadian guidelines were only exceeded during March 2013, with the remainder of the loads being below these guidelines of 1 000 cfu.100 mL<sup>-1</sup> set for irrigation water (Monaghan & Hutchison, 2010). The *E. coli* counts varied between 1 and 206.4 MPN.100 mL<sup>-1</sup> (Fig. 11). It can clearly be seen in Figure 11 that *E. coli* loads decreased from Sampling Point 5 to Sampling Point 6 (after passing through UV) during all of the sampling occasions. These reductions in *E. coli* counts represented decreases ranging between 0.5 and 1.5 log-reductions. The UV was successful in reducing all of the *E. coli* loads to below SAWQG, making it safe for the irrigation of fresh produce that are consumed raw (DWAf, 1996b). Microbial reduction throughout all of the sampling occasions was not constant (0.01 to 1.5 log-reductions). The efficiency of the UV treatment thus varies and this can possibly be ascribed to the fact that the initial counts in the water are extremely high (900 to 142 000 cfu.mL<sup>-1</sup> for ACC, 2 723 to 241 960 MPN.100 mL<sup>-1</sup> for TC

and 195 to 6 867 MPN.100 mL<sup>-1</sup> for *E. coli* at Sampling Point 1), it might be due to biofilms or pieces of biofilm that are present in the water (from the sand filters or the walls of pipes) which are not completely destroyed, incorrect UV dosage or percentage variance in the waters' UVT (Werschkun *et al.*, 2012). Log-reductions ranging from 0.01 to 1.5, 0.01 to 1.5 and 0.5 to 1.5 were achieved for ACC, TC and *E. coli*, respectively, during the UV treatment study. The average overall log-reduction achieved during the UV treatment study was 0.90 to 1.25.

At Sampling Point 7 the ACC ranged from 70 to 13 700 cfu.mL<sup>-1</sup> (Fig. 9). The ACC remained very similar from Sampling Point 6 to Sampling Point 7, with only occasional differences in counts (Fig. 9). On some of the sampling days, an increase in counts occurred, from Sampling Point 6 (after UV) to the pump house (Sampling Point 7) (2 April, 16 April, 28 May, 9 July 2013). This could possibly be attributed to a contaminated piping system or the formation of a biofilm. The TC varied between 6.3 and 6 867 MPN.100 mL<sup>-1</sup> (Fig. 10). A decrease in TC loads could clearly be seen in Fig. 10 from Sampling Point 6 to Sampling Point 7. Even though an additional decrease in TC loads occurred, the Canadian guidelines (1 000 cfu.100 mL<sup>-1</sup>) for the irrigation of fresh crops that are to be consumed raw were still exceeded on 5 and 19 March 2013 (Monaghan & Hutchison, 2010). *Escherichia coli* counts ranged from 1 to 63 MPN.100 mL<sup>-1</sup> (Fig. 11). A decrease in *E. coli* loads could be seen in Fig. 11 from Sampling Point 6 to Sampling Point 7, except on 19 March, 23 April, 28 May and on 11 June 2013. Although these increases occurred, they were relatively small (13.6 to 33.8 MPN.100 mL<sup>-1</sup>) and did not result in any samples exceeding the SAWQG and WHO guideline of 1 000 organisms.100 mL<sup>-1</sup> (WHO, 1989, DWAf, 1996b). These slight increases in *E. coli*, may, however, also be evidence of possible biofilms in the pipe system between Sampling Point 6 (after UV) and Sampling Point 7 (the pump house).

The point of irrigation, Sampling Point 8, was the most critical point in terms of meeting the guidelines for crops being irrigated that are to be consumed raw without any further hurdles implemented to reduce possible microbial loads. The ACC ranged between 44 and 35 000 cfu.mL<sup>-1</sup> (Fig. 9). The ACC remained more or less constant from Sampling Point 7 to Sampling Point 8 (except on 5 March, 2 April, 28 May and 9 July where slight increases occurred). Total coliforms counts varied from 6.3 to 10 462 MPN.100 mL<sup>-1</sup> (Fig. 10) with only very slight increases on 5 March and 19 March. The counts were only above the Canadian guidelines of 1 000 cfu.100 mL<sup>-1</sup> on 5 and 19 March 2013, making the water of the later sampling dates safe to use for the irrigation of fresh crops that are consumed raw (Monaghan & Hutchison, 2010). The *E. coli* counts varied from 1 to 63 MPN.100 mL<sup>-1</sup> (Fig. 11) with slight increases only on 5 March and 2 April. The SAWQG and WHO guideline was met on all of the sampling dates, thus the water could be considered safe for irrigation of fresh crops in terms of *E. coli* counts (WHO, 1989; DWAf, 1996b). During some sampling weeks there was an increase in ACC, TC and *E. coli* loads in the water from the pump house (Sampling Point 7) to the point of irrigation (Sampling Point 8). This could possibly be attributed to a contaminated pipe system or the presence of a biofilm in the pipes between these sampling points. As the irrigation system (from pump house to point of irrigation)

experiences times of non-use, this could provide the opportunity for biofilm formation within the system. The efficiency of the sand filters and UV treatment were improved considerably after the re-sanding of the filters. This can be ascribed to improved filter performance and also an improvement in the UVT (from ca. 56 to 66) which would increase the UV efficiency slightly. UV treatment will still have to be used in conjunction with a pre-treatment such as sand filtration to ensure sufficient decreases in microbial loads.

### CONCLUSIONS AND RECOMMENDATIONS

As mentioned earlier, water used for irrigational purposes always contains some salt (Bauder *et al.*, 2013; Yiasoumi *et al.*, 2005). Since anions and cations were only tested during the UV treatment study, no comparison could be made between the baseline and UV treatment study. Only the sampling months themselves could be compared. According to data recorded the water was suitable for irrigational purposes in terms of fluorine and chloride. Nitrate and phosphate levels recorded during the study, made the water unsuitable for irrigational use since it was above the levels recommended by Yiasoumi *et al.* (2005). Since all of the anions and cations needed to characterise irrigation water were not tested for and testing was performed irregularly, it is not possible to make conclusions on the overall amount of anions and cations found in the water during the different sampling months.

When taking all of the microbial data collected during the baseline study and UV treatment study into consideration it is clear that the water extracted from the Eerste River is not suitable for the irrigation of fresh produce that are consumed raw, since it contains high microbial counts and it does not comply with any of the South African or Canadian guidelines used as set limits. The presence of bacteria of faecal origin such as *E. coli* is definitive evidence that the water is faecally contaminated, most probably due to sewage treatment plants that are not functioning properly. This is even more reason not to use the water for irrigational purposes without receiving any treatment.

Due to several sampling points being monitored throughout the irrigation system, it was possible to monitor the effect of different treatment processes through the irrigation system. It is clear from the results that the first sand filtration step after abstraction from the river was not very effective in lowering the microbial load. This could possibly be due to extended usage, without the filters being re-sanded. It was also observed that the counts generally did not change much through the holding dams, but ACC and TC increased while *E. coli* counts decreased through the sand filters preceding the H<sub>2</sub>O<sub>2</sub> dosing. This could possibly be ascribed to biofilm build-up and clogging within the sand filters. The on-farm dosing of H<sub>2</sub>O<sub>2</sub> was not very effective or consistent. Log-reductions between 0.0 and 1.5 and on one occasion 2.0 were found between Sampling Point 4 and Sampling Point 5, but were mostly below a 1.0 log-reduction. It was also observed that a slight increase in counts occurred when the water was pumped (via the pump house) from the

holding tank to the point of irrigation, indicating the possible existence of biofilms in the pipe system.

During the UV treatment study similar results were obtained as during the baseline study in that the initial sand filtration was ineffective in lowering microbial counts, little change was observed over the holding dams and microbial loads increased after the sand filtration subsequent to the holding dams. The increases in counts after the sand filter were rectified after the re-sanding thereof, highlighting the importance of this practice. A decrease of 0.0 to 1.5 log-reductions was found between samples taken before and after UV. (The average overall log-reduction achieved during the UV treatment study and baseline study was 0.90 to 1.25 and 0.65 to 1.13, respectively.) The efficiency of the UV treatment thus varies and this could possibly be ascribed to biofilms or pieces of biofilm present in the water (from the sand filters or the walls of pipes) which are not completely destroyed, incorrect UV dosage or percentage variance in the waters' UVT (Werschkun *et al.*, 2012). Another possibility is that the environmental strains of bacteria in this river have higher UV dose resistance than expected. This might be due to the fact that the expected UV log-reductions were achieved by testing lab strains. Environmental strains are tougher than lab strains against environmental factors and might thus have resulted in lower log-reductions during treatment. It has previously been shown that several *E. coli* isolates from the Eerste River have increased antibiotic resistance, which may be an indication that they also differ in the resistance to UV (Huisamen, 2012). As a result to achieve the anticipated log-reductions for environmental strains, a higher UV dose would be required.

The counts in the river water during the UV treatment study were mostly higher than during the baseline study. This could possibly be due to an increase in rainfall that occurred from March to July 2013. The baseline study was conducted from October 2012 to February 2013, the summer months in the Southern Hemisphere, while the UV treatment study was done March 2013 to July 2013 (autumn and beginning of winter). According to Bruhn & Wolfson (2007) ultraviolet rays from the sun might kill bacteria on a warm and sunny day, leading to lower than expected counts in the summer months. Heavy storms and rainfall have also been shown to contribute to higher microbial counts in rivers due to storm water overflows, pollution and runoff from pastures and wastewater treatment plant overflows (Kistemann *et al.*, 2002; Hill *et al.*, 2006). This might explain why the counts were higher during the UV treatment study conducted during March to July 2013 (autumn and beginning of winter).

It is difficult to compare the efficacy of the two treatments since several external factors such as ambient temperature and rainfall might have played a role in the results according to literature even though it was not always reflected in the results. However, when all the results are taken into consideration it can be concluded that UV was the most effective treatment method, since the average log-reductions achieved were slightly higher and would have been achieved without the addition of expensive chemical dosing and the risk of producing disinfectant by-products. Not only is UV a slightly better treatment option than H<sub>2</sub>O<sub>2</sub>, it is also the cheaper option

of the two methods when comparing the monthly operating expense (Van Kamp, H. 2013, Winelands UV Technology, East Midlands Water Company, Stellenbosch, South Africa, personal communication, 20 August 2013).

It can be recommended that monitoring of the irrigation system continues, especially to monitor the efficacy of the sand filters. This can be used as an indication of when re-sanding is required. Furthermore, it would be recommended that increased UV dosage be investigated and compared to dosages suggested in literature. It is also important to monitor the effect of biofilms in the irrigation pipe system on the counts at the point of irrigation, especially if the efficiency of the treatment system is enhanced. Otherwise the positive effect of the treatment system could be nullified if recontamination takes place in the subsequent pipe system.

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## CHAPTER 4

### GENERAL DISCUSSION AND CONCLUSIONS

Water is an indispensable natural resource. It is fundamental to life and a crucial component in the environment. It is utilised on large scale in food production, in industrial areas, for hygiene and sanitation purposes and for power generation (Walmsley *et al.*, 1999; Steele & Odumeru, 2004; Paulse *et al.*, 2009; CDC, 2014). As mentioned before, South Africa is a water scarce country facing an undeniable national water crisis, not only in terms of availability, but also in terms of the quality of its fresh water resources. Fresh produce production is an important component of Western Cape agriculture as well as the economic viability of the country (Davies *et al.*, 1993; Gemmell & Schmidt, 2012; Van der Laan *et al.*, 2012).

Several studies performed in the last few years found that the water quality of many South African rivers declined dramatically due to an increase in pollution levels (Paulse *et al.*, 2009; Ijabadeniyi, 2010; Britz & Sigge, 2012). Many farmers in South Africa's agricultural community use water from nearby rivers for crop irrigation, since it is the most affordable and sometimes only source of water available to them. These rivers are often contaminated with high microbial loads and are thus of questionable quality for irrigation. It is thus of utmost importance that the farmers know the quality of the water they use to irrigate crops, since pathogens can be carried over from water onto fresh produce (Ijabadeniyi *et al.*, 2011). With this knowledge in mind this study on different on-farm treatment options was performed to determine which methods would most effectively reduce high microbial contaminant loads in irrigation water as well as the related food safety risk.

A baseline study was performed of the water at Limberlost Farms. The farm irrigates fresh produce with water obtained from the Eerste River. The study was done over a five month period, at six preselected sampling points, to determine the microbiological and chemical parameters of the water so a baseline could be established to compare the results to when the ultraviolet (UV) apparatus was installed. Secondly a UV treatment study was performed over a five month timeline, at two additional sampling points. These included before and after UV.

Overall counts recorded at the river over the two studies varied immensely. Counts reported during the UV treatment study were even higher than the counts reported during the baseline study. According to Bruhn & Wolfson (2007) ultraviolet rays from the sun might kill bacteria on a warm and sunny day, leading to lower than expected counts in the summer months. Heavy storms and rainfall have also been shown to contribute to higher microbial counts in rivers due to storm water overflows, pollution and runoff from pastures and wastewater treatment plant overflows (Kistemann *et al.*, 2002; Hill *et al.*, 2006). This might explain why the counts varied between the two studies and were higher during the UV treatment study conducted during March to July 2013 (autumn and beginning of winter). Counts reported at the river in most instances

exceeded the acceptable levels set out by South African and Canadian guidelines. As a result it was clear that the water had to be pre-treated before it could be used for irrigational purposes.

Since the water was treated in both the baseline and UV treatment study before being used for irrigational purposes, the counts as measured at the point of irrigation were of greater importance than the counts at the river, since the counts present in the river might still decrease to below the guideline levels after passing through sand filters and the addition of hydrogen peroxide (the farm's current mode of treatment) or after passing through the UV in the UV treatment study. The ACC, TC and *E. coli* counts during the baseline study were as high as 8 800 cfu.mL<sup>-1</sup>, 24 196 MPN.100 mL<sup>-1</sup> and 85 MPN.100 mL<sup>-1</sup> at the point of irrigation, respectively. Aerobic colony count, TC and *E. coli* counts as high as 9 600 cfu.mL<sup>-1</sup>, 13 799 MPN.100 mL<sup>-1</sup> and 2 098 MPN.100 mL<sup>-1</sup> were isolated at the river, respectively. The counts at the point of irrigation remained more or less constant and in some instances even increased when compared to counts at the river due to contamination that occurred at the sand filters, making the water unsuitable for irrigation of fresh produce. A possible explanation for the increased microbiological counts noticed in the water after passing through the sand filters could be ascribed to the fact that the sand filters are used for extended periods without being re-sanded or washed. As a result the filters might be clogged and not filtering properly. It is thus of utmost importance that the entire system is maintained in a good, clean and working condition to prevent unnecessary microbiological build up and contamination of water which could not only have major cost implications if left unattended, but could also lead to an increase in the microbiological counts recorded at the point of irrigation.

In the UV treatment study ACC, TC and *E. coli* counts were as high as 35 000cfu.mL<sup>-1</sup>, 10 462 MPN.100 mL<sup>-1</sup> and 63 MPN.100 mL<sup>-1</sup> at the point of irrigation, respectively. Aerobic colony count, TC and *E. coli* counts as high as 142 000 cfu.mL<sup>-1</sup>, 241 960 MPN.100 mL<sup>-1</sup> and 6 867 MPN.100 mL<sup>-1</sup> were isolated at the river, respectively. After treatment with chlorine and re-sanding of the sand filters, no further contamination occurred at the sand filters and the counts decreased to below South African and Canadian guideline limits, making the water safe for irrigational use in terms of all of the microbial parameters, but this was not necessarily due to the effect UV had on the water. Even though this reduction in microbiological counts was visible after treatment, it is difficult to compare or draw conclusions on all points sampled throughout the system since it is not a closed off pilot system but a trial done on a farm with unaccountable variabilities such as not being able to control the volumes of water that was pumped through the entire system, whether the system was working effectively and the entire pipe system is cleaned on a regular basis so as not to contribute to microbiological counts, the irrigation method used or the retention time that the water stood in the holding tank before use.

From both the literature review and the baseline and UV treatment studies that were performed, it was clear that local South African rivers are highly polluted and cannot be used to irrigate fresh produce that is consumed raw. It was thus of great importance to find a treatment that would bring the counts in the water to below the limits required for safe irrigation since

pathogens can be carried over from water onto fresh produce. It is greatly difficult to compare the efficacy of the two treatments with each other since several external factors might have played a role in the results. According to literature these factors include ambient temperature and rainfall. However, when taking all the results into consideration it can be concluded that UV was the most effective treatment method against the heavy microbiological loads in the water, since the average log-reductions achieved were slightly higher (The average overall log-reduction achieved during the UV treatment study and baseline study was 0.90 to 1.25 and 0.65 to 1.13, respectively.) and would have been achieved without the addition of expensive chemical dosing (which leads to a high monthly operating expense) and the risk of producing carcinogenic disinfectant by-products. Not only is UV a slightly more effective treatment option than  $H_2O_2$ , it is also the cheaper option of the two methods when comparing the monthly operating expense since it only requires an initial start-up cost, but thereafter UV treatment is a relatively inexpensive system to sustain. Other advantages of UV include that it only requires a short contact time (seconds) and no holding tanks are required, whereas chemical and other treatments methods can take minutes or even hours to treat water and still might not be effective in reducing all microbiological counts to acceptable levels allowed for the irrigation of fresh produce.

It would be highly recommended that monitoring of the river and irrigation system continue for at least one continuous year to see the effect seasonal changes have on the microbiological counts. It is difficult to account for the inherent variance in microbiological counts due to the fact that the river water compilation differs on a daily basis. This might be attributed to uncontrollable circumstances such as weather, possible bad farming practices or contamination that could have occurred upstream from the point where water is pumped out of the river. It is recommended that the water upstream from the sampling point is investigated and also tested to see whether this will have an impact on the microbiological counts at the point of irrigation. It is also recommended that sampling always occurs at the same time of day to mimic the same conditions and inadvertently reduce inherent variance. For more accurate results samples should be taken in triplicate and more treatments methods must be compared. With the help of the collection of this studies' results, a pilot plant system was made available to the university to further investigate this study field. It is also necessary to monitor the sand filters. This can be used as an indication of when re-sanding is required. It is also important to monitor the effect of biofilms in the irrigation pipe system on the counts at the point of irrigation, especially if the efficiency of the treatment system is enhanced. Otherwise the positive effect of the treatment system could be nullified if recontamination takes place in the subsequent pipe system. Furthermore, it would be recommended that increased UV dosages be investigated and compared to dosages suggested in literature. The entire irrigation system must be monitored and well maintained to ensure effective end results when treating the water for irrigational purpose.

In the meantime, awareness must be raised so consumers are aware of the matter and know to take precautionary measures such as rinsing fresh fruits and vegetables before consuming

it raw. As part of future research, the crop irrigated can also be tested to determine the effect as well as the extent of microbiological carry over from polluted water onto fresh produce.

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