INVESTIGATING THE EFFICACY OF SODIUM HYPOCHLORITE, CALCIUM HYPOCHLORITE AND PERACETIC ACID ON ENVIRONMENTAL *ESCHERICHIA COLI* STRAINS

By

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DECLARATION

By submitting this dissertation electronically, I declare that the entirety of the work contained therein is my own, original work, that I am the sole author thereof (save to the extent explicitly otherwise stated), that reproduction and publication thereof by Stellenbosch University will not infringe any third party rights and that I have not previously in its entirety or in part submitted it for obtaining any qualification.

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ABSTRACT

The intake of enteric pathogens such as *Escherichia coli* (*E. coli)* may lead to serious foodborne illnesses in humans. Previous research has reported high levels of faecal contamination in various Western Cape rivers which make these sources unsuitable for irrigation purposes. This emphasises the urgency for feasible on-farm treatment options to disinfect river water prior to irrigation. Chemical disinfection is a popular choice for general water disinfection. This study, therefore, focussed on the potential application of peracetic acid (PAA) and chlorine in the treatment of irrigation water.

Initially, the efficacy of an emerging water disinfectant, peracetic acid, was investigated. Research was based on the evaluation of PAA disinfection on reference and environmental *E. coli* strains (in saline solution). Environmental *E. coli* strains were more resistant than reference *E. coli* strains to PAA (6 $mg.L^{-1}$ for 5 and 15 min). Strain variation was particularly evident at a contact time of 5 min. The most resistant strain was environmental *E. coli* strain F11.2 (1.54 log reduction) and the least resistant was ATCC 25922 (4.50 log reduction). The effect of lower PAA doses (0.5, 1.5, 3.0, 4.5 and 6.0 $mg.L^{-1}$) and longer contact times (5, 15 and 25 min) were tested against the most resistant strain (*E. coli* F11.2). It was observed that PAA concentrations ranging between 0.5 – 3.0 $mg.L^{-1}$ were ineffective (< 1.5 log reduction) in reducing *E. coli* over a contact period of 25 min and did not reach the 3 log reduction target. Higher PAA doses (4.5 – 6.0 $mg.L^{-1}$) resulted in increased log reductions (4.94 – 5.5 log reduction) after 15 – 25 min of disinfection.

Following this, two sources of chlorine were studied: Granular calcium hypochlorite ($Ca(OCl)_2$) and liquid sodium hypochlorite (NaOCl) (6, 9 and 12 $mg.L^{-1}$ for 30, 60, 90 and 120 min) (in saline solution). Compared to environmental *E. coli* strains (M53, F11.2, MJ56 and MJ58), the ATCC *E. coli* (25922 and 35218) strains were always more susceptible to chlorine. After NaOCl treatment (12 $mg.L^{-1}$, 120 min), ATCC 25922 was totally inactivated compared to MJ58 which showed a reduction of 0.37 log only. The 3 log target reduction level was never reached by any of the environmental strains after chlorine (NaOCl) treatment at 6 – 12 $mg.L^{-1}$ (120 min contact time). The most resistant strain (*E. coli* MJ58) was inactivated (> 4 log reduction) in saline when a chlorine treatment of 24 $mg.L^{-1}$ (NaOCl) was applied (30 min contact time).

The impact of river water quality on chlorine (NaOCl) and PAA disinfection efficiency was also evaluated. Results indicated that the Plankenburg River is severely contaminated with *E. coli* levels exceeding the limit of 1 000 faecal coliforms per 100 mL. Subsequent chlorine (3.0 – 6.0 $mg.L^{-1}$, 120 min) and PAA disinfection (3.0 – 4.5 $mg.L^{-1}$, 25 min) resulted in *E. coli* levels being lowered to within these guidelines. Generally, chlorine disinfection resulted in higher log reductions (heterotrophic microorganisms, total coliforms and *E. coli*) compared to PAA disinfection. The effectiveness of PAA was impacted to a greater extent by water quality compared to chlorine. The microbiological and physico-chemical parameters of river water fluctuated to varying extents on different days. Chlorine was found to be a highly versatile disinfectant as it was efficient within the range of water quality parameters reported in this study.

Chlorine and PAA are considered potential disinfectants for the treatment of river water prior to irrigation. The quality of river water can differ between various river sources. Treatment efficacy should, therefore, be evaluated individually for each specific source of water as the effect water quality has on the chemical disinfection efficiency can vary greatly.

UITTREKSEL

Die inname van ingewandspatogene soos *Escherichia coli* (*E. coli*) kan lei tot ernstige voedselgedraagde siekteuitbrake in mense. Vorige navorsing rapporteer hoë vlakke van fekale kontaminasie in menigte Wes-Kaapse riviere wat hierdie bronne ongeskik maak vir besproeiingsdoeleindes. Dit beklemtoon die dringendheid vir geskikte behandelingsmetodes op plaasvlak om rivierwater te dekontamineer voor besproeiing. Chemiese behandeling is 'n populêre keuse vir algemene water dekontaminering. Die fokus van hierdie studie was daarom gerig op die potensiële toepassing van perasynsuur (PAA) en chloor vir die behandeling van besproeiingswater.

Die effektiwiteit van 'n opkomende behandelingsmiddel, perasynsuur, is aanvanklik bestudeer. Navorsing is gebasseer op die evaluasie van PAA behandeling op verwysingsisolate (ATCC) en omgewingsisolate van *E. coli* (in soutoplossing). Omgewingsisolate was meer weerstandbiedend teen PAA as die ATCC isolate (6 $mg.L^{-1}$ vir 5 en 15 min). Die variasie tussen *E. coli* isolate was veral duidelik by 'n kontaktyd van 5 min. *Escherichia coli* F11.2 (1.54 log reduksie) en ATCC 25922 (4.50 log reduksie) was onderskeidelik die mees weerstandbiedende en mees sensitiewe verwysingsisolate wat getoets is. Die effek van laer PAA dosisse (0.5, 1.5, 3.0, 4.5 en 6.0 $mg.L^{-1}$) en langer kontaktye (5, 15 en 25 min) is getoets teen die mees weerstandbiedende isolaat (*E. coli* F11.2). Daar is waargeneem dat konsentrasies wat wissel tussen 0.5 – 3.0 $mg.L^{-1}$ oneffektief was (< 1.5 log reduksie) in die vermindering van *E. coli* oor 'n kontaktyd van 25 min en het ook nie die 3 log reduksieteiken bereik nie. Hoër PAA dosisse (4.5 – 6.0 $mg.L^{-1}$) het gelei tot verhoogde log reduksies (4.94 – 5.5 log reduksie) na 15 – 25 min van behandeling.

Na aanleiding hiervan is twee chloorbronne bestudeer: Granulêre kalsium hipochloriet ($Ca(OCl)_2$); en natrium hipochloriet (NaOCl) (6, 9 en 12 $mg.L^{-1}$ vir 30, 60, 90 en 120 min) (in soutoplossing). Die ATCC (25922 en 35218) isolate was altyd meer vatbaar vir chloor in vergelyking met omgewingsisolate (M53, F11.2, MJ56 en MJ58). Na NaOCl behandeling (12 $mg.L^{-1}$, 120 min) was ATCC 25922 totaal geïnaktiveer in vergelyking met MJ58 wat slegs 'n reduksie van 0.37 log getoon het. Die 3 log reduksieteiken is nooit bereik nie, selfs na 'n chloor (NaOCl) behandeling van 6 – 12 $mg.L^{-1}$ (120 min kontaktyd), vir enige van die omgewingsisolate nie. Die mees weerstandbiedende isolaat (*E. coli* MJ58) is geïnaktiveer (> 4 log reduksie) in soutoplossing nadat 'n behandeling van 24 $mg.L^{-1}$ (NaOCl) (30 min kontaktyd) toegepas is.

Die impak van rivierwaterkwaliteit op die behandelingsdoeltreffendheid van chloor (NaOCl) en PAA is ook geëvalueer. Resultate het getoon dat die Plankenburg Rivier ernstig besoedel is met *E. coli* vlakke bo die riglyn van 1 000 fekale kolivorms per 100 mL. Die daaropvolgende chloor (3.0 – 6.0 $mg.L^{-1}$, 120 min) en PAA behandelings (3.0 – 4.5 $mg.L^{-1}$, 25 min) het daartoe gelei dat *E. coli* vlakke verlaag was tot onder hierdie riglyn. Chloor behandeling het oor die algemeen gelei tot hoër log reduksies (heterotrofiese mikroorganismes, totale kolivorms en *E. coli*) in vergelyking met PAA behandeling. Die effektiwiteit van PAA is tot 'n groter mate beïnvloed deur die rivierwaterkwaliteit in vergelyking met die effektiwiteit van chloor. Die mikrobiologiese en fisies-chemiese parameters van rivierwater het varieer op verskillende dae. Daar was gevind dat chloor 'n hoogs veelsydige

behandeling is as gevolg van die doeltreffendheid by die reeks waterkwaliteitparameters berig in hierdie navorsing.

Beide chloor en PAA kan beskou word as potensiële behandelings metodes vir rivierwater voor besproeiing. Die kwaliteit van rivierwater kan verskil tussen verskeie rivierbronne. Die effek van waterkwaliteit op die chemiese behandelingseffektiwiteit kan dus varieer, en daarom moet die behandelingsdoeltreffendheid individueel geëvalueer word vir elke spesifieke waterbron.

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CONTENT

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

ABBREVIATIONS

APHA:	American Public Health Association
API:	Analytical Profile Index
ATCC:	American Type Culture Collection
$Ca(OCl)_2$:	Calcium hypochlorite
CDC:	Centres for Disease Control and Prevention
cfu:	colony forming units
COD:	Chemical oxygen demand
DBP:	Disinfection by-product
DWAF:	Department of Water Affairs and Forestry
E. coli:	*Escherichia coli*
H_2O_2	Hydrogen peroxide
HPC:	Heterotrophic Plate Count
HTH	High Test Hypochlorite
Hydroxyl radical:	•OH
L-EMB:	Levine's Eosin Methylene-Blue
NaOCl:	Sodium hypochlorite
NTU:	Nephelometric Turbidity Units
O_3	Ozone
ORP	Oxidation Reduction Potential
PAA:	Peracetic acid
PCA:	Plate Count Agar
ROS	Reactive Oxygen Species
SANS:	South African National Standards
SSS:	Sterile Saline Solution
TC:	Total coliforms
TSS:	Total suspended solids
USEPA:	United States Environmental Protection Agency
UV:	Ultraviolet

UVT%:	Ultraviolet transmission percentage
VRBA:	Violet Bile Red Agar
WHO:	World Health Organisation

CHAPTER 1
INTRODUCTION

Global water supply has decreased dramatically as the earth undergoes annual water withdrawals of more than 6 800 km^3 (IUFoST, 2009). Agricultural activities such as irrigation account for 70% of these withdrawals. In South Africa, rivers are common sources of irrigation. Flowing through multiple areas, they also supply water for use in the mining, domestic and industrial sectors (DWAF, 2004). Strain is placed on water resources, as all sectors strive to maintain growth. Water is a scarce commodity in South Africa, therefore, water quality becomes critically important for agricultural irrigation (DEAT, 2011).

Various studies reveal the deteriorating quality of many South African rivers (Barnes & Taylor, 2004; Olaniran *et al*., 2009; Paulse *et al*., 2009). Research results have shown that some of these rivers are highly polluted with bacteria from faecal origin (Britz *et al*., 2012). Major causes of surface water pollution in South Africa, include, inadequate sewage treatment, illegal waste disposal and the effect of informal settlements (Britz *et al*., 2012). Irrigation with water of a poor quality, can lead to the transfer of pathogenic microorganisms to fresh produce items (Britz *et al*., 2013). Pathogens most frequently associated with fresh produce are *Escherichia coli* (*E. coli*), *Listeria monocytogenes*, *Salmonella* spp. and *Shigella* spp. (Lee *et al*., 2014). These pathogens pose serious food-safety risks and may lead to outbreaks of severe foodborne disease. (Masters *et al*., 2011).

It is cause for concern that studies of South African rivers report high *E. coli* levels (Paulse *et al*., 2009; Lötter, 2010; Ijabadeniyi *et al*., 2011; Gemmell & Schmidt, 2012; Huisamen, 2012), which exceed the irrigation guidelines of ≤1 000 faecal coliforms per 100 mL (WHO, 1989; DWAF, 1996). Lamprecht *et al.* (2014) detected *E. coli* levels of up to 1 000 000 MPN (most probable number) per 100 mL in the Plankenburg River (in the Western Cape), which passes through the industrial and informal settlements of Stellenbosch. As this river is used frequently for irrigation, the possibility of *E. coli* transfer to fresh produce is probable. *Escherichia coli* can cause serious diseases in humans such as haemolytic uremic syndrome (HUS), neonatal meningitis, and urinary tract infections (Masters *et al.*, 2011; Todar, 2012). *Escherichia coli* is used as an indicator of faecal contamination in water resources and is often referred to in water quality guidelines (Campos, 2008).

For these reasons, the introduction of disinfection methods for contaminated water is wide-spread and had become a priority. The process of water disinfection includes chemical, physical and photochemical methods. Chemical disinfection is based solely on the oxidation potential of the chemical itself, and determines the extent of damage towards the cell walls of microorganisms (Randtke, 2010).

Chlorine is the most widely-used disinfectant and its use in water disinfection dates back to 1902 (Schoenen, 2002). The outer-membrane is the sole target of chlorine disinfection in microorganisms. As chlorine reacts with the membrane, it increases the permeability of the layer

causing cell lysis and leads to microbial death (Bitton, 2011). Sodium hypochlorite, available in liquid form, is the most common form of chlorine (Lewis, 2010) and is used for the removal of bacteria, viruses and protozoa (Lazarova & Bahri, 2005). Chlorine also exists in powder form, calcium hypochlorite, providing 65 – 70% ($m.v^{-1}$) available chlorine compared to commercial solutions that provides 12 – 15 % ($m.v^{-1}$) available (Lewis, 2010). Hypochlorites are considered as safer alternatives to chlorine gas for water disinfection (Lewis, 2010). Various studies report on the effectiveness of chlorine on microbial inactivation. Winward *et al*. (2008) and Li *et al.* (2013) reported coliform reductions of 3.8 and 3.5 logs after water was treated with 10 $mg.L^{-1}$ and 0.2 – 3.0 $mg.L^{-1}$ sodium hypochlorite for a contact time of 30 min, respectively.

Chlorine is still a popular disinfectant, due to the low cost associated with its use and its ease of application (Van Haute *et al*., 2013). Chlorine leaves a residual, which prevents recontamination in water after disinfection (Voigt *et al*., 2013). However, the persistence of residuals after disinfection draws negative attention, as these levels may produce harmful disinfection by-products upon reaction with organic particles in water (Bouwer, 2002). The United States Environmental Protection Agency (USEPA, 2004) recommends residual chlorine for 'reclaimed intended for irrigation' of ≤ 1 $mg.L^{-1}$, to prevent the possible formation of by-products in water systems. As some of these products can be carcinogenic and mutagenic towards human beings (Crebelli *et al*., 2005; Sayyah & Mohamed, 2014), the use of chlorine as a fresh produce sanitiser is prohibited in European countries such as Germany, Switzerland, the Netherlands, Denmark and Belgium (Van Haute *et al*., 2013).

Recently, peracetic acid (PAA), an alternative to chlorine, emerged within the wastewater disinfection industry in the late 1980s (Baldry & French, 1989). It is highly effective towards bacteria at low concentrations and for short contact times (Kitis, 2004). The oxidation capability of PAA is higher than that of chlorine and other disinfectants such as hydrogen peroxide and bromine. Research by Koivunen & Heinonen-Tanski (2005) stated that 2 – 7 $mg.L^{-1}$ PAA for a duration of 27 min reduced total coliforms by 3 logs in secondary wastewater. Similar findings by Antonelli *et al*. (2013), reported an *E. coli* reduction of between 4.5 and 5.5 logs, after secondary wastewater was treated with 15 $mg.L^{-1}$ PAA for 38 min. Unlike chlorine, PAA produces little to no by-products, as it decomposes into biodegradable products (Crebelli *et al*., 2005), such as acetic acid and oxygen upon its reaction with water. Thus, water treatment with PAA is often the preferred method for fresh produce farmers. The only disadvantage of PAA is the higher cost involved, when compared to chlorine.

Thus, several factors influence the rate of chlorine and PAA disinfection towards microorganisms. Water quality plays a crucial role during the chemical disinfection of water. Physico-chemical water characteristics such as pH, temperature, chemical oxygen demand (COD), and total suspended solids (TSS) can have a negative influence on the disinfection efficiency of chlorine and PAA (Gehr *et al*., 2003; Koivunen & Heinonen-Tanski, 2005; Zanetti *et al*., 2007; Ayyildiz *et al*., 2009). Together with the microbial load in river water, the COD also exerts a chlorine

and PAA demand in river water, and consequently lowers the available concentration for microbial disinfection.

The unique microbial character of the river plays a significant role during disinfection. The heterogenic population of river water can show various levels of susceptibility toward various chemical disinfectants (Giddey *et al*., 2015). Many studies make use of reference strains during inactivation studies; however, their inactivation kinetics may differ from those of environmental strains (Wojcicka *et al*., 2007) naturally present in the river water systems. Research by Mazzola *et al*. (2006) found that reference strains were more sensitive to chemical disinfection than environmental strains that were isolated from a water purification system. This emphasises the importance of investigating the resistance of environmental strains to chemical disinfectants such as chlorine and PAA, in order to determine the optimum concentrations and contact times needed for adequate water disinfection prior to irrigation.

The development of cost effective methods to treat water prior to crop irrigation, is needed. The most suitable chemical treatment option for contaminated irrigation water is unknown. The overall aim of this study was thus to identify a suitable and effective PAA and chlorine treatment option for contaminated river water, thereby producing water that is safe for the irrigation of fresh produce items. A comparative study between PAA and chlorine was thus conducted against reference *E. coli* as well as environmental *E. coli* strains at laboratory-scale, in order to determine the most resistant strain. The optimum concentration and contact time recommended for river water disinfection was evaluated against the most resistant strain. In addition, the influence of river water quality on chemical disinfection was included in this study.

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CHAPTER 2
LITERATURE REVIEW

A. BACKGROUND

"When the well is dry, we know the worth of water," Benjamin Franklin said.

Water benefits the population in multiple ways. It sustains families; it irrigates fields of commercial farmers; water supports the crops and livestock of rural communities, and also contributes to hydro-electric power for the mining and industrial sectors. In addition, water nourishes the entire ecosystem (DWAF, 2004). This pure, simple molecule is essential to maintain life (DWAF, 2004) and forms part of every person's daily activities.

The Earth's total water supply is estimated at 1385.92 million km^3 per year, of which 96% is oceanic saline water (Anon., 2014a). The remaining supplies are subdivided into freshwater resources (2.5%) (FAO, 2013) such as surface water (rivers, lakes and dams), that is mainly utilised for drinking purposes and crop irrigation (Anon., 2014a), and ground water. The planet experiences an annual water withdrawal of more than 6 800 km^3 (IUFoST, 2009), of which 70% is used for agriculture, 20% within industry and 10% for domestic purposes (FAO, 2013). Global water demand is driven by two main water users: agriculture and human use (IUFoST, 2009). Estimates made on future water supply predict a dramatic decrease, since research proposes a population of 9 billion people by 2050 (UN, 2005). In 2013, the United States Census Bureau (2013) estimated a global population of 7.17 billion. In addition to the steady growth in population, people have more money, so their demands regarding the type of food they consume, become more specific. Therefore, global food demand is expected to rise markedly (UN-Water, 2013), because people are likely to eat more meat, fish, dairy and sugar, all of which use more water for production than grain-derived food products (IUFoST, 2009; De Fraiture & Wichelns, 2010). That water is required for the production of food, remains an undisputed fact. However, the demand for water for non-agricultural purposes (i.e. for industrial and urban uses) results in rising pressures being placed on water that would ordinarily be used for irrigation in the agricultural arena (Hanjra & Qureshi, 2010). Hence, the global water demand is higher than the global water supply, causing this valuable resource to become very scarce.

Three billion people will be living in water-scarce countries by 2025 (Hanjra & Qureshi, 2010) which makes the demand for water highly competitive. The world faces many challenges such as ecosystem degradation, urbanisation driven by poverty, climate change, and hunger (Hanjra & Qureshi, 2010). The poorest of the poor, who usually inhabit rural, informal settlements, are adversely affected by these difficulties. One out of every nine people on earth has access to improved drinking water and one in three people does not have access to proper sanitation. Only 47% of the population living in rural areas has access to sanitation facilities and 3.5 million people die annually due to inadequate water supply, lack of sanitation and poor water quality (UN-Water,

2013). Collectively, these conditions will contribute to an increased demand for municipal and industrial water of a good quality, thereby increasing the need for good-quality irrigation water. However, irrigation is the first sector to lose out on a supply of good-quality water, as it requires large quantity of water (Falkenmark & Molden, 2008).

Sources responsible for poor water quality, include carry-over from human settlements, and water-overflow from industrial and agricultural activities (UN-Water, 2013). Effluent from industrial resources are discarded into near-by rivers and groundwater resources, thereby contaminating the water and posing a significant risk to food safety (Huisamen, 2012). Due to the limited availability of water, the use of wastewater for irrigation in urban and peri-urban regions of developing countries is inevitable (Norton-Brandão *et al.*, 2013). South Africa is a semi-arid area where water scarcity is a reality (Norton-Brandão *et al.*, 2013) and therefore, treatment of wastewater is no longer an option (Gemmell & Schmidt, 2012) but a necessity. Studies reveal that within the last decade, the quality of South African river water has decreased notably (Paulse *et al*., 2009; Ackermann, 2010; Ijabadeniyi, 2010; Lötter, 2010; Kikine, 2011, Gemmell & Schmidt, 2012; Huisamen, 2012). As a result of the increased population growth, people move to the cities for better opportunities and to raise their standard of living. About 58% of the South African population lives in urban areas, and 11.5% in rural areas where basic water services are very scarce (DEAT, 2006). Usually people in rural areas do not have access to clean water and sanitation facilities and are forced to use the nearest river water for their daily needs (Obi *et al*., 2002; Barnes & Taylor, 2004; Gemmell & Schmidt, 2012,).

Presently, farmers are forced to use untreated river water for crop irrigation due to treated water shortages (Gemmell & Schmidt, 2012). Of all food categories, fresh produce is the main recipient of poor-quality irrigation water. The promotion of a healthier lifestyle has led to a marked increase in the consumption of fresh-cut fruit and vegetables (Lee *et al*., 2014). Consequently, the increased consumption of fresh produce is linked to more outbreaks of foodborne diseases, due to faecal contamination of rivers caused by humans and animals (Kikine, 2011). Raw produce irrigated with untreated river water carries a great risk of pathogenic contamination (Pachepsky *et al*., 2011). Pathogenic microorganisms affecting fresh fruit and vegetables, include bacteria (such as enterohemorrhagic *Escherichia coli (E. coli)*, *Campylobacter* spp., *Staphylococcus aureus*, *Listeria monocytogenes*, enterotoxogenic *Bacillus cereus*, *Shigella* spp., *Salmonella* spp., protozoa *Cryptosporidium* spp., *Yersinia enterocolitica*, *Entamoeba histolytica*, *Giardia* spp.) and viruses, in particular rotaviruses, adenoviruses, enteroviruses and noroviruses (Pachepsky *et al*., 2011). Thus, within the South African context, research highlights the unsuitability of river water for the irrigation of fresh fruits and vegetables (Olaniran *et al*., 2009; Paulse *et al*., 2009; Kikine, 2011).

B. FRESHWATER SITUATION IN SOUTH AFRICA

In South Africa, the poverty-stricken are the most adversely affected by the scarcity of water. Even when water is in abundance, the poor still lack water due to insufficient infrastructure that is required

to bring water to where it is needed. In fact, South Africa has enough water to meet future demands provided that water is used sparingly and measures are taken to reduce and avoid pollution (DWAF, 2004).

The amount of water available in South Africa is largely dependent on rainfall and evaporation rates. The world's average rainfall of 860 millimetres per year is almost double that of South Africa's average rainfall of 450 millimetres per annum (DEAT, 2006). However, the country has an annual water supply potential of over 1 100 cubic meters per annum. Freshwater resources are obtained from surface water (77%), groundwater (9%) and return flows (14%) that include effluent and sewage purification waters (DWAF, 2009). South Africa does not have any particularly large rivers, as many of them (such as the Orange, Pongola, Limpopo and Inkomati Rivers) are shared with neighbouring countries (DWAF, 2004). Added to this, South Africa's 320 major dams, each supply over a million cubic meters, and yield a total supply capacity of 32 400 million cubic meters. Groundwater, another source of freshwater, is used particularly in rural and arid regions, where surface water is in short supply. Currently, 10% of the country's water is obtained from groundwater (DEA, 2013); however, its availability is severely limited, because of the hard rock in the underlying surfaces.

Climate change also impacts significantly on the availability of water in South Africa. It is estimated that in the Western Cape, caused by elevated temperatures and fluctuations thereof, will increase the demand for irrigation needed for crop production (DEA, 2013).

Current and future water requirements

An accurate understanding of water use requirements is essential for managing water resources wisely. Water requirements are divided into sectors according to individual needs in terms of quantity, quality, supply and distribution (DEAT, 2006). Of all the water use in this country, irrigation dominates by far. Water is also used in the urban, rural, mining and bulk industrial, power generation and afforestation sectors (DWAF, 2004). Table 1 shows the division of water requirements and percentages for every sector in South Africa for the year 2008. Quantities are standardised at 98%.

Table 1 Total South African water requirements as of 2008 (DWAF, 2009)

	Total for country (m^3/a)	**Calculated percentage**
Agricultural irrigation	7 920	62%
Urban	2 897	23%
Rural	574	4%
Mining and bulk industrial	755	6%
Power generation	297	2%
Afforestation	428	3%
Total requirement	12 871	-

Table 1 shows that irrigation dominates by far and accounts for about 62% of the South Africa's total water requirement while the urban sector requires a significant 23% of water requirements.

The percentage of return flow for sectors is listed in ascending order as follows: rural users (0%), irrigation (9%), urban (33%), and mining/bulk (34%). Thus, only non-consumptive water can be made available for re-use. In the interior regions of South Africa, non-consumptive water is re-used or flows into rivers and is then made available for re-use (DEAT, 2006). In urban and industrial regions like Johannesburg and Pretoria, 50% of the total water requirement is converted into return flow and made available for re-use. However, in coastal cities, like Durban and Cape Town, only between 5 and 15% of required water is re-used. Re-use is strongly recommended, since return flow is a substantial source of water (DEAT, 2006). The quality of return flow is important, especially in the way treatment techniques are applied to ensure safe water. However, water use in irrigation, power generation and within rural areas is mainly consumptive (DEAT, 2006); therefore, little water is available for re-use.

The nature of the economy, living standards and climate change (industrialisation and irrigated agriculture) all influence future water requirements of South Africa (DWAF, 2004). With economic and population growth as the main drivers impacting future water requirements, increases in water requirements are expected to occur more in urban and industrialised areas, than in rural regions (DEAT, 2006). More water requirements are expected to arise in the economically more-favourable urban areas. Strong growth is predicted for the mining sector, with an increase in water demand in the northern region for mineral exploitation (DEAT, 2006).

Imbalances between availability and demand and the degradation of surface and groundwater are often experienced in water scarce regions. Therefore, effective water management in irrigation is required, since the agricultural sector has the highest demand for water, especially in water-scarce areas (Pereira *et al*., 2007).

C. THE AGRICULTURAL SECTOR IN SOUTH AFRICA

South Africa's dual agricultural economy is rooted in subsistence farming in rural regions and developed commercial farming (DAFF, 2012). South Africa is sub-divided into seven climatic regions (DAFF, 2012) thus enabling it to produce a wide range of agricultural products, which include vegetables, grapes, citrus fruit, subtropical fruit, flowers, wool, livestock and game. The fruit sector dominates, contributing 12% of the total earning from agricultural exports (DAFF, 2012). The Western Cape, Eastern Cape and the Langkloof Valley are the main areas where deciduous fruits are grown. Citrus fruit is grown in the irrigation areas of aforementioned regions and pineapples are produced predominantly in northern Kwazulu-Natal and the Eastern Cape (Anon., 2008). Moreover, South Africa is the ninth largest wine exporter in the world, with over 110 000 hectares and 300

million vines being cultivated. With regards to vegetable supply, potato is the leading crop, comprising 50% of the vegetables delivered to fresh produce markets (Anon., 2008).

Twelve percent of South Africa's surface land is used for crop production, while only 22% of this has a high potential for farming. Despite the irregular and unevenly distributed rainfall, 60% of the water is used for agricultural purposes (DWAF, 2004). During winter and high summer rainfall seasons, agricultural activities range from intensive crop production and mixed farming to cattle and sheep farming in arid areas (DAFF, 2012). Not only has South Africa, the ability to be self-sufficient in nearly all agricultural products, but is also a net food exporter (DAFF, 2012). South Africa exports products such as wine, apples, pears, sugar, quinces and grapes and is also the leading exporter of fruits and vegetables to other African countries. The European Union (EU) has an imported market share of 31% for fruits and vegetables, whereas South Africa is the greatest third-world contributor. Except for sub-Saharan African countries, South Africa, Kenya and Cote d'Ivoire account for 90% of international exports with South Africa dominating the field (Ndiame & Jaffee, 2005).

In 2010/11, the agricultural sector yielded R138 904 million compared to R129 833 million from the previous year. The increased value of field crops yielded this growth. Primary agriculture is a vital sector in the South African economy, however, it comprises a small share of the GDP (gross domestic product) (DAFF, 2011). It has increased by 11.8% per year since 1970, an annual growth of 14.9% in the South African economy. The agricultural share in the GDP has declined from 7.1% (in 1970) to 2.5% (in 2010) and from 31 July 2010 to 30 June 2011 an income of R 131 699 million was estimated from all agricultural products (DAFF, 2011). The gross income from horticultural production increased by 23.5% from December 2010 to September 2013 (DAFF, 2013).

Furthermore, the Western Cape is the fastest developing province in the South African agricultural sector (WESGRO, 2006). It accounts for 55 - 60% of South Africa's total agricultural production and it also owns 40% of the country's export market share (WESGRO, 2012). The fruit and vegetable industries are the main drivers of the economy in the Western Cape. Its 8 500 commercial farms and 2 500 newly-settled farms provide employment to 220 000 farm workers (Britz *et al*., 2012). Thus, agricultural activities in the Western Cape contribute significantly towards the South African economy.

The role of irrigation water for agricultural use

Irrigated agriculture (62%) is the largest consumer of water in South Africa (DEAT, 2006). The importance of irrigation water cannot be underestimated, since the country lies within the arid and semi-arid agro-climatologic zone (FAO, 2005). This agro-climatologic zone places a major restriction on the agriculture of South Africa, since the available land is more suitable for livestock farming than crop production. About 1.498 million hectares (ha) of the country's land is utilised and more than 1.3 million ha are irrigated. Irrigation is mostly applied to fodder crops, sugar cane, vegetables, wheat and pulses. For local and export purposes, 25 – 30% of South Africa's crops are produced from irrigated land (Britz *et al.*, 2012). The Western Cape dominates the South African economy

and Table 2 shows that the province utilises the most hectares for commercial irrigation by far, when compared to the other eight provinces (FAO, 2005).

Table 2 Distribution of commercially irrigated area in South Africa per province (FAO, 2005)

Province	**Permanent commercial irrigation (ha)**
Eastern Cape	11 070
Free State	46
Gauteng	18
Kwazulu-Natal	2 747
Mpumalanga	18 498
North West	706
Northern Cape	34 759
Limpopo	58 704
Western Cape	290 204
Total	**416 753**

The economic link between irrigation farming and mainstream agriculture and their impact (directly and indirectly) on the South African economy, are not valued enough (Britz *et al.*, 2012). Irrigated agriculture experiences the same forward and backward economic relations as normal agriculture. Irrigation water utilisation for commercial production of fruits and vegetables has a vital impact on South Africa's economy as it generates foreign exchange. Negative fluctuations in this sector could have a negative impact on employment sustainability, South Africa's trading status and other industries (Lötter, 2010). Also, irrigation has a great impact on food supply, therefore a balance between water supply and demand is essential.

Irrigation is applied in various ways. The soil type, economics, the depth of the water table, costs involved, the slope, and cropping rotations are all determinants of the irrigation methods used. Internationally, three main irrigation methods are applied: Surface irrigation (55 - 65%), mechanised and non-mechanised sprinkler systems (75 - 85%), and localised irrigation (85 - 95%) (FAO, 2005). All rainfall regions are irrigated permanently throughout South Africa. Flood irrigation (32%), sprinkler (54%) and micro-irrigation (12%) are methods commonly used by South African farmers. Some of these methods are also utilised by subsistence and small-scale farmers, who are also familiar with more innovative variations, such as short-furrow irrigation (Britz *et al.*, 2012). Since much water is utilised for irrigation, contamination could arise. Therefore, water quality and safety are the most crucial measures and should be maintained throughout to ensure food that is safe for human onsumption.

D. MICROBIAL INDICATORS OF WATER QUALITY

Indicator and index organisms

Concerns associated with water quality have increased due to regular contamination by waterborne bacteria, protozoan and viral pathogens. A significant number of pathogenic microorganisms can be found anywhere on earth, thereby making it impossible to identify and determine the exact amount of each type of these pathogens (Savichtcheva & Okabe, 2006). This procedure can become labour intensive, therefore microbiological analyses on water quality are based on the identification of microbial indicators. The term 'microbial indicator' is categorised into three groups (Odonkor & Ampofo, 2013):

- general (process) microbial indicators;
- faecal indicators (*E. coli*); and
- index and model microorganisms.

There are distinct differences between the terms 'index' and 'indicator' microorganisms. Index microorganisms are defined as markers that exceed the numerical limits, indicating the possible presence and behaviour of ecologically similar pathogens (WHO, 2001; Busta *et al*., 2006; FDA, 2013; Odonkor & Ampofo, 2013). For instance, *E. coli* is an index for *Salmonella* and F-RNA coliphages, modelling the presence of human enteric viruses (Odonkor & Ampofo, 2013). These organisms have the ability to provide vital information about other pathogens, as their behaviour correlates to other accompanying pathogens (WHO, 2001; Busta, *et al*., 2006).

On the other hand, indicator organisms show the type of contamination that occur. For example, coliforms and *E. coli* are thermotolerant bacterial groups that indicate the presence of faecal matter. Indicator organisms can be characterised as non-pathogenic, low risk microorganisms, indicating that food or water may be contaminated or occur in an environment where growth of pathogens is favourable (Savichtcheva & Okabe, 2006). If indicators are absent, or only present at lower concentration, it means the food or water do not pose any potential threats of contamination and the source is also not exposed to ideal conditions for the growth of pathogens (Busta *et al.*, 2006). These microorganisms strongly correlate to the presence of pathogens and also have similar survival profiles to the pathogens whose presence they confirm (Field & Samadpour, 2007). Cultivation and enumeration of indicator bacteria should be relatively easy and safe under laboratory conditions (Savichtcheva & Okabe, 2006). However, they do not indicate the amount or presence of specific pathogens, but are mainly used to confirm possible contamination (Pachepsky *et al*., 2011), for example faecal contamination.

Indicators of faecal origin confirm faecal pollution and the possible presence of enteric pathogens. Indicator organisms used in water are: total and faecal coliforms, faecal enterococcus, and *Clostridium perfringens.* Internationally, of the coliform group, *E. coli* is regarded as the main recognised indicator of water quality (Field & Samadpour, 2007; Cahoon & Song 2009; Health

Canada, 2012; Odonkor & Ampofo, 2013). The World Health Organization (WHO) and the South African National Department of Water Affairs set guidelines and limits to 1 000 faecal coliforms per 100 mL of water used to irrigate fresh crops (WHO, 1989; DWAF, 1996; DWA, 2013b) (Table 3).

Table 3 International and South African guidelines for indicators present in irrigation water intended for crops and produce eaten raw (DWAF, 1996; Monaghan & Hutchison, 2010; Gemmell & Schmidt, 2013)

International body	Indicator organism	Criteria limits
World Health Organization (WHO) *Unrestricted irrigation*	Faecal coliforms	≤ 1000 cfu per 100 mL
Department of Water Affairs and Forestry (DWAF) (RSA) *Irrigation water guidelines*	Faecal coliforms	≤ 1000 cfu per 100 mL
United Stated Government (USA) *Irrigation of foods consumed raw*	Total coliforms	< 2.2 total coliforms.100 mL
Canadian Council of Ministers of the Environment (CCME) *Irrigation water applied to uncooked vegetables*	Faecal coliforms of *E. coli* and also total coliforms	≤ 100 cfu of faecal coliforms or *E. coli* per 100 mL ≤ 1 000 cfu of total coliforms per 100 mL

cfu – colony forming units

This limit refers especially to irrigation water used on crops eaten raw or minimally processed crops (Table 3). At this limit, the transmission of diseases starts rising and also places farm workers and food handlers at risk of exposure to foodborne diseases (DWAF, 1996; DWA, 2013b). South African standards are more negligent compared to standards from other countries (Britz *et al.*, 2012). Differences in guidelines are a reflection of the country's economic state together with the unawareness of the risk exposed by water contaminated with pathogenic microorganisms (Steele & Odumero, 2004). The guidelines of other countries should also be taken into consideration when wanting to have a share in the export market (Huisamen, 2012). In the past, total coliform bacteria were the main indicators of faecal pollution in water sources. However, this was later proved to be inaccurate as total coliform bacteria also occurred in non-faecal sources such as water and soil (Johannessen *et al.*, 2002). Therefore, *E. coli* is regarded as the most reliable bacterial indicator of faecal contamination of water. The bacterium is also an indication of the bacteriological hygiene in freshwater resources (Johannessen *et al.*, 2002). The detection of this microorganism has proved to be fast, sensitive, affordable and easy to perform (Health Canada, 2012; Odonkor & Ampofo, 2013).

Escherichia coli: a bacterial indicator of faecal contamination

The genus *Escherichia* is a group of gram negative, rod-shaped (length: 2 µm, volume: 0.5 µm), facultative anaerobes belonging the *Enterobacteriaceae* family (Fotadar *et al*., 2005; Todar, 2012).

Theodor Escherich was the first scientist to isolate *E. coli* in 1885 and it was initially known as *Bacterium coli*. Later it was renamed *Escherichia coli* (Todar, 2012). *Escherichia coli* belong to the coliform group and are natural inhabitants in the gut of warm-blooded animals, including humans (Ackermann, 2010; Odonkor & Ampofo, 2013). Their high survival rates within the human gut are because of the acidic (acidophiles: pH ranging from 3.3 to 4.2) and temperarate conditions (37°C) which favour their optimal growth (Fotadar *et al*., 2005; Todar, 2012). When *E. coli* occurs outside its natural habitat, its presence usually suggests the contamination of faecal coliforms in water, food items and processing facilities.

Although most of the *E. coli* strains present in the gut are harmless (non-pathogenic), some of them, such as enterohaemorrhagic *E. coli* O157:H7 (EHEC), may have a certain combination of virulence genes which enables them to cause serious diseases in humans (Vogt & Dippold, 2005) such as haemolytic colitis and bloody or non-bloody diarrhoea. Enteric *E. coli* are categorised into five serological groups according to their serological and virulence properties (Table 4) that cause intestinal diseases in humans. With these genes, they are particularly known to cause serious extra-intestinal infections such haemolytic uremic syndrome (HUS), neonatal meningitis and urinary tract infections in humans (Masters *et al.*, 2011; Todar, 2012). Serious cases of these diseases can prove fatal, especially in the elderly. New *E. coli* strains can develop through the natural biological process of mutation and develop traits that are harmful to future hosts (Odonkor & Amfoko, 2013). The presence of *E. coli* in water does not necessarily imply the presence of pathogenic microorganisms. It does, however, indicate an increased risk of the presence of other pathogenic faecal-borne microbes such as *Salmonella* spp. or hepatitis A virus. For this reason, *E. coli* is an indicator of unacceptable levels of faecal contamination in water (Odonkor & Ampofo, 2013).

Table 4 The five virotypes of *E. coli* which are known to cause intestinal diseases in humans and their target hosts (Todar, 2012)

Type	**Host**
Enterotoxigenic *E. coli* (ETEC)	Causes diarrhoea in humans, pigs, sheep, cattle, horses and dogs
Enteropathogenic *E. coli* (EPEC)	Causes diarrhoea in humans, rabbits, cats, dogs and horses
Enteroinvasive *E. coli* (EIEC)	Only found in humans
Entero Enteroaggregative *E. coli* (EAEC)	Found only in humans
Enterohemorrhagic *E. coli* (EHEC)	Found in humans, goats and cattle

Escherichia coli does not survive for very long periods in surface water or on plant surfaces, therefore its presence is associated with a recent contamination event. Furthermore, Maciorowski *et al.* (2007) mentioned *E. coli*'s general ability to survive when exposed to one environmental stress

and this indicates that *E. coli* has the ability to activate survival mechanisms under stress. They can survive in very acidic conditions as they are able to grow at a pH ranging from 3.3 to 4.2. *E. coli* are facultative anaerobes and there is a direct correlation of their growth to oxygen presence, as they logarithmically increase with oxygen present in the environment, indicating that they have a high chemical oxygen demand (Johnston *et al*., 2006).

E. coli O157:H7 is a fresh produce pathogen responsible for 34% of all *E. coli* outbreaks (Britz *et al*., 2012). None of the outbreaks occurred during preparation, but were traced back to increased levels of *E. coli* present in river systems (Britz *et al*., 2012). Faeces from livestock on agricultural lands wash into river systems after heavy rainfall (Monaghan & Hutchison, 2010) and may contain one or more virulence genes. Therefore, runoff from agricultural areas and sewers may result in the occurrence of pathogenic *E. coli* strains (Masters *et al.*, 2011). These microorganisms can also find their way into river systems when wastewater treatment plants overflow after heavy rainfall, thereby causing further contamination of surface water (Monaghan & Hutchison, 2010).

E. THE QUALITY OF RIVERS IN THE WESTERN CAPE AND THEIR POLLUTION

Water quality is defined as the physical, chemical and biological properties of water reflecting its suitability for various use (DWAF, 2014). Freshwater quantity and quality are major concerns facing South Africa and other countries. Of all the river ecosystems in South Africa, 60% are threatened and of these, 25% are critically endangered. Also, 65% of wetlands are threatened, of which 48% are critically endangered (DEAT, 2011).

Degradation of water quality increases with the growing water demand, the impact of extreme events, and climate change. Irrigated agriculture contributes to poor water quality, however, irrigation also requires good water quality (CSIR, 2010). South Africa faces water scarcity that is caused by many factors, including low rainfall and high evaporation rates, a growing economy and increasing population, all of which place pressure on the utilisation of natural resources. Therefore, water quality becomes a critical component of agricultural supplies, especially for irrigation purposes in water-scarce countries (DEAT, 2011).

Britz *et al.* (2012) mention the need for scientific solutions to the problems of contaminated water sources containing hazardous microbial organisms from human activities. Pandey (2006) reports that 80% of the world's diseases originate from contaminated surface water (rivers and dams), especially in developing countries. Waterborne diseases are the main result of poor water quality and can be prevented by adequate sanitation, water treatment and waste disposal (Britz *et al.*, 2012). This is a cause for concern, as South Africa sources 77% of its water from surface water (Kikine, 2011). There is a need for risk assessment posed by pollution to control the distribution and transport of pathogenic microorganisms into freshwater resources, thereby preventing potential disease outbreaks such as cholera, diarrhoea, skin infections, and dysentery (Kikine, 2011).

The common problem that arises, is the virtual impossibility to test for every potential hazardous organism. Therefore, indicator organisms are used to point out the possible presence of pathogenic organisms that have the ability to cause these diseases in humans. *Escherichia coli* is found in the gut of warm-blooded animals and belongs to the coliform group of bacteria and is specifically used to indicate faecal contamination in rivers (Britz *et al.*, 2012). If considerable amounts of *E. coli* are identified, presumptions can be made that such water is contaminated with faecal waste. At the same time, assumptions can be made for the presence of disease-causing microorganisms and/or pathogens. Exact safe limits are used to ensure safe use of water prior to irrigation (≤ 1000 faecal coliforms per 100 mL) (WHO, 1989; DWAF, 1996). Studies have reported that many of South Africa's rivers are not suitable for irrigation due to the high contamination levels of faecal coliforms (*E. coli*) (Barnes & Taylor, 2004; Germs *et al*., 2004; Olaniran *et al*., 2009; Paulse *et al*., 2009).

Barnes & Taylor (2004) investigated the Plankenburg river water quality in the Western Cape (Stellenbosch) and reported high pollution levels. This river flows through Stellenbosch and passes through the dense rural settlements of Kayamandi, which is situated on the banks of the river. During a four year study, the faecal coliform pollution of the Plankenburg river reached a high of 12 000 000 *E. coli* per 100 mL water. The allowable limit then, 2 000 faecal coliforms per 100 mL water (DWAF, 1996), was exceeded 95% of the time (Barnes & Taylor, 2004).

Another study done by Paulse and co-workers (2009) on the Plankenburg River from June 2004 to June 2005 tested the most probable number (MPN) and reported high counts for faecal coliforms and *E. coli* were 3 500 000 microorganisms per 100 mL water. Paulse *et al.* (2009) conducted a study on the Diep River in the Western Cape (Plumstead) from March 2005 to November 2005 and found the highest counts for both faecal coliforms and *E. coli* was 1 600 000 microorganisms per 100 mL of water. This sampling site was polluted with effluent waste from residential and industrial areas. Consequently, the results did not comply with stipulated regulations for most of the research period. An earlier study performed in the Boland region on the Berg River in the Western Cape (Paarl), found most probable numbers (MPN) for faecal coliforms of 35 000 000 microorganisms per 100 mL water of which 17 000 000 were identified as *E. coli* (Paulse *et al.*, 2007). Sampling was done in Mbekweni (Paarl) where effluent, human and household waste, flow into the river.

In addition, Ackermann (2010) conducted a study on the microbiological condition and water chemistry of the upper Berg and Plankenburg Rivers. Over a four month sampling period, Ackermann (2010) reported faecal coliform counts ranging from 540 to 1 700 000 colony forming units per 100 mL (cfu.100 mL^{-1}). Counts from the Plankenburg River ranged from 490 to 160 000 cfu.100 mL^{-1}. According to the Health Canada regulations (2002), faecal coliforms are directly related to *E. coli;* therefore, faecal coliform counts can also be taken as the load of *E. coli* present in the water. Ackermann (2010) mentioned that the state of these two rivers was found to be unacceptable, most of the time, for water intended for human consumption and irrigation of crops.

The microbial loads in the Mosselbank River were investigated by Lötter in 2010. The Mosselbank River is situated North West of the Kraaifontein sewage works, about one kilometre downstream from the treated effluent discharge area (Lötter, 2010). This river is frequently used for irrigation. However, Lötter (2010) detected faecal coliform counts as high as 160 000 microorganisms per 100 mL water. Thereafter, Kikine (2011) assessed the microbial quality of the Plankenburg and Eerste Rivers and reported *E. coli* loads of 1 400 000 cfu.100 mL^{-1} and 79 000 cfu.100 mL^{-1}, respectively. The Eerste River is located upstream and eventually merges with the Plankenburg River, therefore lower *E. coli* counts were expected. Huisamen (2012) also investigated the microbial contamination of the Plankenburg and Eerste Rivers and found counts as high as 7 000 000 cfu.100 mL^{-1} for both faecal coliforms and *E. coli.*

From previous investigations (Barnes & Taylor, 2004; Paulse *et al*., 2007; Paulse *et al*., 2009; Ackermann, 2010; Lötter, 2010; Kikine, 2011; Huisamen, 2012) , it can be concluded that results did not comply with the South African water quality guidelines for irrigation water in most of the cases. These results regularly exceeded the allowable limit set by the DWAF (1996) and WHO (1989) of ≤ 1 000 faecal coliforms per 100 mL water used. The condition of South African (Western Cape) rivers is unacceptable and can be attributed to the failing infrastructure needed to treat municipal wastewater and effluents from informal settlements, and consequently risks are posed to both the consumer and the agricultural industry (Kikine, 2011).

F. OUTBREAKS ASSOCIATED WITH CONTAMINATED IRRIGATION WATER AND FRESH PRODUCE ITEMS

Escherichia coli O157:H7 and *Salmonella* spp are pathogens that are most often associated with foodborne diseases from fruit and vegetables (CDC, 2014). Major pathogenic strains like *E. coli* O157:H7 have been identified as causing foodborne outbreaks and dominate world literature on EHEC (Müller *et al*., 2001). Transmission of this strain occurs via contaminated foods, humans, contact with animal faeces, and through the consumption of fruits and vegetables irrigated with water contaminated with *E. coli* O157:H7 (Abong'o *et al.*, 2007; CADE, 2011). *Escherichia coli* O157:H7 has a lower infectious dose than other pathogenic *E. coli* strains and could cause infections of between 2 and 2 000 cells. This is because these bacteria are acidophiles (pH growth range of 3.3 - 4.2) that can withstand the gastric acid of the human stomach (Ackermann, 2010). Pathogenic *E. coli* strains pose a great risk to humans consuming contaminated fruits and vegetables. Numerous outbreaks of enterohemorrhagic O157:H7 illnesses due to consumption of mixed vegetables, salad mixes, lettuce, cilantro, coriander and celery have been reported (Johnston *et al.*, 2006; Lynch *et al.*, 2009; Ijabadeniyi, 2010). Therefore, the contamination of rivers with pathogenic *E. coli* strains has led to increased numbers of disease outbreaks and consequent deaths around the world (Masters *et al.*, 2011).

Many reported outbreaks caused by the transmission of foodborne pathogens are from animal origin. However, recent studies show fruit and vegetables to be significant sources of these disease outbreaks (Berger *et al*., 2010; Pachepsky *et al.*, 2011). This is due to the increased consumption of fresh produce (Berger *et al*., 2010; Jung *et al*., 2014) since world-over, the people have become more health conscious. A total of 48 million people in USA get sick from foodborne illnesses, 128 000 are hospitalised and 3 000 die annually (CDC, 2011). Painter *et al*. (2013) stated that of all foodborne diseases reported in the USA, for the period 1998 to 2008, 46% were related to fresh produce consumption.

Developed countries such as USA and Europe may have higher reported cases of foodborne outbreaks than developing countries, like South Africa, as these third-world countries lack sufficient surveillance systems and updated data on recent foodborne disease outbreaks (Lynch *et al*., 2009). South Africa is a country where foodborne illnesses are likely to occur. However, there is very little literature available to substantiate the existence of these foodborne diseases.

History's largest outbreak on fresh produce, traces back to 1996 in Japan, and led to more than 6 000 *E. coli* cases on sprout consumption. Sprouts were prepared in central kitchens where the pathogen was transmitted and thousands of people, mostly children, became ill and at least 12 people died. Sprout related diseases are still reported, all over the world, in countries such as the United Kingdom, Finland, Denmark, Sweden and Canada (Buck *et al.*, 2003).

In December 2006, 216 South Africans were hospitalised in Kwazulu-Natal with symptoms of gastroenteritis caused by *Salmonella* (Table 5). A student ate a meal at a local primary school which contained beef, stew, rice, coleslaw, pumpkin, chakalaka, fruit juice, pineapple, tomatoes, kidney bean salad, beetroot and chicken. The meal contained many fresh ingredients and one specific food vehicle could not be identified (Niehaus *et al.*, 2011).

Greene *et al.* (2008) examined a *Salmonella* outbreak on tomatoes in the USA (Table 5) and found that the isolated strain was from pond water used to irrigate tomatoes. This outbreak caused illness to 1 300 people. In an *E. coli* O157:H7 outbreak in Sweden (Table 5), contaminated lettuce was found to be irrigated from a small stream of water. The same strain that was identified in infected humans, was also found in cattle located upstream from the irrigation point (Söderström *et al*., 2008). In another lettuce outbreak of *E. coli* O157:H7, investigators found that well-water, generally used for irrigation, was accidentally mixed with water from a manure-infested lagoon (Pachepsky *et al.*, 2011).

Germany and France experienced a massive outbreak on *E. coli* O104:H4 in 2011 (Table 5). Over 4 000 people became ill and 50 people died (Griffith, 2011; Hyde, 2011). Consequently, more than a 1 000 cases of HUS were reported and fenugreek seeds were identified as the cause of the outbreak. Contamination occurred during sprout production and this was one of the most severe outbreaks linked to fresh produce (Griffith, 2011; Hyde, 2011).

In 2012, USA experienced an *E. coli* O157:H7 outbreak related to spinach and spring mix whereby 33 persons were infected. Forty-six percent of them were hospitalised and no deaths were

reported. In the same year, another *E. coli* outbreak on lettuce was reported in 10 states of America; however, distribution of the product ceased before it reached the retail stores. During this time people were advised not to consume any fresh produce items so that the risk of disease outbreaks (CDC, 2014) could be limited.

Table 5 Reported foodborne illnesses associated with fresh produce items from 2006 to 2014

Food item	Year	Pathogen	Country	Reported cases
Sprouts	1996	*E. coli*	Japan	12 deaths
Fresh produce	2006	*Salmonella*	South Africa	216 hospitalised
Tomatoes	2008	*Salmonella*	USA	1300 illnesses
Lettuce	2008	*E. coli* O157:H7	Sweden	-
Fenugreek seeds	2011	*E. coli* O104:H4	Germany and France	4000 illnesses, 50 deaths
Spinach and spring mix	2012	*E. coli* O157:H7	USA	33 illnesses
Raw clover sprouts	2014	*E. coli* O121	USA	-

A common water pathogen, cholera, is commonly associated with drinking water and from 2011 to the end of March 2012 the Democratic Republic of Congo (DRC) reported 8 000 cases of cholera with 120 deaths. Cholera is an intestinal infection caused within humans, as a result of contact with contaminated food and water (DeCapua, 2012).

The main causes of water pollution in South Africa

The main causes of surface water pollution and degradation in South Africa are urbanisation, deforestation, damming of rivers, destruction of wetlands, industries, mining, agriculture and energy use. The resulting effects caused a dramatic decrease in freshwater quality in the past few years (Rietveld *et al*., 2009).

Urbanisation is the result of more people moving into cities. Pollution is caused by physical disturbance of land (as new houses are constructed), poor sewage management system, and the increased use of fertilisers, due to an increased demand for food (Rand Water, 2014). Urban areas are mainly located on river banks and rivers flowing past these areas transport waste material. Fresh water resources (dams and rivers) located downstream of metropolitan areas have become critically contaminated during the last few years (Oberholster & Ashton, 2008). The sewage from big cities and towns is also a major problem, due to the failing sewage disposal systems leading to large amounts of sewage being discharged into rivers. Usually the sewage is from inadequate sanitation in low-income areas, poor maintenance of sewage reticulation systems or insufficient wastewater treatment infrastructure (Oberholster & Ashton, 2008; Britz *et al.*, 2012).

Industries are also a big concern because the production of waste-containing chemicals, could change the pH, colour and the amount of nutrients in the water (Rand Water, 2014). These waste products are sometimes discharged directly into the nearest rivers, wetlands and sewers (CSIR, 2010). The production of waste could also result in temperature fluctuations that may favour the possible growth of microorganisms (Rand Water, 2014). This has a significant impact on the urban, industrial and agricultural water users.

Agriculture utilises the most water of all the economic sectors in South Africa, but also contributes to decreasing water quality. Farming causes soil erosion through physical disturbance of soil during overgrazing, road building and even ploughing. This affects the amount of salts and minerals in the water (Rand Water, 2014). The use of fertilisers increases the amount of nutrients present in the soil and leads to excessive quantities of nitrites and phosphates in the water which ultimately causes eutrophication (CSIR, 2010; Rand Water, 2014)

People living in informal settlements near rivers, also contribute to elevated contamination levels (Rand Water, 2014). During the past 20 years, the number of un-serviced informal settlements has increased. The proportion of South Africans (population: 51.77 million people) living in rural areas in 2011 can be categorised as follows (DWA, 2013a):

- 6% of the population (3.10 million people) lives in small towns situated in rural areas and
- 35% of the population (18.12 million people) lives in rural villages and scattered settlements.

These impacts on water quality lead to significant consequences affecting every segment of the South African society, as well as the ecosystems dependent on freshwater resources. South Africa's outdated infrastructure as well as unskilled operators contribute to insufficient water treatment (Rietveld *et al*., 2009). The result is high microbial contaminant loads in river water that pose a risk to human health and safety.

G. POSSIBLE SOURCES OF CONTAMINATION RELATED TO FRESH PRODUCE

Contamination of fresh produce can take place anywhere along the farm-to-fork chain (Jung *et al*., 2014) and most pathogens that caused recent fresh produce outbreaks are related to faecal contamination (Ravaliya *et al*., 2014). Contamination of fresh produce, particularly, has higher food safety risks than the contamination of other food types. Other food products usually undergo heat treatments prior to packaging, which lowers the food safety risk by eliminating the presence of potential pathogens (Kikine, 2011). Fresh produce is not processed; therefore, present pathogens would not be eliminated (Jung *et al*., 2014). Instead, fresh produce often undergoes a washing and cleaning step, but is sometimes eaten without any washing or cleaning steps involved. The contamination of fresh produce should, therefore, be controlled throughout, in order to prevent disease outbreaks (Kikine, 2011).

Food pathogens are the main causative agents that have been identified with raw and minimally processed food and the question is *how* these organisms got onto the product. Every step

of the process, from planting up to consumption, can have a significant impact on the microbiological safety of the product. Contamination of fresh produce can occur during pre- or post-harvest conditions (Beuchat, 2006). Table 6 displays the pre-harvest and post-harvest sources of contamination. The main pre-harvest sources of contamination include: manure (of wild and domestic animals), irrigation water, inadequate sanitation facilities, and sewage from informal settlements (Beuchat, 2002; Buck, 2003; Steele & Odumeru, 2004; Johnston *et al*., 2006; Jones *et al*., 2014).

Table 6 Pre-harvest and post-harvest sources of contamination related to fresh produce containing pathogenic microorganisms (Beuchat, 2002; Steele & Odumeru, 2004; Johnston *et al*., 2006)

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

Farmers use compost from animal manure (Table 6), on a regular basis to enhance the quality and production of fruit and vegetables (Beuchat, 2006) as it makes the soil fertile as a result of the nitrogen presence (Blommenstein, 2012), but these may contain *E. coli.* Pathogens such as

E. coli O157:H7 have the ability to survive for weeks in manure (Nwachuku & Gerba, 2008). Therefore, it is important to treat manure properly to reduce pathogens that may be present (Solomon *et al*., 2002).

Moreover, fresh produce grown in a field where livestock (Table 6) graze, is likely to be contaminated by enteric pathogens (Panigrahy *et al*., 2011). Apart from farm animals that are commonly known to transmit faecal microorganisms, wild animals, such as birds and reptiles also carry some of these pathogenic microorganisms. Domestic and wild animals can contaminate the soil and ultimately fresh produce items (Beuchat, 2006). Pathogens from faeces filter through the soil at rates dependent on factors such as the soil type, the management thereof and rainfall. Some strains have the ability to survive for months or even years within soil (Beuchat, 2006). Therefore, the transport of pathogenic microorganisms from animals and/or manure across distant locations thereby causing contamination of river streams and fresh produce, is a concern.

Irrigation water (Table 6) is considered as one of the most common ways by which enteric pathogens are directed onto vegetable crops (Parke & Fisher, 2012) and is probably the main source of pre-harvest contamination of fresh produce (Beuchat, 2002; Steele & Odumeru, 2004; Johnston *et al.*, 2006; Panigrahy *et al*., 2011; Ijabadeniyi & Buys 2012; Jung *et al*., 2014). Surface water, especially river water, is the main source of irrigation in South Africa and other developing countries, and, especially in rural areas, it is used for irrigation of vegetable crops (Ijabadeniyi & Buys, 2012; Jung *et al*., 2014). It is likely that water does not undergo any purification steps due to insufficient facilities. This will result in high microbial loads, that include potential pathogens mainly originating from faecal contamination, and when consumed, illnesses can occur in humans.

H. MANAGEMENT AND CONTROL OF PRODUCE CONTAMINATION WITH PATHOGENS FROM IRRIGATION WATERS

It is nearly an impossible task to control the contamination of water sources in areas where crops are irrigated. There are too many variables involved in the contamination of river water and the carry-over of pathogens to fresh produce. However, Buck *et al*. (2003) suggests four major ways to reduce the introduction of pathogenic microorganisms into irrigation water:

- Knowing the origin and distribution of irrigation water;
- Knowledge on the history of the land;
- Maintaining irrigation wells;
- Monitoring of all irrigation sources for human pathogens.

Produce-associated outbreaks usually occur after harvesting and then it is very difficult to identify the source of contamination. Therefore, practical control measures should be implemented. Hazard Analysis Critical Control Points (HACCP), Good Manufacturing Practices (GMPs) and Good Agricultural Practices (GAPs) programmes have been incorporated into various stages of the fresh produce production process to prevent contamination (Bihn & Gravani, 2006; Ijabadeniyi & Buys;

2012). The proper design, construction and protection of water sources may minimise the contamination of irrigation water sources (Ijabadeniyi & Buys, 2012).

As the behaviour and view of food safety differ greatly among food suppliers, the only options to control contamination of fresh produce are to avoid fields where animals have grazed, and to use water free from pathogens for irrigation (Yiannas, 2009; Ijabadeniyi & Buys, 2012). It is very important for fresh produce suppliers to realise that once the produce has been contaminated with pathogenic microorganisms, sanitisers are unable to decontaminate the food item completely (Ijabandeniyi, 2010). Instead of trying to decontaminate the food product, contamination should be prevented right from the start (Beuchat, 2006), even before the water is used at the point of irrigation. If all the above-mentioned preventative options are not possible or are difficult to manage, on-farm treatments should be considered as a means to disinfect contaminated irrigation water (Lynch *et al*., 2009). Farmers should be aware that they have a great responsibility towards consumer safety when supplying fresh produce to the industry, while keeping in mind that the selection of disinfection treatments is dependent on contamination levels of the water, costs, the irrigation mode, the environment, and the education of farm workers and consumers (Britz *et al.*, 2012).

I. ON-FARM DISINFECTION TREATMENTS FOR IRRIGATION WATER

Disinfection of irrigation water encompasses the removal, deactivation or reduction of pathogenic microorganisms (LENNTECH, 2014). When the microorganisms are destroyed, the regrowth or reproduction of microorganisms is limited. As a result, it reduces the risks that are exposed to fresh produce by contaminated irrigation water. The reason disinfection, and not sterilisation, is used, is that the latter kills both harmful and harmless microorganisms present in the water (LENNTECH, 2014). The intention of disinfecting irrigation water is not to produce potable water, but to irrigate crops with water that complies with national guidelines. Treatment of irrigation water is particularly low in developing countries and the use of contaminated irrigation water is a common occurrence. The call for disinfection has increased with the need to reduce the health risks related to fresh produce (WHO, 2010).

Water disinfection can be accomplished through physical (sand filtration, ultrafiltration), photochemical (ultraviolet light (UV) and ultrasound) and chemical methods (bromine (Br_2), ozone (O_3), hydrogen peroxide (H_2O_2), peracetic acid ($C_2H_4O_3$) and chlorine sources). The choice of the most effective treatment available is determined by the following factors: the nature and concentration of the disinfection system; the amount and type of microorganisms present in the water; suspended solids content; organic matter within the water; water pH and temperature as well as the contact time (NHMRC, 2004). In addition, the disinfection capability of the treatment and its toxicity at high levels may affect water, soil and crops. The use of some disinfectants may lead to the possible formation of disinfection by-products (DBPs) when they react with components in the

water. Other factors involving the choice of a suitable disinfectant, are safety and the costs associated with the particular disinfection method (Lazarova & Bahri, 2005).

Every decision is unique according to a specific farm-setup and its financial implications. However, the goal is to utilise irrigation water that will not pose risks to consumers and which complies with national water quality standards.

J. PHYSICAL/MECHANICAL DISINFECTION METHODS

Physical methods have been used for many years and are the oldest technologies available for water disinfection (Kesari *et al*., 2011a). These methods are commonly applied to wastewater for purification and recycling (Kesari *et al*., 2011a). Physical treatments are primarily referred to as filtration methods and are based solely on the separation of solids from liquids (LENNTECH, 2014). The microorganisms are held back by mechanical retention, due to incorporated sand or synthetic membranes in the system (Acher *et al*., 1997; Yiasoumi *et al*., 2005; LENNTECH, 2014). There are various filtration techniques that are widely used for specific applications. These include the use of slow sand filtration as well as ultrafiltration.

Slow sand filtration

Background

This method was the first successful water treatment technology for municipal water (Huisman & Wood, 1974; Langenbach *et al*., 2010). The effectiveness of slow sand filtration (also known as biological filtration') has been proven. The invention and first demonstration of slow sand filtration by John Gibb is traced back to 1804 when he built the filter for his water treatment business in Scotland following an improvement where the method was first amended for public supply in 1829 by James Simpson (Huisman & Wood, 1974). The use of this method spread and in 1892, the most convincing proof of water filtration effectiveness was achieved by Hamburg and Altona (Huisman & Wood, 1974). The use of slow sand filters became so popular, that by 1940, the United States had over 100 filters supplying around 52.6 million gallons per day. The World Health Organization (WHO) claims that "Under suitable circumstances, slow sand filtration may be not only the cheapest and simplest, but also the most efficient method of water treatment" (Huisman & Wood, 1974).

Mode of action

Sand filters typically consist of a tank, a filter media and a controller to enable backflow (LENNTECH, 2014). The filter is simply a bed of sand (60 - 120 centimetres (cm)) with particle sizes varying from 0.15 and 0.35 millimetres to remove various types of microorganisms (Huisman & Wood, 1974; Hendricks, 2006). However, Hugo & Malan (2006) found that the sand bed was unable to remove nematodes from irrigation water due to the big pores of the sand bed's membranes. Other studies on tertiary wastewater showed faecal coliform removal of 2 log-units (Keraita *et al*., 2008).

Moreover, Figure 1 shows that the sand bed is supported by a 30 - 50 cm gravel layer that contributes to uniform filtration with the underdrains at the bottom of the filter to remove the filtered water (Fig. 1) (Campos, 2002). As the water enters the top layer, it remains within the reservoir (1 to 1.5 meters) above the sand bed for 3 - 15 hours, depending on the drainage velocity. During this time, heavier constituents within the water start to settle and lighter particles are united with one another. On the surface of the sand bed is a thin, slimy layer of organic material, called the *schmutzdecke* (filter skin), the main pathogen control in the filter (Huisman & Wood, 1974; Steward-Wade, 2011). This layer builds up on the surface of the sand bed as suspended solids are mechanically strained out of the water and embedded onto the *schmutzdecke* (Campos, 2002), thereby decreasing the permeability of the sand filter. In addition to this physical removal, the removal of pathogens is controlled by a biofilm of beneficial microorganisms. These biofilms are formed over time on the surfaces of sand grains, resulting in the removal of pathogens through antagonistic interaction or competition (Campos, 2002; Zheng & Dunets, 2014). The *schmutzdecke* contains numerous forms of life, including bacteria, fungi, nematodes and protozoa (Steward-Wade, 2011). When the water has passed the filter skin, it gradually enters the filter bed (sand bed) and passes through the pores between the sand grains. This process usually takes several hours (3 – 15 hours) to result in purified water (Huisman & Wood, 1974; Campos, 2002; Zheng & Dunets, 2014).

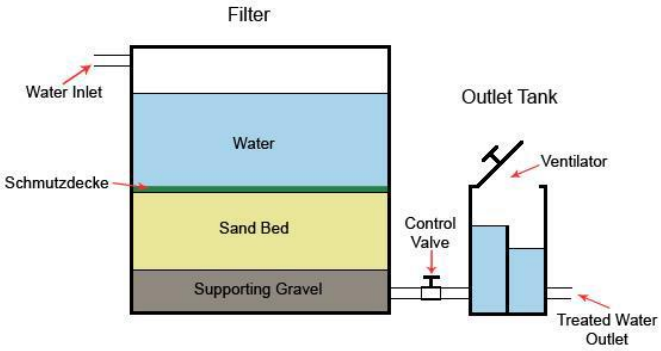


Figure 1 The components of a slow sand filtration system (Zheng & Dunets, 2014).

Positive and negative aspects

There are definite advantages of slow sand filtration's usage for irrigation water treatment. Sand filters are capable of removing algae, bacteria, viruses, protozoa (*Giardia & Cryptosporidium*) and *Phytophthora* species from drinking and irrigation water (Hijnen *et al*., 2006). It is a very simple technology where no harmful chemicals are used, limiting the formation of DBPs (Langenbach *et al*., 2010). No risks are exposed to workers, fresh produce or the environment. Operating costs are low since the filter predominantly consists of biological material and little technical monitoring is required (Huisman & Wood, 1974; Zheng & Dunets, 2014). Slow sand filtration is commonly used as a pre-treatment in other filter technologies and can also be very effective when combining it with other treatments such as ultraviolet light.

When the land is very restricted and expensive, the use of this filter may be eliminated completely as it takes up a large amount of space, consequently adding to the capital costs of the method (Huisman & Wood, 1974; Zheng & Dunets, 2014). The filtration performance is greatly influenced by the uneven pores due to the large variability of sand particles. After several days or months of filtration, sand particles need to be replaced due to build-up on the *schmutzdecke* and water of lesser quality will be delivered. When using sand filters on irrigation water containing high levels of particles, the filter's pores may plug too frequently and, therefore, regular maintenance and a pre-filtering step are required (Campos, 2002; Zheng & Dunets, 2014).

In conclusion, sand filters have the ability to remove algae, bacteria, viruses, protozoa (*Giardia & Cryptosporidium*) and *Phytophthora* species from drinking and irrigation water. However, this process is time-consuming, with retention times of 3 – 15 hours and may not be viable with irrigation where a lot of water will be sent through the filter. Without regular replacement of the sand particles, the filtration process is not uniform, due to build-up in the sand bed which results in delivering water with differing qualities.

Ultrafiltration

Background

Ultrafiltration was first introduced by Bechold in 1907 and is simply based on forcing solutions at pressures through various membranes constituted from filter paper with acetic acid (Nath, 2008). During this process, hydrostatic pressure is used to force a liquid against a semipermeable membrane. The implementation of this membrane has increased and is used for the removal of bacteria and other microorganisms, particles and organic material in water (Nath, 2008). Aitken *et al.* (2006) did a study on the operation of an ultrafiltration plant for an irrigation scheme to produce class A recycled water. Ultrafiltration was the main method used in combination with other treatments and has received positive feedback regarding water quality for both the community and the environment, as it removed all colloidal particles from water as well as some of the largest dissolved contaminants (Aitken *et al*., 2006).

Mode of action

The physical removal of substances is a pressure driven membrane separation process. Substances with low molecular weights and water pass through a thin layer semipermeable membrane while other larger particles and macromolecules are retained (Cotterill, 2000; Nath, 2008). The particles are removed by size rejection, however, the electrical characteristics and surface chemistry of both substances and membranes may influence the purification efficiency (Cotterill, 2000; Nath, 2008). There are many membrane varieties available in tubes or fibres and all of them function with similar mechanisms. The asymmetric structure of the membrane can be viewed under a microscope with pores appearing as inverted conical-shaped holes. At the one end of the membrane, the diameter is narrow (ranging between 0.002 - 0.1 µm) (Betancourt & Rose,

2004) comparing to the wider one on the other end (Cotterill, 2000). Ultrafiltration used in water treatment processes use membranes with pore sizes ranging from 0.01 – 0.5 µm. This pore is small enough to remove protozoan oocysts that have a larger diameter of 4 – 15 µm (Betancourt & Rose, 2004). The narrow end of the membrane is better known as the 'skin' and the pores at this surface are so small, they allow only the permeation of water and small constituents therefore, pressure is required to force these particles through the membrane (Cotterill, 2000). Ultrafiltration membranes can become clogged as particles push through the membrane and the use of backwashing could correct this problem as well as chemical cleaning of the membranes (Cotterill, 2000).

Positive and negative aspects

Ultrafiltration provides a strong barrier against the following: particles, bacteria, high molecular weight substances and colloids (Nath, 2008). Some microorganisms, such as *Cryptosporidium and Giardia* are resistant to chemical disinfectants and can rather be removed by ultrafiltration due to their large size (Betancourt & Rose, 2004). Ultrafiltration can also be used in conjunction with other disinfection methods and is carried out at ambient temperatures avoiding the thermal and oxidative degeneration of water (Nath, 2008).

The major drawback limiting the use of ultrafiltration, is its high operating and capital cost (Freese *et al*., 2003). In addition, not all pathogenic microorganisms and viruses are removed from irrigation water as some of them have smaller diameters than the membrane pore size (GHD, 2005). The application of an additional disinfection procedure is suggested after ultrafiltration for total removal of microorganisms. However, this may add to the high cost of the ultrafiltration procedure.

In conclusion, some of the particles are smaller than the membrane's pores, allowing the permeation of possible pathogens through the membrane (GHD, 2005). Therefore, an additional post-treatment will be required for the maximum removal of pathogens. The high operating and capital costs, together with an additional treatment, will not be feasible on farms. Instead, farmers seek the most effective and affordable treatment for the disinfection of irrigation water.

K. ALTERNATIVE / PHOTOCHEMICAL DISINFECTION METHODS

The use of non-chemical treatment methods have increased notably (Broekman *et al*., 2010) and the main attribute that distinguishes photochemical from chemical treatment, is that the former does not produce DBPs. According to Broekman *et al.* (2010) there is an increased trend in the urgency to implement and develop water treatment technologies that are more environmentally responsible, thereby lowering the impact of chemicals in effluent water. Common photochemical treatment methods include ultrasound and ultraviolet light that can be considered to remove pathogenic microorganisms from irrigation water effectively.

Ultrasound

Background

Ultrasound is a cyclic sound pressure of mechanical vibrations with a frequency greater than the upper limit of human hearing, 20 Hz (Hunter, 2008; Oyib, 2009; Naddeo *et al*., 2014). Ultrasonic vibrations were first discovered by Pierre and Jacques Curie in 1881 (Hunter, 2008). In the late 1920s, the first ultrasound application for the inactivation of microorganisms was reported (Harvey & Loomis, 1929). This study was related to ultrasound's good disinfection action, however it was recommended to restrict its application due to high expenses (Harvey & Loomis, 1929). During the 1970s, another study presented good disinfection performance using ultrasound for the removal of heat resistant bacterial spores (Burgos, 1972).

Thereafter, many studies have been performed on the commercialisation of ultrasound for the bacterial inactivation of wastewater. Hunter (2008) observed a 4 log reduction in viable bacterial cells and other studies noted reductions of total aerobic bacterial counts and free bacterial counts (*E. coli* and *Enterococci*) in irrigation and wastewater (Hulsmans *et al.*, 2010). The extensive use of ultrasound on wastewater treatment has shown that pollutants and pathogenic bacteria were reduced successfully (Hulsmans *et al*., 2010). Cui *et al.* (2011) conducted a study on the disinfection of *E. coli* in primary sludge and found a 90% reduction at a high dose of 20 Hz.

The applications of this treatment are numerous. Typically, it is used to penetrate a medium to measure the reflection signature or for the removal of trapped gasses, cleaning of microscopic contamination, ultrasonic humidifier as well as the disruption of biological cells (Oyib, 2009).

Mode of action

The physical ultrasonic inactivation of bacterial cells is caused by a phenomenon called cavitation (Hunter, 2008; Kesari *et al*., 2011a). This is caused at high frequencies ranging from 20 - 100 kHz and is better known as 'power ultrasound' (Kesari *et al*., 2011a). Cavitation can be defined as the formation, growth and subsequent collapse of microbubbles over a very short period of time (Hulsmans *et al.*, 2010; Kesari *et al*., 2011b). The high pressure (50 000 kPa) shock-wave generated during bubble collapsing, is the main inactivation technique in microorganisms (Hunter, 2008) as this produces free radicals (OH, HO_2 and O) (Furuta *et al*., 2004) with strong oxidative powers. At the same time, high temperatures (5 500°C) are generated during bubble collapsing; however, increased temperatures are not the main cause of cell inactivation (Hunter, 2008). The structural design of microorganisms plays a significant role in the inactivation efficiency of ultrasound. Hulsmans *et al.* (2010) mentioned that the exact method of bacterial inactivation is unknown, but the above-mentioned information suggests three main antimicrobial inactivation steps: mechanical, chemical and heat effects caused by cavitation as summarised below (Kesari *et al*., 2011b; Naddeo *et al*., 2014):

- Pressure gradients from collapsing bubbles cause bacterial cell wall damage due to mechanical fatigue;
- The chemical structures of the cells are oxidised by radicals, causing complete disintegration of the cell wall.

The thicker cell wall of gram-positive microorganisms compared to gram-negative microbes' make the former less effective to ultrasound treatment.

Positive and negative aspects

Ultrasound is an alternative to chemical disinfectants that neither leads to the generation of DBPs nor contributes additional chemical compounds (Kesari *et al*., 2011b; Naddeo *et al*., 2014). From an operational point of view, ultrasound is a simple method with high bacterial inactivation yields (Naddeo *et al*., 2014). It removes a wide variety of microorganisms during water and wastewater disinfection. The efficacy can be improved by combining it with other disinfection treatments such as chlorine, ozone and ultraviolet (Naddeo *et al*., 2014). Ultrasound can effectively eliminate faecal coliforms such as *E. coli*. Hulsmans *et al.* (2010) did a study on water contaminated with *E. coli* (with initial loads of 4.8×10^4 and 2.0×10^4 cfu.mL^{-1}) and after 180 minutes of ultrasonication, a 2 log reduction (>99%) was observed. Another review done by Naddeo *et al*. (2014) on water disinfection found that *E. coli* and total coliforms were optimally removed at low frequencies (20 - 40 kHz), high densities and sonication times of 3 - 15 min. Adding to this, the ultrasonic inactivation of bacteria and protozoa like *Cryptosporidium* and *Giardia* from irrigation water, are possible because it is widely applied to various types of wastewater (Sangave & Pandit, 2004; Mahamuni & Adewuyi, 2010).

There are drawbacks though. Despite the research already done on laboratory scale and the potential of ultrasound for water disinfection, little is known about its application at industrial scale (Gibson *et al*., 2008). The energy demand for ultrasound is high and therefore, it is rather not recommended for big volumes of water (Hulsmans *et al*., 2010; Naddeo *et al*., 2014). Ultrasound has the ability to remove all pathogens during disinfection, however this requires high ultrasonic intensities that will lead to increased costs and extended contact times consequently limiting its use for large-scale disinfection (Hulsmans *et al*., 2010; Naddeo *et al*., 2014). Researchers have found ultrasound cooperates more effectively with combined treatment options like ultraviolet and heat treatment (Hunter, 2008).

In conclusion, disinfecting large amounts of irrigation water at farm-scale is thus not feasible as it will be very expensive due to the high energy requirement and a long disinfection time. For effective elimination of microorganisms, ultrasound is rather recommended as a pre-treatment in disinfection processes as it woks more effectively in combined disinfection processes. Considering all the above-mentioned factors, the ultrasonication of irrigation water would not be feasible.

Ultraviolet (UV) irradiation

Background

The use of UV radiation dates back to 1877 when the first germicidal effect of UV was discovered by Downes and Blount (1877) and years later, in 1903, Niels Finsen was given a Nobel prize for killing *Tuberculosis* in the skin with UV light (Hunter, 2008). Ultraviolet light is defined as wavelengths ranging between 4 and 400 nm, below visible light in the electromagnetic spectrum (Oppenländer, 2003; Hunter, 2008). It is classified into three types (UV-A, UV-B and UV-C) according to their wavelengths and germicidal effects (Oppenländer, 2003; Hunter, 2008). The most lethal wavelengths responsible for killing microorganisms range between 200 nm and 280 nm (UV-C spectrum) (Hunter, 2008). Within this spectrum, microbes absorb most of the energy which leads to microbial inactivation (Newman, 2004).

This photochemical reaction efficiently inactivates a wide range of human pathogens such as bacteria (Wong, 2002), viruses, algae, fungi, moulds and protozoa like *Giardia* and *Cryptosporidium parvum* (Hijnen *et al*., 2006; Meunier *et al.*, 2006; Trombert *et al*., 2007; Bolton & Cotton, 2008). According to Poepping *et al*. (2014), UV disinfection is well-known by the water industry and has been widely applied to water and wastewater treatment facilities, especially during recent years. The main motivation for its increased use is that it does not produce DBPs that are usually observed with chemical treatments such as ozone, chlorine and other chemical disinfectants (Hijnen *et al*., 2006).

Mode of action

UV-C is most effective between 254 - 260 nm for the inactivation of microorganisms (Betancourt & Rose, 2004; Gurol, 2005). Within this range, inactivation occurs via oxidation processes in the cell, also known as photolysis, when UV light is absorbed by the pyrimidine bases in RNA and DNA (Bolton & Linden, 2003). These nucleotide bases are known as thymine or cytosine in DNA, and cytosine or uracil in RNA. As UV light is absorbed by the cell, chemical pyrimidine dimers are formed between two bases (Poepping *et al*., 2014) and inhibits the formation of new DNA or RNA chains (Bolton & Linden, 2003). These dimers interfere with cellular processes such as DNA replication during cell production (mitosis) as well as transcription of DNA to RNA for protein synthesis (Bolton & Linden, 2003; Eischeid & Linden; Hunter, 2008; Rodriguez *et al.*, 2014). Cyclobutane pyrimidine dimer (CPD) is the main photoproduct formed during photolysis from two neighbouring thymine bases (Fig. 2) (Eischeid & Linden, 2007).

UV disinfection makes use of monochromatic, low pressure and medium pressure lamps and the latter produces much higher UV intensities than low pressure lamps (Wong, 2002). Low pressure and medium pressure lamps emit light within the UV-B (280 - 315 nm) and UV-C range (200 – 280 nm). DNA absorbs UV light significantly at a maximum of 260 nm therefore, both medium pressure and low pressure lamps can be used for disinfection (Eischeid & Linden, 2007).

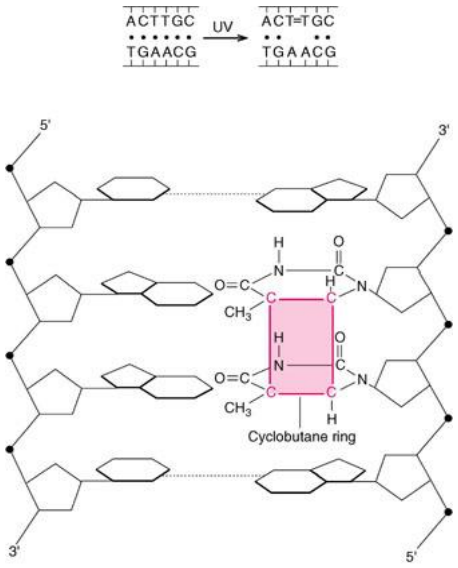


Figure 2 Neighbouring thymine base pairs joined together during UV light exposure leading to the formation of a pyrimidine dimer called cyclobutane pyrimidine dimer (Khan, 2014)

The UV dose requirements of bacteria, bacterial spores, viruses and protozoa vary and the term 'dose' can be defined as follows (Bolton & Linden, 2003):

$$Dose\ (mJ.cm^{-2}) = UV\ intensity\ (\mu W.cm^{-1}) \times UV\ time\ (seconds)$$

Currently, UV dose can only be manipulated in bench scale experiments at laboratory scale using a collimated beam device (Bolton & Linden, 2003). The output of the lamp is directed onto a horizontal surface where the sample is placed for irradiation (Bolton & Linden, 2003). The most common doses used for the elimination of pathogens are 16mJ.cm^{-2}, 30mJ.cm^{-2}, 40mJ.cm^{-2} and higher. Two types of UV apparatus are mainly used on industrial scale: Flow through also called 'open channel systems' are used for wastewater disinfection and 'in-pipe, closed systems' are used for drinking water and discharge effluents (Acher *et al*., 1996; Lazarova & Bahri, 2005). The UV dose is significantly affected by the flow rate and water quality. Only a few seconds of UV light exposure are required for the inactivation of microorganisms (Hunter, 2008); however, this is greatly influenced by the flow rate of water travelling through the UV chamber. High flow rates are directly correlated to shorter exposure times and low UV doses and vice versa (EWP, 2014). Also, the effect of water quality on UV disinfection is complex as various water characteristics may influence UV efficiency, such as UV transmittance, suspended solids, temperature, pH and water hardness. Most importantly, UV transmittance is the predominant influence on UV efficiency, as it affects the light penetrating pathogens (EWP, 2014). Other factors such as BOD (biochemical oxygen demand), COD (chemical oxygen demand), suspended solids and turbidity are also coupled to UV transmittance and lower the extent of UV light water penetration (EWP, 2014).

Positive and negative aspects

Numerous studies have shown that UV disinfection effectively eliminates the presence of enteric bacteria, bacterial spores, viruses and oocysts without producing any DBPs or other chemical residues that may lead to DBP formation (Rajala *et al*., 2003; Bolton & Cotton, 2008; Spellman, 2014). The absence of DBP formation is one of the main advantages of UV compared to traditional disinfectants that release by-products into the water. Ultraviolet disinfection alters the water quality by degrading the natural organic matter (NOM) and micro-pollutants present in the water (Meunier *et al*., 2006). When UV is compared to exposure times needed for chemical disinfection, much shorter contact times, in fact, only a few seconds are needed for effective disinfection (Spellman, 2014)

Ultraviolet irradiation is a physical process, therefore, it eliminates the generation, transport and storage of toxic or hazardous chemicals consequently representing lower costs compared to chemical disinfection (Spellman, 2014). Ultraviolet units, as well as the installation thereof, are expensive but the operational cost to sustain the apparatus is fairly low as little much maintenance is required. The application of UV disinfection is suitable for small- as well as industrial-scale water disinfection facilities.

Despite the advantages associated with UV irradiation, there are also some drawbacks regarding its use (Spellman, 2014). The initial implementation cost of a UV system is expensive. The fluid should be penetrable (low organic content) to UV light otherwise, penetration of the UV rays will not be effective to reduce high bacterial numbers (Hunter, 2008; Spellman, 2014). Similarly, non-homogenous fluids containing certain amounts of suspended solids greatly affect the efficiency of UV light as it directly relates to water turbidity that associates negatively with effective disinfection (Freese & Nozaic, 2004; Gurol, 2005; Hunter, 2008; Spellman, 2014). Spellman (2014) stated that UV disinfection using low pressure lamps is not as effective when suspended solids levels in the water exceed 30 $mg.L^{-1}$. In the literature, it was stated that the possibility of photoreactivation or dark repair of microorganisms, occurring at sub-lethal UV doses under the desired conditions, may occur (Guo *et al*., 2011; Vélez-Colmenares *et al*., 2011).

In conclusion, ultraviolet disinfection is a very effective method of killing a wide variety of microorganisms. However, its greatest limitations are associated with water quality (Gurol, 2005), especially water turbidity (Freese & Nozaic, 2004). Therefore, applying a pre-treatment, such as filtration (sand filters), is strongly recommended (Newman, 2004). Ultraviolet installation cost is high which can limit the use of UV disinfection by non-commercial farmers. However, comparing this to the continual costs associated with chemical disinfection, UV disinfection can be recommended. Ultraviolet light disinfection is used in multiple European countries and from a South African point of view, the application of this method for water disinfection has potential in the coming future.

L. CHEMICAL DISINFECTION METHODS

Chemical disinfection started when Pasteur and Koch presented the germ theory of diseases (Yusaf & Al-Juboori, 2014). Koch discovered the bactericidal properties of chlorine in 1881 and in 1902, the first water disinfection attempt, using chlorine, occurred (Arrojo *et al*., 2008). Thereafter, chemical disinfection spread (Richardson, 2003). Chemical treatments on water systems have been applied for more than a century and are still being used by multiple water industries. Numerous chemicals are available to enhance the microbiological quality of water such as ozone (O_3), bromine (Br_2), chlorine (Cl_2), chlorine dioxide (ClO_2), chloramine (RNHCl), hypochlorites (ClO^-), peracetic acid ($C_2H_4O_3$) and hydrogen peroxide (H_2O_2) (Acher *et al*., 1997).

Chemical disinfection is based solely on the oxidation potential of the chemical itself that harms the cell walls of microbes resulting in lethal damage (Acher *et al*., 1997). The term 'oxidation' refers to the increase in the positive oxidation number, simultaneously resulting in a loss of electrons (Newman, 2004). Each chemical has an oxidation potential that reflects its effectiveness of disinfection (Table 7). However, the choice of disinfection agents remains difficult when considering other factors (Acher *et al*., 1997) such as water quality that may affect their disinfection efficiency. These factors may include suspended solids, oxidisable organic and inorganic material, temperature and pH (Acher *et al*., 1997). Together with water quality parameters, very importantly, the dose ($mg.L^{-1}$) and exposure time (minutes) are great determinants of disinfection efficiency (Acher *et al*., 1997; Yiasoumi *et al*., 2005; Ali, 2010).

Table 7 Oxidation potential of commonly used chemical disinfectants (Acher *et al*., 1997; Newman, 2004)

Chemical disinfectant	**Oxidation potential (mV)**
Ozone	2.07
Peracetic acid	1.81
Hydrogen peroxide	1.78
Sodium hypochlorite	1.36
Bromine	1.07

Although chemical disinfectants are effective in treating contaminated water sources, modern analytical techniques indicated that they release DBPs into the water (Acher *et al*., 1997; Yiasoumi *et al*., 2005). During disinfection, chemical substances react with compounds present in the water leading to the formation of DBPs (Voigt *et al*., 2013; LENNTECH, 2014). During the 1970s, DBPs (trihalomethanes) were discovered by gas chromatography (LENNTECH, 2014) when water containing organic compounds, was chlorinated (Freese & Nozaic, 2004). Organic particles in the water react with substances from chemicals, forming by-products. Typical DBPs such as di-trichloroacetic acids, trihalomethanes and 3-chloro-4-(dichloromethyl)-5-hydroxy-2(5H)-furanone are considered carcinogenic or mutagenic (Tate & Arnold, 1990; Woo *et al*., 2002; Bitton, 2005).

These may have detrimental effects on human health (Kobylinski & Bhandari, 2010), however, the risk humans are exposed to, are much smaller than the risks associated with inadequate disinfection (Bitton, 2005; Voigt *et al*., 2013).

Although chemical disinfectants are an unavoidable need of the water industry, there are also disadvantages associated with their application. Every chemical treatment, functions according to its own chemical characteristics, properties and management issues. Regarding the latter, human health is one of the main considerations (Connellan, 2013). Each have its own drawbacks, but the following are combined disadvantages: the manufacturing, transport and storage of chemical disinfectants pose a risk to anyone who works with them and can also be harmful to the environment (Acher *et al*., 1997; Yiasoumi *et al*., 2005). After the application of chemical disinfectants, residuals still remain in water, especially if used at high concentrations. Therefore, excessive concentrations in water should be controlled to prevent high toxicity levels exposed to plants/crops. The reuse and recycling of water or accidental overdosing may lead to excessive concentrations of chemicals in irrigation water (Gurol, 2005). Disinfection of irrigation water is of great importance as it prevents and controls the growth of bacterial pathogens in irrigation systems to reduce the risk of introducing diseases onto farms, and eventually onto fresh produce (Yiasoumi *et al*., 2005; Pehlivanoglu-Mantas *et al*., 2006).

Ozone (O_3)

Background

Ozone is the most powerful oxidising agent compared to other chemical disinfectants for water and wastewater treatment (Table 7) (Venosa *et al*., 1984; Gurol, 2005; Burns, 2010). It was first discovered by Martinus van Marum in 1785 when a characteristic odour was noticed. Years later, in 1886, the first O_3 water disinfection occurred in de Meritence (Hunter, 2008; Burns, 2010). From this point forward, the effective use of O_3 against viruses, bacteria, fungi and moulds was known (Hunter, 2008). Ozone is a powerful disinfectant for the elimination of bacteria and viruses such as coliforms, *E. coli* and *Giardia lambila* as well as *Cryptosporidium* oocysts (Burns, 2010). Ozone disinfection has been utilised for more than a century (Burns, 2010; Voigt *et al*., 2013) and currently, thousands of municipal water treatment plants use O_3 for disinfection (Burns, 2010).

Ozone generation

Water treatment plants generate the O_3 on-site by passing dried oxygen gas through an electrical field where oxygen molecules are split by an electrical current. The unstable oxygen molecules adhere to the other available oxygen molecule forming O_3 (Newman, 2004). Ozone is then injected into irrigation water to inactivate potential pathogens by disrupting their cell membranes and constituents of nucleic acids (Newman, 2004; Voigt *et al*., 2013).

Mode of action

Literature states that O_3 decomposes in three different pathways, however, the exact mechanisms of these phases are debatable (Gehr *et al*., 2003). As O_3 reacts with water, it decomposes into free radicals, hydroperoxyl (HO_2) and hydroxyl (•OH) that have great oxidising properties and serve as intermediates of the reaction (Staehelin & Hoigne, 1982; Tomiyasu *et al*., 1985; Voigt, 2013). The effectiveness of this reaction relies on the O_3 concentration and contact time together with the susceptibility of target microorganisms. The exact disinfection pathways and targets for O_3 are poorly understood (Gehr *et al*., 2003). Researchers stated two disinfection pathways: some stated that ozone alters the protein bonds in cell membranes and others say that it affects the DNA (Gehr *et al*., 2003). The mechanism whereby the DNA is affected during cell wall disintegration is called cell lysis (Hunter, 2008; Voigt *et al*., 2013) and disruption of the cell wall is caused through lipid peroxidation. When O_3 eventually enters the cell, it keeps on oxidising and destroying DNA, RNA and proteins that are essential components for cell survival (Hunter, 2008). However, Hunt & Marinas (1997) did a study on O_3 disinfection of *E. coli* and found that noticeable changes within the cell only took place after most of the cells became non-viable. This confirms that, in most cases, the cells are destroyed due to the inactivation of the cell membrane followed by DNA damage (Gehr *et al*., 2003).

Positive aspects and negative aspects

Compared to other disinfectants such as chlorine, O_3 requires much lower dosages and shorter contact times (10 - 30 minutes) due to its high oxidation potential (Table 7) (Wong, 2002; Hunter, 2008; Voigt *et al*., 2013). Added to the fact that O_3 is a better disinfectant than chlorine, some other advantages include the reduction of colours, odours and the removal of suspended solids (Masten & Davies, 1994). When water is overdosed with O_3, it is not a concern as it decomposes rapidly back into oxygen, leaving no residual that will need to be removed post-treatment.

Several disadvantages are linked to the use of O_3, in particular the high capital cost, since O_3 gas should be generated on-site (Masten & Davies, 1994; Voigt *et al*., 2013), as it is unstable during storage and also requires highly skilled staff to manage and operate generation facilities. If not operated properly, health problems may occur during exposure due to leakages (Freese *et al.*, 2003; Gurol, 2005). The rapid decomposition of biodegradable ozone residuals may require an additional disinfectant to control the regrowth of microorganisms or the aseptic transport of the disinfected product to the point of use (Hunter, 2008). Unfortunately, the formation of DBPs from O_3 disinfection has been reported: non-halogenated by-products (aldehydes, ketones and carboxylic acids) and bromates are formed when O_3 reacts with the natural organic matter in the water (Wong, 2002; Freese *et al*., 2003; Bitton, 2005; Hunter, 2008).

In conclusion, as a result of the high cost and dangers that are associated with O_3 disinfection, farmers would not find this technique feasible. Ozone can cause air in the pipes that will restrict proper irrigation and therefore, an additional pump to clear the air in pipes will be required,

causing further financial outlays. Although O_3 is a very powerful oxidant, it is a very expensive disinfectant. Taking all the above into consideration, the use of O_3 disinfection on water intended for irrigation will be a difficult and costly procedure.

Hydrogen peroxide (H_2O_2).

Background

Hydrogen peroxide was discovered by a scientist, Louis Jacque Theard in 1818 (Schumb *et al*., 1955; Spaulding *et al.*, 1977) and was first used as a disinfectant by B.W. Richardson in 1891 (Linley *et al*., 2012). Hydrogen peroxide is a combination of oxygen and hydrogen atoms with the following chemical formula: H_2O_2. The two oxygen atoms are joined with a single bond $(O\text{-}O)^{2-}$ to form the peroxide ion (O_2^{2-}) (Newman, 2004). In 1950, the first application of H_2O_2 occurred through the disinfection of drinking water (LENNTECH, 2014). Hydrogen peroxide is one of the most versatile oxidants and has a wide application that can be used both alone and in combination with other disinfection treatments (Vargas *et al*., 2013). For the last two decades, H_2O_2 has been used for wastewater disinfection (Ronen *et al*., 2010; Vargas *et al*., 2013) and has also been applied in air, water and soils (LENNTECH, 2014). Hydrogen peroxide controls colours, tastes and corrosion in polluted sources, destructs residual chlorine, reduces the chemical and biochemical oxygen demand and inhibits microbial growth (Vargas *et al*., 2013). A commercial form of H_2O_2, hydrogen dioxide (XeroTol), has the ability to kill bacteria, fungi, algae, yeasts and viruses and is often used as an irrigation water disinfectant (McDonnell & Russell, 1999; Newman, 2004).

Mode of action

The bonds between the hydrogen and oxygen atoms in H_2O_2 are unstable making the molecule susceptible to break along the oxygen-oxygen bond. Hydrogen peroxide is a strong oxidant that easily enters the cell membrane of microorganisms and releases free hydroxyl radicals (•OH) and superoxide radicals (O_2^-) also known as reactive oxidative species (ROS) (Labas *et al*., 2008; Vargas *et al*., 2013; Zheng *et al*., 2014). The damaging effects of H_2O_2 are referred to as oxidative stress and the radicals, particularly •OH radicals that have the greatest potential to destroy (Labas *et al*., 2008), attack components of the cell membrane ultimately followed by the destruction of DNA, proteins and lipids (McDonnel & Russel, 1999; Vargas *et* al., 2013; LENNTECH, 2014). The lethal and sub-lethal effects of these radicals lead to changes in the physical bacterial structure that delay cell growth due to cell membrane oxidation (Vargas *et al*., 2013). The disinfection performance of H_2O_2 is determined by factors such as concentration, contact time, pH, catalysers as well as temperature (LENNTECH, 2014). Labas *et al*. (2008) stated that effective H_2O_2 disinfection depends on the concentration and exposure time.

Positive and negative aspects

Hydrogen peroxide disinfection poses little danger to the environment as it degrades into hydrogen and oxygen leaving no residual (EPA, 2002; LENNTECH, 2014; Zheng *et al.*, 2014). It is effective at a wide pH range (Fisher, 2011) and has a higher oxidation potential than chlorine and chlorine dioxide (Newman, 2004; LENNTECH, 2014).

The following are disadvantages associated with its use: Its reaction with organic particles in irrigation water via oxidation decreases the disinfection efficacy (Zheng *et al*., 2014) and Newman (2004) suggested a pre-treatment step such as filtration to eliminate some of these organic particles. This is one of the main observed drawbacks of using H_2O_2 for irrigation water disinfection. Also, peroxides are highly unstable and corrosive, therefore proper safety measures should be taken during handling and storage (LENNTECH, 2014; Zheng *et al*., 2014). Fisher (2011) states that the handling and storage of H_2O_2 are problematic and ultimately, costly. The reason for being costly is attributed to high concentrations that are required for effective pathogen reduction (Newman, 2004). A study done by Labas *et al*. (2008) investigated the effect of H_2O_2 on *E. coli* ATCC 8739 at concentrations ranging from 15 – 300 ppm and long contact times and only resulted in very low inactivation levels. Another study showed that a very high concentration of 350 $mg.L^{-1}$ H_2O_2 resulted in a 2.06 *E. coli* reduction after 120 min (Giddey *et al*., 2015).

In conclusion, although H_2O_2 is a very versatile disinfectant, it is very unstable and easily influenced by water quality characteristics restricting effective pathogen removal (Vargas *et al*., 2013). When farmers use H_2O_2, proper safety measures should be in place to reduce the risk and hazard it can pose to workers (Zheng *et al.*, 2014). Furthermore, low concentrations of H_2O_2 are insufficient for effective pathogen reduction, while higher concentrations may lead to phytotoxicity (Newman 2004; Sichel *et al*., 2009). If used at very high concentrations or in conjunction with other treatment options it may become quite costly. Long contact times and low quality water treatment will not be a feasible option for irrigation water disinfection.

Chlorine

Background

Chlorine, the most common and widely applied water disinfection method in the world was first discovered in 1774 in its gaseous state by Mark Steele in Sweden (Lazarova & Bahri, 2005; Momba *et al*., 2008). In 1886, the first chlorine disinfection occurred when it was applied to combat a typhoid fever epidemic (Schoenen, 2002). This led to the first application of chlorine for water disinfection in 1902 in Middelkerke, Belgium. To date, chlorine has various applications (Schoenen, 2002) compared to other chemicals. It is extremely versatile in water and wastewater treatment with various applications such as disinfection, control of microorganisms, removal of ammonia, control of taste and odour, colour reduction, destruction of organic matter, hydrogen sulphide oxidation and iron and manganese oxidation.

As mentioned in numerous studies, chlorine is extremely effective against bacteria and to a lesser extent, against viruses and protozoa (*Cryptosporidium* and *Giardia*) which requires higher chlorine doses for elimination (Cheremisinoff, 2002; Wong, 2002; Lazarova & Bahri, 2005). Chlorine exists in three common forms: chlorine gas, hypochlorite (sodium hypochlorite or calcium hypochlorite) and chlorine dioxide (Newman, 2004; Ivey & Miller, 2013). Several studies have evaluated the efficacy of these three forms, serving as sanitisers for wash water and recycled irrigation water to eliminate human and plant pathogens (Warriner *et al*., 2009). They are generated by different chemical reactions in water (Table 8) and recently, hypochlorites (Table 8) have gradually become alternatives for chlorine gas and chlorine dioxide in water and wastewater disinfection industries. Hypochlorites are commercially available in dry and liquid forms and are considered much safer than other chlorine sources such as chlorine gas and chlorine dioxide (Lewis, 2010).

Table 8 Different sources of chlorine and their reactions in water (Newman, 2004)

Sources of chlorine	**Formula**	**Reaction in water**
Chlorine gas	Cl_2	$Cl_2 + H_2O \rightarrow HCl + OCl$
Sodium hypochlorite	NaOCl	$NaOCl + H_2O \rightarrow NaOH + HOCl$
Calcium hypochlorite	$Ca(OCl)_2$	$Ca(OCl)_2 + 2H_2O \rightarrow Ca(OH)_2 + 2HOCl$
Chlorine dioxide	ClO_2	$HOCl + HCl + 2NaClO_2 \rightarrow 2ClO_2 + 2NaCl + H_2O$

Sodium hypochlorite (NaOCl)

Sodium hypochlorite, also known as liquid bleach, has been used since the 1930s (Newman, 2004). Besides being the active ingredient in household bleach, it was first used as commercial disinfectant to whiten textiles (Newman, 2004). From all the available hypochlorites, sodium hypochlorite is most commonly used within industry for domestic, industrial and commercial water applications. Although the transport of NaOCl takes up more space and is more costly to distribute over long distances than dry chlorine, it is far safer to handle and the maintenance is relatively low (Lewis, 2010).

Moreover, NaOCl is produced by the reaction displayed below: the addition of Cl_2 to caustic soda (NaOH) produces sodium hypochlorite (NaOCl), water (H_2O) and salt (NaCl) (LENNTECH, 2014). This occurs in the presence of heat and is regarded as a highly exothermic reaction (Newman, 2010; Lewis, 2010).

$$Cl_2 + 2NaOH + \rightarrow NaOCl + NaCl + H_2O$$

Commercial NaOCl solutions are available in 10-15% (trade percent), with 12.5 % trade percent (Table 9) most commonly used in water and wastewater treatment (Newman, 2004; Lewis, 2010). Trade percentage does not reflect the precise chlorine concentration in NaOCl solutions. Therefore, Table 9 displays the relation between the trade percentage and the actual available chlorine for disinfection.

The disinfection reaction of NaOCl in water (Table 8) produces hypochlorous acid (HOCl) that contains an oxygen atom with very strong oxidising properties (LENNTECH, 2014). Since NaOCl is very effective against bacteria, it is extensively used for water disinfection to eliminate indicator organisms and pathogenic microorganisms such as faecal coliforms and *E. coli* (Veschetti *et al*., 2003). Sodium hypochlorite is less effective against viruses, protozoa and helminths and not effective against *Cryptosporidium* oocysts and toxoplasma oocysts (Voigt *et al*., 2013). Since this chemical is in liquid form, it is easy to adapt to greenhouse systems for irrigational purposes (Newman, 2004).

Table 9 Relationships between trade percentage and actual available chlorine of NaOCl solutions (Lewis, 2010)

Trade %	**Available Cl_2 (wt %)**	**Available Cl_2 ($g.L^{-1}$)**
0.8	0.8	8
2	1.93	20
4	3.77	40
6	5.51	60
8	7.17	80
10	8.76	100
12	10.27	120
12.5	10.64	125
15	12.44	150

wt % - weight percentage
$g.L^{-1}$ – grams per litre of available chlorine in NaOCl solutions

Calcium hypochlorite ($Ca(OCl)_2$

Calcium hypochlorite, another form of hypochlorite, is available in the form of powder, tablets or granules (Lewis, 2010). The production of $Ca(OCl)_2$ involves the addition of chlorine gas to a solution containing calcium oxide (lime) and sodium hydroxide (NaOH) (Wong, 2002; Lewis, 2010) as demonstrated in equation below.

$$2Ca(OH)_2 + 2Cl_2 \rightarrow Ca(OCl)_2 + CaCl_2 + 2H_2O$$

The most common form exists in a powder called HTH (High Test Calcium Hypochlorite) typically containing 65 – 70% available chlorine, 4 – 6% lime and calcium carbonate (Lewis, 2010). The disinfection reaction of $Ca(OCl)_2$ in water is indicated in Table 8 where hypochlorous acid formed in water dissociates into the hydrogen ion and hypochlorite (OCl^-). Since two hypochlorous acid molecules are produced from one $Ca(OCl)_2$ molecule, this disinfectant is considered a strong oxidant (Lewis, 2010). This allows $Ca(OCl)_2$ to be very effective against bacteria, algae, slime, fungi and other microorganisms (Newman, 2004). Granular $Ca(OCl)_2$ is soluble in water ideally at room

temperature (Lewis, 2010) and is easier to store than NaOCl since it does not require large spaces for bulk tanks. Yet, great care should be taken during storage using corrosion-resistant materials.

Mode of action

Chlorine causes significant injury in pathogens such as *E. coli*, *Salmonella thyphimurium*, *Yersinia enterocolitica* and *Shigella* spp. (Leyer & Johnson, 1997; Bitton, 2011). There are various cell locations that are targets for cell injury and ultimately, cell death. Chlorine attacks the bacterial cell membrane, consequently leading to decreased cell permeability and disruption of many other functions. When cells are exposed to chorine their DNA, RNA and proteins leak out of the cells restricting protein and RNA synthesis as well as potassium uptake, ultimately causing cell death (Bitton, 2011). Chlorine also causes destruction to bacterial nucleic acids and enzymes such as catalase and dehydrogenases (Bitton, 2011). A study on the whole genome of *Staphylococcus aureus* showed that exposure to hypochlorous acid results in repression of the transcription of genes that control membrane transport, cell wall synthesis, protein synthesis and primary metabolism (Bitton, 2011). Additional effects of chlorine include the disruption of nutrient transport, inhibition of cell respiration, damage to ion sulphur centres and oxidation of sulfhydryl groups causing the disability of cells to maintain an adequate energy charge to ensure cell viability (Leyer & Johnson, 1997; Bitton, 2011). Generally, gram negative microorganisms like *E. coli* are more fragile towards chemical disinfectants than gram positive microorganisms (i.e. *Listeria monocytogenes*) due to the intracellular space between the two peptidoglycan layers in gram positive organisms providing more resistance to inactivation.

Disinfection by-product (DBP) formation

The presence of chlorine residuals after disinfection provides both positive and negative consequences. Residuals include the prevention of pathogen regrowth (Voigt *et al*., 2013) and also protect irrigation pipes against slime and algae growth. However, chlorine residuals may also have detrimental effects causing the formation of DBPs when applied in high concentrations. Disinfection by-products and residuals are the result of the reaction with organic and inorganic particles naturally present in water sources and can be a great concern for crop safety and consumers of fresh produce (Bouwer, 2002). Water regulations and guidelines set by international and national organisations regulate the presence of chlorine residuals to reduce the risk of DBP formation in water sources (Table 10).

The occurrence of DBPs was first detected by Bellar *et al*. (1974) in the USA and Rook (1974) in the Netherlands. They noted four trihalomethanes (THMs) in water following chlorination: chloroform, monochlorodibromomethane, dichlorobromomethane and bromoform (Bitton, 2011). Thereafter, Richardson (2002) discovered over 600 DBPs and typical forms include THMs such as chloroform ($CHCl_3$), bromodichloromethane ($CHBrCl_2$), dibromochloromethane ($CHBr_2Cl$) and bromoform ($CHBr_3$) and haloacetic acids (HAA) such as monochloroacetic acid, monobromoacetic, dichloroacetic acid, dibromoacetic acid and trichlors acetic acid. Chloroform is the most commonly

found THM as a result of chlorination and is also a known carcinogen (Freese & Nozaic, 2004; Sayyah & Mohamed, 2014).

Chloroform can cause cancer if one is exposed to high concentrations over a long period of time (Freese & Nozaic, 2004). However, Kobylinski & Bhandari (2010) mentioned that chloroform is not hazardous to humans when present in water at low concentrations. Despite the intense studies done on THMs and their health effects on humans, no evidence has been found proving that THMs are harmful in the quantities normally found in water (Freese & Nozaic, 2004). Most of these studies are based on rats, mice and rabbits and the highest dose causing no adverse health effects range from 34 500 to 43 000 $mg.kg^{-1}$ chloroform per day (Ruddick *et al*., 1983). For instance, a man weighing between 70 and 90 kg would have to drink eight glasses of water, each containing 1 500 to 2 000 $mg.L^{-1}$ chloroform (Freese & Nozaic, 2004). This threat is very unlikely to cause adverse effects in humans and the same could be argued for other THMs such as bromoform.

Table 10 Residual chlorine guidelines produced by international and national organisations (WRC, 1998; USEPA, 2004; WHO, 2004; DWA, 2013b)

Organisation	Residual chlorine limits
Water Research Commission (1998) *Guideline for Domestic water supply*	0.3 – 0.6 $mg.L^{-1}$
US Environmental Protection Agency (2004) *Reclaimed water for irrigation*	$\leq$ 1 $mg.L^{-1}$
World Health Organisation (2004) *Guidelines for drinking water quality*	$\geq$ 0.5 $mg.L^{-1}$
Department of Water Affairs (2013) *Wastewater intended for irrigation*	$\leq$ 0.25 $mg.L^{-1}$

The reaction in surface water between natural organic particles (humic and fulvic acids) leads to the formation of DBPs containing volatile and non-volatile compounds with probable mutagenic or carcinogenic activity (Crebelli *et al*., 2005; Sayyah & Mohamed, 2014). Wastewater provides a good substrate for DBP formation due to the high organic content present (Crebelli *et al*., 2005). Therefore, residual chlorine concentrations of 0.1 $mg.L^{-1}$ (special limit) and 0.25 $mg.L^{-1}$ (general limit) are set by the Department of Water Affairs (DWA, 2013b), for wastewater intended for irrigation, to limit the formation of DBPs that might be harmful for human health. A study done by Freese *et al.* (2003) to eliminate parasitic oocysts (*Giardia* and *Cryptosporidium*), viruses (coliphages) and bacterial indicators (*E. coli* and coliforms) from secondary wastewater resulted in chlorine residual concentrations that did comply with DWA standards. In fact, the wastewater was adequately disinfected leaving no residual (Freese *et al.*, 2003).

Factors influencing disinfection efficiency:

pH

Chlorine's activity is measured as 'free' residual chlorine and when it dissolves in water, chlorine exists in equilibrium as hypochlorous acid (HOCl) and hypochlorite (OCl^-) (Newman, 2004). A low ratio of HOCl to OCl^- is explained when the pH of a certain solution is also low. Below a pH of 7.5, the predominant species of chlorine exists as HOCl which is regarded as a very strong oxidiser in water (Fig. 3) (Newman, 2004; Bitton, 2005). Above pH 7.5, the dominant species is OCl^- having a much lower oxidative capacity than HOCl. The disinfection efficiency at this point will be markedly decreased (Wong, 2002). In fact, HOCl is 80 times more effective against *E. coli* than OCl^- (Newman, 2004; Lewis, 2010; Bitton; 2011). Figure 3 displays the relationship between HOCl and OCl^- over a wide pH range and the steepest area of the curve is observed between pH 7 and 8 (Anon., 2014b). Only a small change of 0.1 units can cause an adjustment in the HOCl and OCl^- ratio (Anon., 2014b). Therefore, the most active form of free chlorine should be maintained and the pH of a solution should be kept between 7.4 and 7.6 (Newman, 2004). Within this pH range, the ratio between oxidative species are suitable to deliver a maximum germicidal effect (Anon., 2014b)

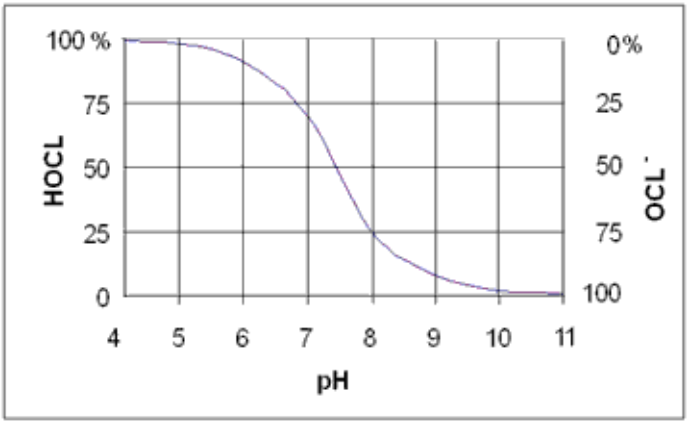


Figure 3 The ratio of HOCl to OCl^- as a function of pH expressed as percentage available chlorine (Bitton, 2005; Anon., 2014b).

The oxidation reduction potential (ORP) of chlorine is also influenced by pH. The ORP is an indication of the disinfectant's oxidising capability and higher ORP values indicates stronger oxidising potential (Park *et al*., 2004). Park *et al.* (2004) examined the effect of different chlorine concentrations (1.0 – 5.0 $mg.L^{-1}$) and pH values (3.0, 5.0 and 7.0) on the elimination of *E. coli* O157:H7 and *Listeria monocytogenes*. Lower *E. coli* populations were observed at lower pH values. Moreover, these results indicated that a low pH leads to increased sensitivity of these pathogens at aforementioned chlorine concentrations. It is suggested that stronger bactericidal activity at low pH values could be due to the higher ORP. However, *E. coli* was effectively reduced at a wide range pH values (between 2.6 and 7.0) (Park *et al*., 2004). Therefore, pH is a very important factor to

consider when using chlorine for disinfection. A pH lower than 8.0 is recommended for chlorine disinfection which falls within the range of pH values characteristic to surface waters (pH 6.5 - 8.5).

Temperature

Disinfection of chlorine is decreased at lower water temperatures (EPA, 1999; Pickard, 2006) and higher chlorine doses and longer exposure times are needed for effective disinfection (Bitton, 2011). In general, a temperature decrease of 10°C will lower the disinfection rate by 50 – 60%. The inactivation of parasites and pathogens increases at higher temperatures (Bitton, 2011). Therefore, it can be assumed that river water representing lower temperatures in winter months may yield decreased disinfection compared to disinfection achieved during summer months.

Organic matter and turbidity

Components naturally present in water interfering with chlorine disinfection are organic and inorganic particles as they also exert a chlorine demand. Free chlorine residuals react with organic content in water that lead to the formation of DBPs (Wong, 2002; Pickard, 2006; Sayyah & Mohamed, 2014). Nonetheless, the TSS and organic load (COD, DOC (dissolved organic content) and NOM (natural organic matter)) present in water may lower chlorine efficiency to inactivate pathogenic microorganisms (Ayyildiz *et al*., 2009).

Organic and inorganic particles increase water turbidity and also protect microorganisms (coliform bacteria) from free chlorine disinfection, a process known as 'particle association' (Pickard, 2006; Ayyildiz *et al*., 2009; Bitton, 2011; Van Haute *et al*., 2013). Protection is provided through the stabilisation of cell membranes whereby access to key components for cellular inactivation is restricted for coliform reduction (Winward *et al*., 2008). A study done on the shielding effect that particles have on chlorine disinfection found that particle agglomeration and clumping may have significant effects on chlorine disinfection (Pickard, 2006). Therefore, particle associated microorganisms are more resistant to inactivation by chlorine than free-swimming organisms (Winward *et al*., 2008; Bitton, 2011). The implementation of a pre-filtration step is suggested to eliminate suspended particles in order to enhance the disinfection effectiveness (Winward *et al*., 2008). Ayyildiz *et al.* (2009) found that the reduction of total coliforms and *E. coli* increased 1.5 - 2 times when COD levels were decreased by 50% using a filter compared to reductions achieved without filtration. Similar results were found by Van Haute *et al.* (2013), proving that COD had a detrimental effect on *E. coli* inactivation. Therefore, the dose of chlorine applied for water disinfection is determined by the water quality, in particular the COD load (Van Haute *et al*., 2013).

Concentration (dosage) and contact time

There are numerous studies done at different chlorine dosages and contact times for the inactivation of pathogenic microorganisms (Wong, 2002; Veschetti *et al*., 2003; Freese *et al*., 2003; Koivunen & Heinonen-Tanski, 2005a; Winward *et al*., 2008; Li *et al*., 2013). Dosages and exposure times differ due to the varying water qualities. A study conducted on secondary wastewater (Freese *et al*., 2003)

showed that 6 mg.L^{-1} chlorine (NaOCl) was adequate to obtain a 2 – 3 log reduction for most bacterial indicators after a reaction time of 30 min. Winward *et al.* (2008) studied the affect of chlorine on grey water and observed coliform reductions of approximately 3.8 logs after a 30 min disinfection period and 10 mg.L^{-1} chlorine. A similar study done on reclaimed water evaluated the effect of 0.2 - 3.0 mg.L^{-1} chlorine for 30 min and observed coliform reductions of 3.5 logs (Li *et al*., 2013).

Type of microorganism

There is variation in the susceptibility of microorganisms to chemical disinfectants (Veschetti *et al*., 2003; Bitton, 2011; Li *et al*., 2013). Resistance can differ among non-spore forming bacteria and also within strains of the same species (AWWARF & USEPA, 2005; Bitton, 2011; Cherchi & Gu, 2011). Many studies mentioned the use of reference strains, however, their inactivation kinetics are not always the same as those observed with environmental strains (Wojcicka *et al*., 2007).

A study by Li *et al.* (2013) found that *Salmonella* was more resistant to chlorine disinfection than total coliforms and *Enterococcus.* When NaOCl was compared to peracetic acid disinfection it was observed that NaOCl was more effective at reducing resistant organisms such as faecal streptococci, bacteriophages and anti-*E. coli* (Veschetti *et al*., 2003). Freese *et al.* (2003) observed 2 - 3 log reductions for bacterial indicators, however, coliphages showed more resistance at the same disinfection parameters. Van Haute *et al.* (2013) conducted a study on NaOCl disinfection of *E. coli* O157:H7, *Salmonella* spp. and *Listeria monocytogenes*. It was found that *E. coli* O157:H7, a gram-negative microorganism, was much more susceptible to chlorine than *Listeria monocytogenes* which is classified as gram-positive. This is due to structural differences in the bacterial membrane and cell wall composition between gram-negative and gram-positive microorganisms. A study by Mir *et al*. (1997) found that inactivation by chlorine was lower for gram-positive microorganisms than gram-negative microorganisms.

Several factors contribute to microbial resistance against disinfectants (Cherchi & Gu, 2011). Together with adaptation and genetic modifications of bacterial strains, the following factors also contribute to chlorine resistance:

- inadequate chlorine residual levels due to the high organic content in the water;
- physico-chemical properties of the water;
- shielding affect provided by organic particles and
- operating conditions contributes to observed resistance

Positive and negative aspects

The following advantages can be summarised relating to the use of chlorine. It is the most commonly used disinfectant around the world (Koivunen & Heinonen-Tanksi, 2005b; Van Haute *et al*., 2013) and is very effective against a broad range of microorganisms (Eckert, 2013). The strong oxidising capacity of chlorine also reduces odour and taste problems, prevents slime and algal growth and

maintains the water quality in distribution systems (Wong, 2002). Chlorine also leaves a residual that prevents microbial recontamination in water systems.

Chlorine is a recommended treatment option for irrigation due to the low installation and operating costs and reliable variability (Freese & Nozaic, 2004; Van Haute *et al.*, 2013). It is relatively easy to handle and required simple dosing (Freese & Nozaic, 2004). Both sodium hypochlorite and calcium hypochlorite can be used in large-scale operations to treat irrigation water and to date, no other disinfectant has been found to compete against this disinfectant's overall versatility (Freese & Nozaic, 2004; Voigt *et al*., 2013).

The main drawback concerning the use of chlorine is the formation of DBPs (THMs) that are considered as carcinogenic and mutagenic, although little evidence is available proving the effect on human health specifically (Freese & Nozaic, 2004; Koivunen & Heinonen-Tanski, 2005b). Chlorine disinfection is very dependent on water quality and DBP formation occurs due to the reaction between remaining chlorine residuals and organic substances present in the water (Wong *et al*., 2002; Crebelli *et al*., 2005). Therefore, the use of chlorine for fresh-cut produce washing is permitted in European countries such as Switzerland, Germany, Netherlands, Belgium and Denmark (Van Haute *et al*., 2013).

Hypochlorite solutions are highly unstable since degradation takes place upon heat and light exposure (Freese *et al*., 2003; Newman 2004). Therefore, safety measures should be in place during storage. Granular hypochlorites are much more stable than liquid hypochlorites (Newman, 2004), however, combustion can occur when the latter are exposed to heat or readily oxidisable organic matter (Freese *et al*., 2003). With regards to disinfection area, good ventilation should be maintained throughout dosing as inhalation by operators may lead to harmful health effects.

In conclusion, chlorine has been used for more than a century as it is a very effective and the most popular disinfectant for water decontamination. The main drawback concerning the use of chlorine is the possible formation of DBPs especially in low quality irrigation water. This disadvantage will be linked to the current state of South African rivers, since rivers are the main source of irrigation applied by farmers. However, the public health benefits provided by chlorine utilisation greatly exceeds the dangers caused by THMs. Chlorine is very effective at eliminating a broad range of bacteria such as *E. coli* and total coliforms that are general indicators of water quality in South Africa. The ability to disinfect water with chlorine at farm-scale may be feasible since chlorine can be applied on large scale to make water suitable for use prior to irrigation.

Peracetic acid (PAA)

Background

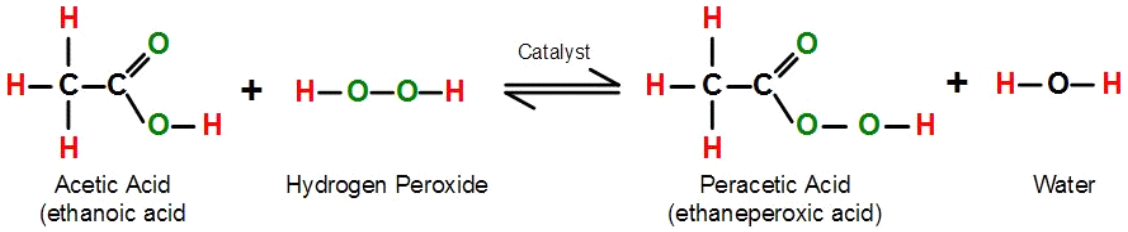
Freer and Novy were the first to discover the germicidal effect of PAA in 1902 when mentioning 'the excellent disinfection and cold sterilisation actions of PAA (Kitis, 2004). In 1946 it was used as a disinfectant in gnotobiotics (Reyniers, 1946) and in 1949 Hutchings & Xezones (1949) proved that PAA was the most effective against *Bacillus thermoacidorans* when tested amongst 23 other

germicides. Two years later, Greenspan & MacKeller (1951) determined PAA's bactericidal, fungicidal and sporicidal concentrations at 0.001%, 0.003% and 0.3% ($v.v^{-1}$), respectively.

Apart from water disinfection, there are also a few environmental disinfection applications of PAA such as cooling towers, ion exchangers, membrane hollow fibres as well as combined sewer overflows (Kitis, 2004). Industrial disinfection applications of PAA are commonly found within the beverage, medical, pharmaceutical and food processing industries (Kitis, 2004; Zanetti *et al*., 2007) and only within the last 20 years PAA's efficiency towards water/or wastewater disinfection has been discovered (Dell'Erba *et al*., 2007). Peracetic acid disinfection of wastewater was first published by Baldry & French (1989) and Baldry *et al*. (1991) in the late 1980's. Previous studies showed that PAA effectively deactivated the presence of indicator and pathogenic microorganisms in wastewater (Stampi *et al*., 2001, Stampi *et al*., 2002; Salgot *et al*., 2002; Wagner *et al*., 2002) and another study used PAA for growth control of water pathogens in irrigation water (Parke & Fisher, 2012). Due to the fact that PAA is a recently adopted disinfectant, literature cites the use of PAA as a wastewater disinfectant noticeably more than irrigation water disinfection (De Luca *et al*., 2008).

Mode of action

Peracetic acid ($C_2H_4O_3$) is a combined mixture of acetic acid (CH_3COOH) and hydrogen peroxide (H_2O_2) in a watery solution (illustrated in equation below) (Block, 1991; Profaizer *et al.*, 1997; Dell'Erba *et al*., 2007; LENNTECH, 2014):



The reaction may occur in the presence of a catalyst such as sulphuric acid (Blok, 1991) and at a pH below 2, this colourless and bright solution exhibits a sharp odour mainly due to the acetic acid component (Kitis, 2004; LENNTECH, 2014). On commercial level, PAA is available in quaternary equilibrium solutions consisted of the following: CH_3COOH, H_2O_2 and PAA in water (Gehr *et al*., 2003; Koivunen & Heinonen-Tanski, 2005a; De Luca *et al*., 2008; Luukkonen *et al*., 2014). The commercial form of PAA (10 - 15%) is much more stable than PAA solutions with higher- and lower strength solutions (Kitis, 2004). The commercial form of PAA is used in many water disinfection studies (Profaizer *et al*., 1997; Koivunen & Heinonen-Tanski, 2005a; Luukkonen *et al*., 2014).

PAA has great disinfection capability against enteric bacteria and to a lesser extent in descending efficiency, against viruses, bacterial spores and protozoan cysts (Stampi *et al*., 2001; Stampi *et al*., 2002; Koivunen & Heinonen-Tanski., 2005a). It has a high oxidation potential of 1.81

electronic volts (eV) and is a stronger disinfectant than hydrogen peroxide, chlorine dioxide, chlorine and bromine (Newman, 2004; LENNTECH, 2014).

There is limited research available on the exact mode of PAA disinfection, but the reaction takes place in a similar way to peroxides and other oxidants (Block, 1991; Gehr *et al*., 2003). Peracetic acid's disinfection capability is based on the generation of ROS, such as superoxide radicals (O_2^- or HO_2) and hydroxyl radicals (HO) (Flores *et al*., 2014) which conduct the oxidative stress within the microorganisms, ultimately aimed at the disruption of the DNA molecule.

First, the chemiosmotic function of the lipoprotein cytoplasmic membrane is disrupted by means of dislocation or cell wall rupture (Leaper, 1984; Baldry & Fraser, 1988). Only gram-positive microorganisms have this lipoprotein membrane, but the action is also executed in gram-negative microbes since it has equal effectiveness against outer membrane lipoproteins (Leaper, 1984). Peracetic acid binds and penetrates these lipids causing the oxidation (denaturation) of sensitive sulfhydryl and sulphur bonds in proteins, enzymes and lipids (Block, 1991; Gehr *et al*., 2003), subsequently disintegrating the cell membrane (Block, 1991; Gehr *et al*., 2003). Furthermore, it is also possible for intracellular PAA to oxidise essential enzymes to damage active transport across membranes, vital biochemical pathways and intracellular solute levels (Fraser *et al*., 1984). The bases of DNA molecules are targeted by PAA (Tutumi *et al*., 1973) restricting transcription and translation processes, ultimately leading to mutations.

Disinfection by-product (DBP) formation

Many studies suggested the use of PAA as disinfectant rather than chlorine sources to avoid drawbacks caused by chlorine and its DBPs (De Luca *et al*., 2008). Peracetic acid is readily decomposed into harmless by-products such as acetic acid, oxygen and water (Koivunen & Heinonen-Tanski, 2005b; Zanetti *et al*., 2007; Kobylinski & Bhandari, 2010; LENNTECH, 2014) and does not release significant amounts of mutagenic or toxic DBPs (Booth & Lester, 1995; Monarca *et al*., 2000; Veschetti *et al*., 2003; Crebelli *et al*., 2005; Koivunen & Heinonen, 2005b). However, the possibility of their occurrence could not be ruled out (Zanetti *et al.*, 2007). Monarca *et al.* (2001) isolated the by-products from river water after being treated with PAA and predominantly detected the presence of non-mutagenic carboxylic acids. The latter are formed when PAA oxidises organic particles present in water (Monarca *et al*., 2002) and sometimes the formation of aldehydes also occur, but are eventually broken down into carboxylic acids and carbon dioxide (Booth & Lester, 1995; Crebelli *et al*., 2005; Dell'Erba *et al*., 2007). Research done on municipal wastewater indicated that high dosages of PAA will introduce significant amounts of genotoxic by-products into the water that may be hazardous for human and environmental exposure (Zanetti *et al*., 2007). Although few studies mentioned the formation of DBPs after PAA treatment, much lower levels are formed in relation to other chemicals such as chlorine and ozone (Kitis, 2004). Also, PAA is very effective at low concentrations which also limits significant DBP formation or chemical residues in effluents (Veschetti *et al.*, 2003; Crebelli *et al*., 2005; Koivunen & Heinonen-Tanski, 2005a). Koivunen &

Heinonen-Tanski (2005a) detected PAA residues of 1 – 2 $mg.L^{-1}$ in water following disinfection but stated that low residual concentrations do not cause any harmful ecological effects since these residues are diluted rapidly after disinfection.

Factors influencing disinfection efficiency:

pH

Although PAA's activity has a low dependence on the pH, it is more effective at a lower pH (Kitis, 2004). The undissociated form (CH_3COOOH) of PAA initiates the biocidal activity towards microorganisms (Colgan & Gehr, 2001; Kitis, 2004). Peracetic acid has a pKa of 8.2 (i.e. pH above 9) and its dissociated form (CH_3COOO^-) mainly occurs at alkaline conditions which has shown to decrease its disinfection efficiency (Baldry & French, 1989; Sanchez-Ruiz *et al*., 1995; Tutumi *et al*., 1973). However, at a pH from 5 – 8 it was shown that the disinfection efficiency of PAA was not affected and Sanchez-Ruiz *et al.* (1995) observed that coliform removal at pH = 7 was 2 – 3 logs greater than at pH = 10. Likewise, PAA performance against coliforms was greater at neutral or mild acidic conditions (Baldry & French, 1989).

Temperature

PAA has strong antimicrobial properties functioning over a wide range of temperatures (0 – 100°C) and its disinfection capability increases with temperature (Profaizer *et al*., 1997). This is illustrated by laboratory and full-scale experiments performed on wastewater in Brazil and Italy where it was shown that PAA disinfection efficiency was higher than NaOCl at warm temperatures (Baldry *et al*., 1995; Stampi *et al*., 2001). Similarly, results by Profaizer *et al.* (1997) have shown bacterial inactivation at 20°C was 1.7 times greater than at 10°C.

Organic matter

There is evidence in literature indicating that high organic contents (including BOD, COD and TSS) leads to decreased PAA efficiency in treated wastewater (Shanchez-Ruiz *et al*., 1995; Colgan & Gehr, 2001; Stampi *et* al., 2001; Gehr *et al*., 2003; Kitis, 2004; Koivunen & Heinonen-Tanski, 2005a; Zanetti *et al*., 2007, Flores *et al*., 2014). A five-day research done on wastewater only showed two days of significant PAA disinfection. During the other 3 days the COD, BOD and TSS levels were too high and influenced PAA disinfection (Gehr *et al*., 2003). Julio *et al*. (2014) investigated the effect of TSS on PAA efficiency before (TSS = 15.08 $mg.L^{-1}$) and after filtration (TSS = 0.95 $mg.L^{-1}$) in wastewater. It was reported that PAA efficiency was 91% before filtration compared to 99% after filtration (Julio *et al*., 2014).

Contrary to previous findings, Lazarova *et al.* (1998) and De Luca *et al.* (2008), have found that PAA disinfection remained constant with low levels of TSS ranging between 11 – 40 $mg.L^{-1}$. Similar results were also found by Stampi *et al.* (2001) where TSS levels up to 100 $mg.L^{-1}$ resulted in good PAA disinfection. Despite contradicting results found by researchers, a pre-treatment such as filtration is recommended prior to PAA disinfection (Luukkonen *et al*., 2014) to remove substances

such as organic material and TSS in the water. These provide protection to microorganisms causing increased consumption of PAA in the water matrix (Koivunen & Heinonen-Tanski, 2005a).

Concentration (dosage) & contact time

Gehr *et al.* (2002) noted that PAA dosages of 2 – 6 $mg.L^{-1}$ removed faecal coliforms from primary effluents to below 1000 $cfu.100\ mL^{-1}$ after 60 minutes. After 120 minutes, PAA was consumed and also, Gehr *et al.* (2003) observed similar results. Although a disinfection period of 120 min was allowed, studies have proved that most disinfection takes place within the first 60 min. Exposure times exceeding 60 min would not have beneficial consequences due to the small amount of PAA residuals remaining after 60 min of disinfection (Gehr *et al*., 2002). Julio *et al*. (2014) applied 10 – 20 $mg.L^{-1}$ PAA to primary treated effluent and obtained a 5.1 faecal coliform log reduction after a reaction time of 15 min. Koivunen & Heinonen-Tanski (2005a) disinfected primary effluent with 5 – 15 $mg.L^{-1}$ PAA for 27 min and reduced total coliforms with 3 – 4 logs (initial counts – 4.4×10^4 $cfu.100\ mL^{-1}$).

Compared to primary wastewater treatments (removed gross, suspended and floating solids) discussed above, secondary wastewater (reduced contaminants or growths that remained after primary treatments) required lower PAA concentrations of 0.6 – 4.0 $mg.L^{-1}$ to achieve faecal coliform reductions to below 1 000 $cfu.100\ mL^{-1}$ (Gehr *et al*., 2002). Koivunen & Heinonen-Tanski (2005a) also found that 2 – 7 $mg.L^{-1}$ PAA and 27 min reduced total coliforms by 3 logs (< 500 $cfu.100\ mL^{-1}$) in secondary wastewater (initial coliform counts – 4.8×10^5 $cfu.100\ mL^{-1}$). Similar results showed a maximum total coliform and *E. coli* reduction, ranging between 4.5 – 5.5 logs, after 15 $mg.L^{-1}$ PAA was added to secondary wastewater for 38 min (Antonelli *et al*., 2013). For tertiary wastewater treatment, lower doses and contact times are required for disinfection. Koivunen & Heinonen-Tanski (2005a) also disinfected tertiary wastewater with 2 – 7 $mg.L^{-1}$ PAA for 27 min and reduced total coliforms by 3 logs (initial coliform count – 5.4×10^4 $cfu.100\ mL^{-1}$). Luukkonen *et al*. (2014) has found that a dose of 1.5 – 2.0 $mg.L^{-1}$ and a contact time of 10 – 15 min was acceptable for effective bacterial reduction in tertiary wastewater and a PAA concentration of 1.5 – 2.0 $mg.L^{-1}$ is deemed economically viable (Profaizer *et al*., 1997).

A South African research study by Freese *et al*. (2003) investigated wastewater treatment options and noted that *E. coli* was reduced form 17 700 $cfu.100\ mL^{-1}$ to less than 100 $cfu.100\ mL^{-1}$ at a PAA concentration and exposure time of 5 $mg.L^{-1}$ and 30 min. Peracetic acid is fast reacting, therefore one can say that concentration is more influential regarding PAA disinfection efficiency than exposure time (Azzellino *et al*., 2011) because most bacterial reductions occur within the initial stages of the disinfection period (USEPA, 2012). For instance, a 10 fold reduction of faecal coliforms was experienced within 8 – 10 min of PAA addition showing that PAA is a fast acting disinfectant (USEPA, 2012). However, longer contact times (i.e. 30 min) ensure that more resistant pathogens are being destroyed due to the initial resistance to PAA diffusion through the cell membrane (Rossi *et al*., 2007). PAA is rarely used in high concentrations, except if short contact times are applied.

The active concentration of PAA is the main determinant for effective microbial reduction while the effect of longer contact times will be greater at low dosages (Koivunen & Heinonen-Tanski, 2005a; Zanetti *et al*., 2007).

South African standards for irrigation water (≤ 1 000 faecal coliforms.100 mL^{-1}) (DWAF, 1996) are the key determinants of the correct concentrations and exposure times for desired disinfection. Most studies found that PAA was able to achieve a reduction level of 3 – 5 logs in total coliforms, faecal coliforms and *E. coli,* with PAA concentrations ranging from 5 – 10 $mg.L^{-1}$ applied for different contact times.

Type of microorganism

Microorganisms are uniquely designed according to their cellular structure, composition and physiology which determines their susceptibility towards disinfectants. Peracetic acid disinfection efficiency differs among microorganism species and has been shown to be effective against the following in descending order: enteric bacteria, viruses, phages, bacterial spores and protozoan cysts (Stampi *et al*., 2001; Stampi *et al*., 2002; Koivunen & Heinonen-Tanski, 2005b). Typical water quality indicators include faecal coliforms, *E. coli* and coliphages (Lin & Ganesh, 2013) and each of them show different levels of resistance in the way they react towards chemical treatments. A study by De Luca *et al.* (2008) investigated the action of PAA at 1.5 – 2.0 $mg.L^{-1}$ on microorganisms which resulted in reduced presence of total and faecal coliforms and *E. coli*, however the abatement of phages and enterococci were much lower. Phages and enterococci are indicators of the probable presence of enteric viruses. Of all the microbes investigated in this study, *E. coli* appeared to be the most sensitive to PAA (De Luca *et al*., 2008). Kitis (2004) obtained similar results, stating that higher PAA dosages, up to 150 $mg.L^{-1}$ PAA, were required for resistant viruses such as *F*-specific bacteriophage MS2 (Koivunen & Heinonen-Tanski, 2005b). Zanetti *et al.* (2007) observed *E. coli*, faecal and total coliform log reductions of 2.43, 1.77 and 1.71, respectively. At the same time, only 0.58 and 0.66 log reductions were noted for coliphages and enterococci (Zanetti *et al*., 2007)

From evidence, it is clear that various species react differently to PAA disinfection. However, it is very important to keep in mind that resistance of microorganisms vary within certain strains of the same species (AWWARF & USEPA, 2005). With reference to previous studies conducted on *E. coli*, environmental strains may show different behaviour towards PAA disinfection than environmental strains as they are physiologically better adapted to survive in adverse conditions (Wojcicka *et al*., 2007).

Positive and negative aspects

The main advantage represented by PAA is that it produces little to no DPBs when compared to other chemicals like chlorine or ozone (Monarca *et al*., 2000; Veschetti *et al*., 2003; Kitis, 2004; Crebelli *et al*., 2005) but rather is decomposed into harmless by-products, oxygen and acetic acid. Peracetic acid leaves low levels of residuals that prevent the regrowth of pathogenic microorganisms after disinfection (Freese *et al*., 2003) and Rossi *et al*. (2007) stated that microorganisms could not

repair after PAA damage, at least not within the first five hours of disinfection. The disinfectant has strong bactericidal properties functioning over wide pH ranges, temperatures and solids concentrations (Profaizer, 1997). It is a relatively stable disinfectant when stored under appropriate conditions, easy to handle and does not require expensive capital investment (Freese *et al*., 2003; Kitis, 2004). The use of PAA as a disinfectant is economically feasible as it is very effective at low concentrations and short contact times (Kitis 2004).

The following are negative aspects associated with the use of PAA. Acetic acid is a decomposition product of PAA. Acetic acid is part of the PAA mixture and formed after PAA decomposition. The decomposition of PAA to acetic acid may increase the organic content leading to increased COD levels. This compound serve as a food source for microorganisms that may result in microbial regrowth. The latter will not occur if PAA residuals are present (Kitis, 2004). Another drawback concerning PAA disinfection is the high cost, partly due to the limited availability. The high cost of PAA is estimated to decrease with increased demand and mass production capacity, especially within the water disinfection industry (Freese *et al*., 2003; Kitis, 2004). However, the application of PAA as disinfectant for water is has increased since 2003. Lastly, the organic content of solutions influence PAA efficiency, therefore a pre-treatment step prior to disinfection is strongly required.

From the information given above, an overall conclusion can be made: its wide antimicrobial activity (sporicidal, fungicidal, virucidal and bactericidal) is drawing increased attention towards the water disinfection industry. The application of PAA in low to moderate dosages offers advantages in terms of cost and insignificant by-product formation. Altogether, its broad spectrum of activity, functioning over a wide pH range, the absence of toxic residues and short contact times, are respectable reasons to consider the investment of such a disinfectant for water disinfection (Kitis, 2004). Therefore, the disinfection of irrigation water is feasible as short contact times yield an effective disinfection leading to less productive time wasted during farming.

M. RESISTANCE TO CHEMICAL DISINFECTANTS

The term ’resistance’ can be seen in the context of a strain that is not susceptible to a disinfectant concentration used in practice, or not deactivated at the concentration that usually inactivates the majority of strains of that microorganism (Russell, 1998; Cloete, 2003). After discovering that bacterial responses to disinfectants are influenced by a number of factors, bacterial resistance is categorised into two mechanisms: intrinsic (resistance can be a natural property of the microorganism) or acquired resistance (by mutation of acquisition of plasmids or transposons) (Russell, 1998; McDonnell & Russell, 1999; SHENIHR, 2009). Common resistance mechanisms exhibited by microorganisms are listed as follow (Cloete, 2003):

- Enzyme mediated resistance;
- Interaction between antimicrobial compound and biofilm matrix;

- Genetic adaptation;
- Outer membrane structure;
- Efflux pumps;
- Diffusion of biocides are limited through the biofilm matrix.

Intrinsic resistance

Intrinsic resistance is the natural chromosomal characteristic which enables microorganisms to resist disinfection (McDonnell & Russell, 1999; SHENIHR, 2009). Initially, biocides gain access through the cell membrane in order to reach their target. The nature of the organism and the thickness of the outer layer prevents the biocide from intrusion. The permeability of the cell envelope in the *Enterobateriaceae* family (Gram-negative microorganisms) decreases and limits biocide access to the cell, consequently decreasing the effective disinfectant concentration. Likewise, lipopolysaccharides (LPS) as well as the thickness of peptidoglycan layers in Gram-negative microorganisms act as permeability barriers that influence biocide entrance into the cell (McDonnell & Russell, 1999; SHENIHR, 2009). Other microorganisms such as *Staphylococci* have increased sensitivity towards disinfectants due to their lower peptidoglycan content (McDonnell & Russell, 1999). There is a possibility that the cytoplasmic membrane of bacteria may also contribute to acquired resistance mechanisms. This membrane is composed of lipoprotein that will limit the diffusion of hydrophilic compounds into the cell (McDonnell & Russell, 1999). There have also been other reports on reduced disinfection performance caused by structural components of the outer membrane including phospholipids (Boeris *et al*., 2007), fatty acid composition (Méchin *et al*., 1999) and proteins (Winder *et al*., 2000).

Moreover, the charge presented by the cell and the presence of efflux pumps also plays a role in bacterial resistance. Efflux pumps are prevalent among bacteria causing intracellular biocide degradation and is long recognised as a resistance mechanism against disinfectants (SHENIHR, 2009). Lastly, the modification of target sites, also an intrinsic resistance mechanism developed by bacteria, is not likely to occur often (SHENIHR, 2009).

Phenotypic adaptation is also an intrinsic resistance mechanism developed within bacteria. This mechanism is dependent on environmental conditions where both nutrient limitation (starvation) and reduced growth may change the bacterium's susceptibility toward disinfectants (Russell, 1998). These conditions are seen in greater context when the development of biofilms arises. Biofilms appear as microorganisms organise themselves onto solid surfaces consequently causing an extensive exopolysaccharide polymer called the glycolcalyx (Russell, 1998). Microorganisms exist within different parts of the biofilm, therefore, reduced growth rates are likely to occur. This is due to the growth-limiting biocide concentrations. The underlying cells could not be accessed by biocides which ultimately lowers the disinfection efficacy of disinfectants (Russell, 1998).

Acquired resistance

Acquired resistance developed within bacteria is a result of genetic changes in cells caused either by a mutation or the acquisition of plasmids or transposons (Russell, 1998). Non-plasmid resistance occurs when the bacteria are exposed to increased biocide concentrations. The concern with regards to acquired resistance is that the bacterium was previously susceptible to a disinfectant and then became insusceptible to that certain compound (Russell, 2002). Plasmid-mediated resistance in Gram-negative bacteria is related to the plasmid-encoded changes that takes places in proteins of the outer membrane leading to decreased susceptibility to formaldehyde in *E. coli* (Russell, 1998). The plasmid (plasmid R124) alters the surface of *E. coli* making them more resistant to cetrimide and other biocides (Russell, 1998).

Moreover, the development of resistant genes is well-documented and might contribute to cross or co-resistance (Poole, 2004). Evidence on the effect that biocides have on the transfer of genes is limited. However, one study showed some disinfectants applied at sub-lethal concentrations promoted genetic transfer while the other inhibited genetic transfer (Pearce *et al*., 1999). Intrinsic and acquired mechanisms are both experienced by bacteria, however intrinsic resistance is predominant (Russell, 1998).

Bacterial (*E. coli*) response to chlorine disinfection

Microbial resistance to chlorine disinfection has been observed in multiple research investigations and this occurrence is still widely studied. For instance, the ability of *E. coli* O157:H7 to remain viable and develop resistance in water systems poses significant health and public implications (Lisle *et al*., 1998). Aerobic microorganisms have the ability to cope with reactive oxygen species (H_2O_2, •OH, O_2^-) formed during chlorine disinfection (Zheng *et al*., 1998; Saby *et al*., 1999). Lisle *et al*. (1998) mentioned that chlorine resistance develop through a biphasic process where the extrinsic components of the cell membrane are first targeted by oxidants followed by the inducement of intrinsic components (heat shock proteins and redox regulon) to repair injury. Components of the cell membrane provide a certain chlorine demand and gradually decrease the disinfectant concentration initially presented to the membrane (Lisle *et al*., 1998).

Starvation is one of the main factors influencing bacteria's sensitivity and in an investigation by Lisle *et al*. (1998), the ability of starved (29 days) *E. coli* O157:H7 cells to resist HOCl disinfection was investigated. The starvation of *E. coli* cells alone led to an injury-resistant membrane and indicated that this organism can adapt to starvation conditions, similar to those presented by water systems, consequently increasing its resistance to sub-lethal damage by chlorine up to 0.5 $mg.L^{-1}$. The sub-lethal resistance of *E. coli* O157:H7 to chlorine is a result of the reaction between oxidants and sulfhydryl groups as well as capsule layers embedded in the bacterial membrane. Once the oxidant passed the cell membrane, intracellular resistance mechanisms are carried out by *E. coli*. Phagocytosis, also defined as oxidative burst, occurs in macrophage (Albrich & Hurst, 1982). *Escherichia coli* use certain mechanisms to repair and repel these oxidants at sub-lethal chlorine

disinfection concentrations (Storz *et al*., 1990) followed by the induction of specific heat shock (dnaK*,* grpE*, and* lon*)* regulon and redox (soxRS*)* regulon genes (Dukan *et al*., 1996). Dukan *et al*. (1996) stated that *E. coli* resistance is cell mediated and is a result of injury-induced protein synthesis.

Chesney *et al*. (1996) hypothesised that the main intracellular thiol compound present in *E. coli*, glutathione, could present a defence mechanism against HOCl attack. Glutathione is a key component of all cell systems and its redox status is remained by the thiol equilibrium inside the bacterial cell. Results showed that the intracellular glutathione defended *E. coli* from chlorine compounds and is a process that does not require the co-operation of intracellular enzymes (Chesney *et al*., 1996; Saby *et al*., 1999). The glutathione metabolism plays a significant role in *E. coli* to resist oxidation by chlorine compounds. This is established through the rapid reaction with the HOCl oxidants and, when less-reactive chlorine compounds are present, more protection is provided by recycling oxidised glutathione to glutathione (Chesney *et al*., 1996).

N. CONCLUDING REMARKS

Although water is essential for everyday life, it is an undeniable fact that various parts of the world, including South Africa, suffer from water scarcity as concerns continue to rise with regard to the limited availability and deteriorating quality of freshwater resources. Eighty percent of the world's diseases are caused by contaminated surface waters (Pandey, 2006) as substantiated by recent reports on foodborne disease outbreaks linked to fresh produce items that were traced to faecally contaminated irrigation water. In developing countries such as South Africa, due to water shortages, water is mainly extracted from rivers for the irrigation of fruit and vegetables. South African river water quality is deteriorating rapidly and various South African studies have reported high levels of faecal coliforms and *E. coli* present in river water sources used to irrigate crops (Barnes & Taylor, 2004; Paulse *et al*., 2009, Ackermann, 2010; Lötter, 2010; Kikine, 2011; Huisamen, 2012). High levels of *E. coli* in water indicate faecal contamination of water. *Escherichia coli* is an internationally adopted indicator organism of water quality and faecal contamination and some intestinal pathogenic *E. coli* species have the ability to cause serious illnesses in humans.

The treatment of irrigation water in developing countries is essential to eliminate pathogens to reduce the risk exposed to consumers by fresh produce items. Chemical (such chlorine, peracetic acid, hydrogen peroxide and ozone), physical (slow sand filtration, ultrafiltration) and alternative (UV light and ultrasound) approaches could potentially all be used to eliminate harmful pathogens present in irrigation water, thus preventing them from contaminating the surfaces of fresh crops. Of all the mentioned treatments, chemical disinfection, by means of chlorine, is the oldest and most commonly applied around the globe. Another disinfectant receiving increasing interest within the water industry, is peracetic acid and its first use only occurred in 1974. Each disinfectant functions according to its operational and environmental requirements that reflect several advantages and

drawbacks. Environmental parameters in the water are known to play a significant role in the disinfection performance and factors including pH, temperature, organic matter, suspended solids, water turbidity and more importantly, the disinfection concentration and reaction time, are main contributors influencing disinfection performance of chemical disinfectants (NHMRC, 2004). The disinfection mode executed by these chemicals eliminate or inactivate pathogenic microorganisms through a process called 'oxidation'.

Microorganisms exhibit different inactivation kinetics after being exposed to repetitive disinfection events as they have the ability to develop resistance within their cell structure (Wojcicka *et al*., 2007). Besides the difference in inactivation kinetics between species, variability may also occur between strains of the same species (Wojcicka *et al*., 2007). Reference culture strains (ATCC) are normally used in disinfection studies, but their behaviour may not be the correct representation of environmental strains naturally found in water distribution systems due to physiological differences (Wojcicka *et al*., 2007). This leads to a tough decision with regards to the most suitable and most cost effective treatment method to reduce the high microbial contaminant loads in water. South Africa is a developing country and, therefore, the treatment of water prior to irrigation is not often observed. Fresh produce consumption has increased over the past few years and so have the outbreaks of foodborne illnesses related to them. This emphasises the urgency for effective treatment methods to reduce the prevalence of pathogenic strains in water distribution systems. The implementation of chemical disinfection on farms primarily considers the costs, storage of chemicals as well as the concentration and contact time needed for sufficient disinfection.

The risks involved with use of contaminated water for fresh produce irrigation are well documented. The Water Research Commission project No. K5/2174//4 initiated a project entitled *'Scoping study on different on-farm treatment options to reduce the high microbial contaminant loads of irrigation water and related food safety risk on the treatment of contaminated river water'.* As part of the scoping study (K5/2174//4) on river water treatment prior to irrigation, this thesis focussed on PAA and chlorine disinfection at laboratory-scale, also taking into account river water characteristics that may influence disinfection.

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CHAPTER 3

EVALUATING THE EFFICACY OF PERACETIC ACID DISINFECTION ON ENVIRONMENTAL *ESCHERICHIA COLI* STRAINS AT LABORATORY-SCALE

SUMMARY

Evidence suggests that many South African rivers are unsuitable for irrigation as they are highly contaminated with microorganisms of faecal origin. *Escherichia coli* (*E. coli*) is an internationally recognised water quality indicator and has been associated with many foodborne outbreaks related to fresh produce items. To consider peracetic acid (PAA) as a potential disinfectant for contaminated irrigation water, the effect thereof was studied on various *E. coli* strains. Both environmental and reference *E. coli* strains were analysed during this study. Environmental strains were generally more resistant to PAA disinfection than reference strains. Among the six strains investigated, *E. coli* F11.2 was identified as the most resistant strain. *Escherichia coli* F11.2 was further tested against 0.5, 1.5, 3.0, 4.5 and 6.0 $mg.L^{-1}$ PAA for time intervals of 5, 15 and 25 min. Differences were seen at different PAA concentrations over a contact time of 25 min. Peracetic acid concentrations ranging from 0.5 – 3.0 $mg.L^{-1}$ were ineffective and resulted in reductions lower than 1.5 log after 25 min. Significantly higher ($p < 0.05$) reductions were achieved against 4.5 and 6.0 $mg.L^{-1}$ PAA at a 25 min contact time (4.5 $mg.L^{-1}$ – 4.94 log; 6.0 $mg.L^{-1}$ – 5.51 log). Reductions observed after a contact time of 5 min were much lower than those after 15 and 25 min of disinfection, therefore a contact time of at least 15 min is recommended for effective disinfection. The effect of water quality on PAA disinfection was determined by inoculating river water with an *E. coli* strain prior to PAA treatment at concentrations of 4.5 and 6.0 $mg.L^{-1}$. Disinfection was not influenced at low levels of physico-chemical parameters, however, higher levels of particularly the alkalinity (100.0 – 137.5 $mg.L^{-1}$ $CaCO_3$), mainly influenced PAA disinfection and resulted in *E. coli* reductions < 0.88 log. In conclusion, PAA showed high efficiency at low concentrations and contact times. This indicates that PAA disinfection is feasible for river water disinfection. However, river water quality can severely influence PAA disinfection efficiency.

INTRODUCTION

Rivers and canals are the main water sources for agricultural irrigation in South Africa (CSIR, 2010). Various studies have, however, reported the deteriorating water quality of many of these rivers (Obi *et al*, 2002; Lötter, 2010; Ackermann, 2010; Kikine; 2011; Gemmell & Schmidt; 2012; Huisamen, 2012). River water can be contaminated by faecal matter and pathogenic microorganisms as a result of inadequate sewage treatment at wastewater treatment works, as well as waste discarded into rivers from informal settlements (Britz *et al*., 2012). *Escherichia coli* and *Salmonella* spp. are enteric pathogens generally detected in contaminated river water (Aijuka, 2013). The use of contaminated water for irrigation of fresh produce may pose a threat of bacterial contamination to

humans that can lead to foodborne disease outbreaks if pathogens are not eliminated by post-harvest processing (Aijuka, 2013). Numerous enterohemorrhagic *E. coli* O157:H7 reported illnesses around the globe were linked to the consumption of mixed vegetables, salad mixes, lettuce, cilantro and celery (Johnston *et al.*, 2006; Lynch *et al.*, 2009; Ijabadeniyi, 2010). Also, the presence of pathogenic *E. coli* in river water systems contributes to increased disease outbreaks and deaths reported around the world (Masters *et al.*, 2011).

The treatment of contaminated irrigation water is limited in developing countries although the need for disinfection has increased to reduce the health risks associated with contaminated fresh produce (WHO, 2010). Chemical disinfection has been applied for many years to eliminate the amount of pathogenic microorganisms in contaminated water systems. Only within the last 20 years, peracetic acid (PAA) has been used as a water or wastewater disinfectant (Dell'Erba *et al*., 2007). Peracetic acid has been evaluated more frequently in the past few years due to its promising disinfection properties and is therefore regarded as an alternative to chlorine (De Luca *et al*., 2008; Kobylinski & Bhandari, 2010). Peracetic acid is widely applied as a sterilising agent in laboratories and as disinfectant in the medical, food and beverage industries (Kitis, 2004). Commercially, it is sold in quaternary solutions consisting of acetic acid, hydrogen peroxide and PAA (Dell'Erba *et al*., 2007). The use of PAA as disinfectant includes many advantages. As a result of its strong oxidising properties it is highly effective against pathogenic and indicator microorganisms (Koivunen & Heinonen-Tanski, 2005a). It is also readably stable under the right storage conditions (Freese *et al*., 2003). The main benefit of its application is that it does not produce significant amounts of disinfection by-products (no halogen-containing by-products) during water treatment (Monarca *et al*., 2002). Peracetic acid is also effective over broad pH and temperature ranges (Stampi *et al*.; 2001; Kitis, 2004).

Nevertheless, peracetic acid disinfection can be influenced by a number of factors such as the pH, temperature, concentration and contact time as well as the organic matter present in water. Although it has a low dependence on pH, a pH range of 5 – 8 is more favourable for effective PAA disinfection (Sanchez-Ruiz *et al*., 1995). It has also been reported that the organic content, expressed as chemical oxygen demand (COD), can influence the disinfection efficiency of PAA. This includes the presence of suspended solids that could decrease the disinfection efficacy of PAA (Zanetti *et al*., 2007, Flores *et al*., 2014).

Another advantage of PAA is its efficacy at low dosages and short contact times. Promising results have been reported for the use of PAA in wastewater treatment. Primary wastewater that was treated with 5 – 15 $mg.L^{-1}$ PAA for a contact time of 27 min resulted in total coliform reductions between 3 – 4 logs (Koivunen & Heinonen-Taski, 2005a). Julio *et al*. (2014) obtained a 5.1 log faecal coliform reduction after primary wastewater was treated with 10 – 20 $mg.L^{-1}$ PAA for 15 min. Secondary wastewater treatment requires lower PAA dosages due to differences in water qualities. A concentration of 2 – 7 $mg.L^{-1}$ PAA for 27 min was used to disinfect secondary wastewater and resulted in a 3 log reduction in total coliforms (Koivunen & Heinonen-Taski, 2005a).

Peracetic acid is effective against a broad range of microorganisms in descending order: Bacteria, viruses, bacterial spores and protozoan cysts (Liberti & Notarnicola, 1999). For instance, the elimination of faecal coliforms and *E. coli* by PAA is more effective than the disinfection of coliphages and enterococci indicating that disinfection differs between species (Lin & Ganesh, 2013). Importantly, resistance to PAA disinfection can also vary within the same species (AWWARF & USEPA, 2005). Wojcicka *et al*. (2007) observed that reference strains showed equal, in some cases better, resistance towards PAA disinfection than environmental strains. Another study done by Mazzola *et al*. (2006) reported opposite results by showing that ATCC reference strains were more sensitive toward chemical disinfection than environmental strains isolated from a water purification system.

With regards to the microbial loads reported in river water by previous studies, adequate disinfection is aimed at a reduction between 3 – 4 logs. This will produce water that conforms to standard guidelines for irrigation water (≤ 1 000 faecal coliforms.100 mL^{-1}) (WHO, 1989; DWAF, 1996). The optimum dosage and contact time combination to consider PAA as a potential treatment option for contaminated irrigation water is unknown. The purpose of this study was to investigate the efficacy of different PAA treatments against reference and environmental *E. coli* strains at laboratory-scale. Firstly, the sensitivity of two environmental *E. coli* strains against different PAA dosages were compared to those concentrations recommended for commercial application to determine at which concentration *E. coli* becomes resistant. Also, *E. coli* strains were evaluated against selected PAA concentrations and contact times to determine the optimum dosage potentially needed for river water disinfection. Ultimately, the effect of water quality on PAA efficacy was evaluated to determine its potential efficiency within a river water environment.

MATERIALS AND METHODS

Experimental design of disinfection experiments

In this research study, the resistance of six *E. coli* strains was investigated during PAA inactivation trials. *Escherichia coli* isolates were exposed to various PAA concentrations and contact times. Initial selection of concentrations and contact times was based on preliminary experiments and literature data available on PAA performance. Note that all disinfection trials were conducted in triplicate and 'no growth' was recorded as 30 colony forming units per millilitre (cfu.mL^{-1}) at the lowest dilution investigated. SigmaPlot 13 (Systat Software, Inc.) to construct graphs.

In Study 1, the sensitivity of two environmental *E. coli* strains to PAA concentrations, lower than those recommended for commercial application, was investigated. The recommended PAA concentration for commercial applications is up to 50 mg.L^{-1} for 5 min for fresh produce, where PAA is to serve as a sanitising agent for example in washing water. Environmental *E. coli* strains M53 and F11.2 were selected to test against the commercially recommended PAA concentration of 48 mg.L^{-1} and also against lower doses of 6, 12 and 24 mg.L^{-1}. Strains were enumerated after 5 min

of exposure to PAA in 0.85% ($m.v^{-1}$) sterile saline solution (SSS). The selection of the lower concentrations of PAA investigated in this study was based on previous studies on wastewater disinfection (Freese *et al*., 2003; Veschetti *et al*., 2003; Dell'Erba *et al*., 2004; Koivunen & Heinonen-Tanski, 2005a; Luukkonen *et al*., 2014).

In Study 2, strain-to-strain variation between environmental and reference *E. coli* strains (Table 1) was evaluated in SSS. All *E. coli* isolates were exposed to 6 $mg.L^{-1}$ PAA and enumerated at time intervals of 5, 15 and 25 min.

In Study 3, the optimisation of PAA treatments in terms of treatment concentrations and contact times was studied in saline solution. The effect of longer contact times and lower PAA concentrations was investigated. The most resistant strain from Study 2 was selected for this trial and was tested against 0.5, 1.5, 3.0, 4.5 and 6.0 $mg.L^{-1}$ PAA. *G*rowth was evaluated after 5, 15 and 25 min.

Study 4 focussed on the influence of river water quality on PAA efficiency during two separate trials (Trial 1 & Trial 2). This was done by using both sterile river water as well as flocculated river water. The river water samples (untreated and flocculated river water) were inoculated with environmental *E. coli* F11.2 and treated with a PAA dose of 4.5 $mg.L^{-1}$ (Trial 1) for a contact time of 25 min, as well as a PAA concentration of 6 $mg.L^{-1}$ (Trial 2) for an exposure time of 25 min. Trial 1 and Trial 2 were conducted on separate days.

General materials and methods

Escherichia coli strains

Six *E. coli* strains were evaluated during this study. Two of the six strains (ATCC strains) served as reference strains. These were compared to four environmental strains that had been previously isolated form different environmental sources (Table 1). Table 1 includes their antibiotic resistance profiles.

Table 1 Six *E.coli* strains accompanied with their isolation sources and their antibiotic resistance profiles

Strains	Source	Resistance	Study
ATCC 25922	Reference (ATCC)	None	2
ATCC 35218	Reference (ATCC)	Amp, C, STR	2
M53	River water	T; TM; Amp; STR	1 & 2
MJ56	Parsley	None	2
F11.2	River water	T	1, 2, 3 & 4
MJ58	Parsley	None	2

T - Tetracylcine; TM - Trimethoprim; Amp - Ampicillin; STR – Streptomycin, C – Chloramphenicol

API (Analytical Profile Index)

The biochemical profiles of the *E. coli* test strains (Table 1) were obtained to confirm *E. coli* identification. The API Rapid 20E identification system (Biomérieux, South Africa) was used according to the step-wise instructions compiled by the manufacturers (Biomérieux, South Africa). The Rapid 20E is specifically designed for the profiling of *Enterobacteriaceae*. Confidence levels were obtained using the API Database (V4.0) program (Biomérieux, South Africa).

Preparation of *Escherichia coli* strains

Escherichia coli cultures were maintained at -80°C in 40% glycerol (v/v %). For resuscitation, 5 mL nutrient broth (NB) (Biolab, South Africa) was inoculated with 100 µL of the defrosted bacterial suspension followed by incubation for 24 h at 37°C. Thereafter, a loop full was streaked out on Levine's eosin-methylene blue (L-EMB) (Oxoid, South Africa) agar that was prepared according to manufacturer's instructions followed by inverted incubation for 20 h at 37°C. *Escherichia coli* colonies have a metallic green sheen on L-EMB plates (Merck, 2005).

***Escherichia coli* enumeration**

Violet Red Bile Agar (VRBA, Biolab, Merck) was selected as growth medium during this study and plating was done in duplicate followed by an incubation period for 18 – 24 h at 36°C. *Escherichia coli* growth was identified as red colonies surrounded by a red halo (Merck, 2007). Colonies were counted following standard guidelines' instructions (SANS 4832, 2007). The result was recorded as $cfu.mL^{-1}$.

Solutions

A commercial form of PAA was used: Tsunami 100, composed of 31% acetic acid, 15% peroxyacetic acid and 11% hydrogen peroxide (Ecolab, South Africa). Sterile saline solution (SSS) (0.85% NaCl ($m.v^{-1}$)) served as test medium for all experiments, except when mentioned otherwise. A sterile sodium thiosulfate ($Na_2S_2O_3$) (Merck, South Africa) stock solution (1%) ($m.v^{-1}$)) was used to quench the action of PAA ensuring the exact contact time was reached. One millilitre of the stock solution was added to 8 mL of SSS and this was added only to the 10^{-1} dilution (Mazzola *et al*., 2006).

PAA treatments

Figure 1 illustrates the exact disinfection procedure followed for PAA disinfection trials. The efficacy of PAA was investigated at time intervals up to 25 min. Initially, one typical *E. coli* colony from an L-EMB plate was transferred to 5 mL NB and incubated for 20 h at 36°C. Thereafter, 1 mL of the *E. coli* inoculum was transferred to 50 mL SSS to obtain a cell density equal to the 0.5 McFarland standard (Fig. 1). A dilution series ranging from 10^{-1} to 10^{-6} was prepared and control plates (time = 0 min) were poured, aseptically, in duplicate (10^{-4} to 10^{-6}) using VRBA (Fig. 1). Then, the *E. coli* suspension was treated with PAA and *E. coli* growth was investigated at different time intervals of 5,

15 and 25 min (10^{-1} – 10^{-6}). In between each time interval evaluated, a dilution series (10^{-1} – 10^{-6}) and also an undiluted sample (10^{0}), were prepared and plated in duplicate using VRBA (Fig. 1). This was followed by inverted incubation at 36°C for 18 – 24 h and colonies characteristic of *E.coli* were counted and recorded as cfu.mL^{-1} (Merck, 2005). Triplicate tests were conducted for each disinfection treatment and 'no growth' was recorded as 30 cfu.mL^{-1} (2.48 log cfu.mL^{-1}) at the lowest dilution investigated.

Statistical analysis

The data obtained was analysed using Statistica 12.5 (Statsoft, 2014). One way, two way and mixed model repeated measures ANOVA were used as required. During Study 1, the *E. coli* log reductions obtained at various PAA concentrations were analysed using the two way ANOVA. This was followed by the mixed model repeated measures ANOVA analysing strain differences (Study 2) as well as the log reductions obtained at various PAA concentrations and contact times (Study 3). Lastly, the one way ANOVA was applied to compare the log reductions obtained in SSS, untreated and flocculated river water (Study 4). Fisher LSD (least significance difference) post hoc tests were used. A 5% ($p < 0.05$) significance level was used as a guideline for significant results.

Site selection and sampling (Study 4)

River water was sampled from the Plankenburg River (33°56'15.4''S, 18°50'53.0''E) at a pilot water treatment plant site situated on the bank of the river. Sampling was done according to the standard methods (SANS 5667-6, 2006). Prior to sampling, river water passed through a commercial sand filter. River water samples were transported in a cooler box and analysed within two hours.

Flocculation (Study 4)

During each trial, river water was flocculated prior to PAA exposure. The flocculent used, ZetaFloc 533L, was supplied by ZetaChem (South Africa). Prior to flocculation, a 700 mg.L^{-1} flocculent stock solution was prepared. The stock solution (25 mL) was added to 2.5 L river water to obtain a final flocculent concentration of 7 mg.L^{-1}. For effective distribution of the flocculent, the water was mixed for 2 min at 100 rpm followed by an additional rotation of 15 min at 40 rpm by means of a Heidolph mixer (Labotech, South Africa). The water was then allowed to settle for another 15 min before filtration through a Whatman No. 1 filter. Physico-chemical analyses (pH, COD, alkalinity, total suspended solids (TSS), electrical conductivity, turbidity and UVT%) were performed on both the untreated river water as well as the flocculated river water after flocculation. Water samples were autoclaved and stored at 4°C until needed for disinfection.

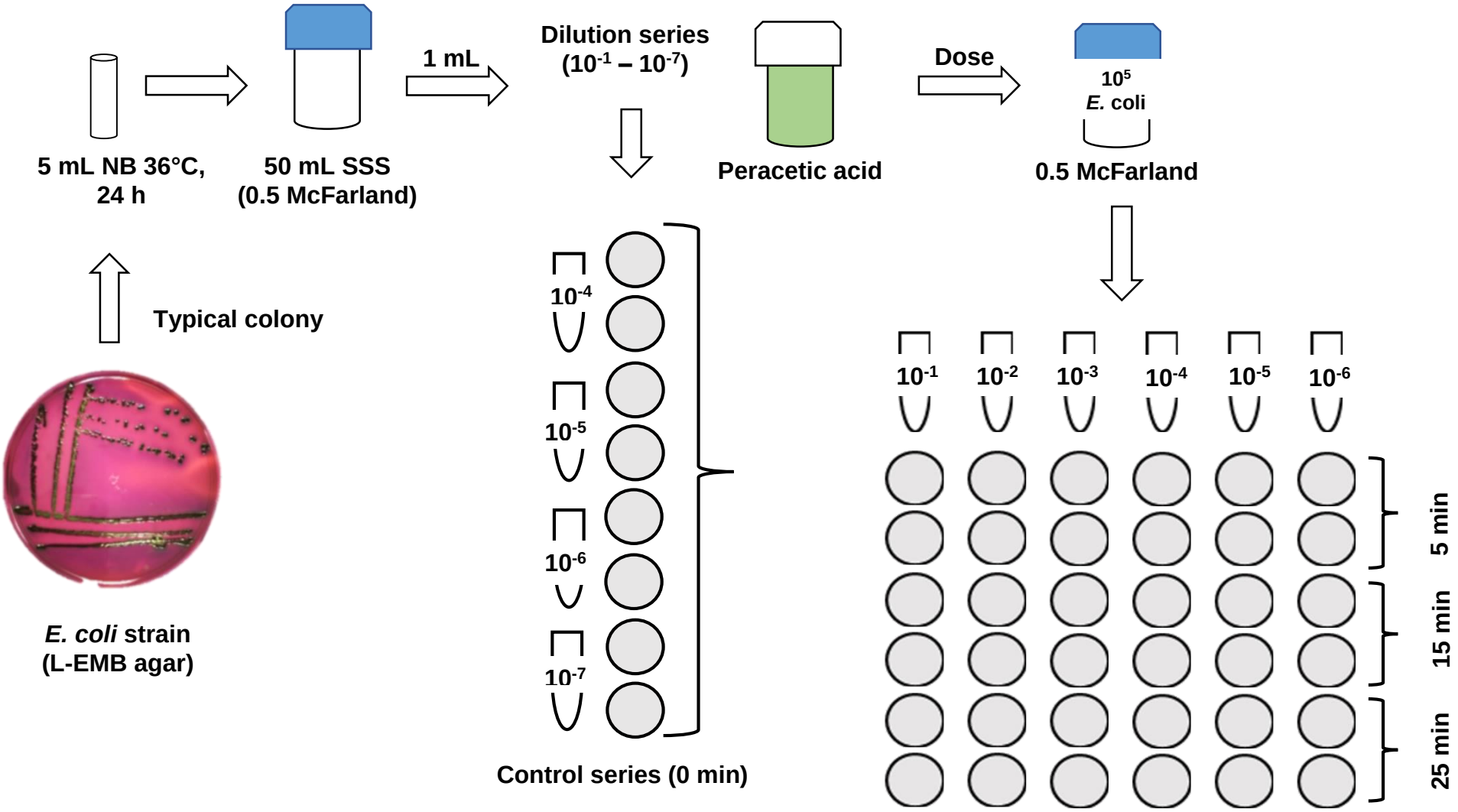

Figure 1 General protocol followed during PAA disinfection studies. Note that the diagram represents the inactivation of one *E. coli* strain at one PAA concentration.

NB – nutrient broth; SSS – Sterile saline solution

Physico-chemical analyses (Study 4)

COD, TSS and alkalinity

These parameters were determined according to standard methods (APHA, 2005). The COD ($mg.L^{-1}$) of river water was determined colorimetrically using a DR 2000 HACH spectrophotometer (Hach Co. Loveland, CO) at 585 nm. Reagents specific for a COD range between 10 – 150 $mg.L^{-1}$ were used. The suspended solids content determination was based on total water removal at 105°C and was expressed as $mg.L^{-1}$. Alkalinity indicates buffer capacity and generally reflects the water's capacity to neutralise acidic solutions. Alkalinity is expressed as $mg.L^{-1}$ $CaCO_3$ and determined by titrating river water against 0.1 M H_2SO_4.

Turbidity, electrical conductivity and ultraviolet transmission percentage (UVT%)

The pH of water was measured using a 320 pH meter (WTW, Germany). Water turbidity was measured using an Oreon AQ3010 turbidity meter (Thermo Scientific, USA). Water turbidity rates the ability of light passing through a liquid expressed as nephelometric turbidity units (NTU). Electrical conductivity was measured using a HI 8711 conductivity meter (Hanna Instruments, USA) and was expressed as $mS.m^{-1}$. The UVT% of river water was measured by a hand held, Sense™ Ultraviolet Transmittance Monitor (Berson, Germany). Deionised water was used to calibrate the meter prior to measurement.

RESULTS AND DISCUSSION

API results

Table 2 indicates the biochemical profiles for all *E. coli* isolates investigated during this study determined by the API 20E test for *Enterobateriaceae.* The most common biochemical activities of microorganisms include fermentation of sugars (carbohydrates), production of certain fermentation products and the utilisation of carbon sources (Reiner, 2013). Some *E. coli* strains expressed similar biochemical profiles. Interestingly, ATCC 25922 and environmental *E. coli* M53 (isolated from river water) showed the same biochemical profile (5144552) at a confidence level of 99.9% (Table 2).

Environmental *E. coli* strains F11.2 (isolated from river water) and MJ58 (isolated from parsley) also correlated in terms their API results (5044552) (Table 2). The biochemical profile of ATCC 25922 and M53 differed with one test from F11.2 and MJ58. *Escherichia coli* F11.2 and MJ58 yielded negative ornithine decarboxylase results. An *E. coli* microorganism with a biochemical code of 5144572 (MJ56) has the ability to ferment saccharose compared to a code of 5144552 (ATCC 25922 & M53) which showed negative test results for saccharose. Strains having the ability to ferment saccharose may stand a better chance to survive on fresh produce if transfer occurs via irrigation (Janezic *et al*., 2013).

Table 2 API codes and percentage confidence levels for each *E. coli* strain investigated

Strain	API code	% Confidence
ATCC 25922	5144552	99.9
ATCC 35218	5144570	99.5
M53	5144552	99.9
MJ56	5144572	99.5
F11.2	5044552	99.9
MJ58	5044552	99.9

STUDY 1: Efficacy of commercial and lower PAA concentrations on *E. coli* inactivation

Manufacturers recommend high concentrations of up to 50 mg.L^{-1} PAA for short contact times to sanitise fresh produce items prior to consumption. Figure 2 shows the log reductions of two environmental *E. coli* strains (M53 and F11.2) after treatment with four different PAA concentrations for 5 min. The reductions obtained at 6 mg.L^{-1} for M53 and F11.2 were 1.95 log and 1.59 log, respectively (Fig. 2).

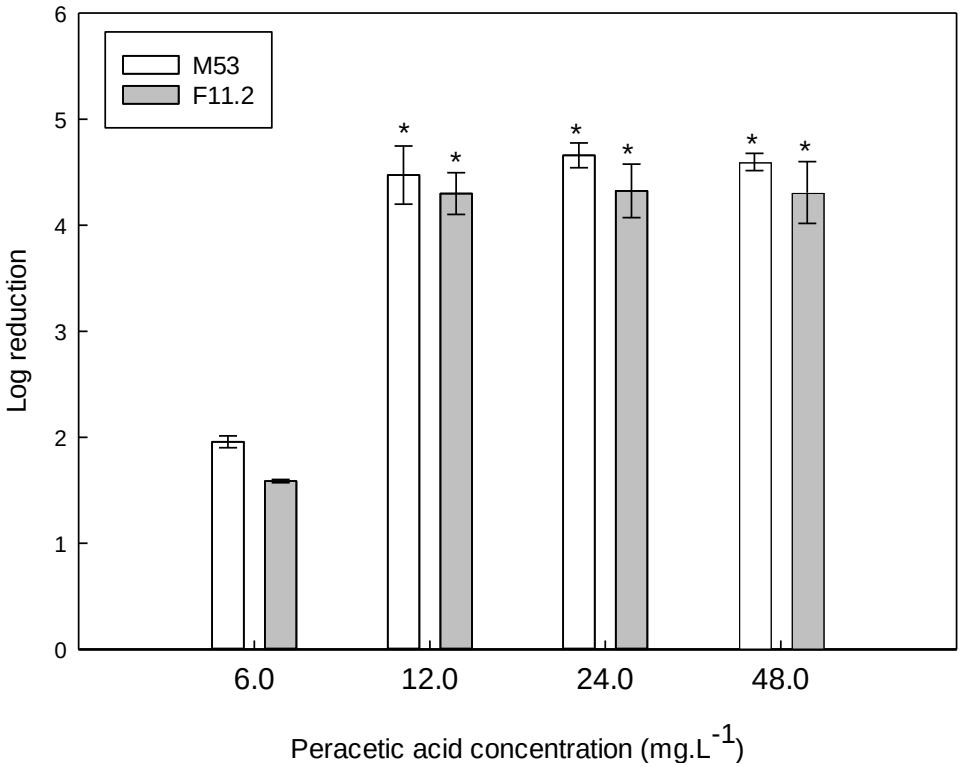

Figure 2 Log reductions observed after 5 min at different PAA concentrations for environmental *E. coli* strains M53 and F11.2 in saline solution. Error bars represent standard deviation calculated at 95% confidence level. * - No growth detected at lowest dilution (10^{-1})

It was clear that PAA concentrations ranging from 12 – 48 mg.L^{-1} were very effective against the two

E. coli strains as no growth was observed, indicating log reductions > 4 log, after a contact time of 5 min (Fig. 2). The log reductions obtained at 6 $mg.L^{-1}$ PAA differed significantly ($p < 0.05$) from the reductions observed at 12 – 48 $mg.L^{-1}$. At the lowest dose (6 $mg.L^{-1}$), both strains were more resistant to PAA disinfection. There was a 2.52 log difference in reductions between 6 $mg.L^{-1}$ and 12 $mg.L^{-1}$ for *E. coli* M53 (Fig. 2). Also, the inactivation of *E. coli* F11.2 showed a 2.71 log difference in reductions between 6 $mg.L^{-1}$ and 12 $mg.L^{-1}$ (Fig. 2). Neither of the two strains could resist the high oxidative stresses provided by PAA above 6 $mg.L^{-1}$ during 5 min exposure. It is however unknown at which point between 6 $mg.L^{-1}$ and 12 $mg.L^{-1}$ the test strains became increasingly sensitive to PAA.

The two environmental *E. coli* strains were thus very sensitive to commercial concentrations, but less so at the lower concentrations of 6 $mg.L^{-1}$ for 5 min. A longer exposure time to 6 $mg.L^{-1}$ PAA could possibly result in higher log reductions. Higher PAA concentrations would increase the cost of dosing, while longer contact times would require larger storage capacity in full-scale systems. The effect of longer contact times at lower concentrations (i.e. 6 $mg.L^{-1}$) would have to be ascertained.

STUDY 2: Strain-to-strain variation between environmental and reference *E. coli* strains

Figure 3 shows the effect of 6 $mg.L^{-1}$ PAA on four environmental *E. coli* strains and two *E. coli* ATCC reference strains (Table 1). The graph illustrates the initial microbial load before disinfection (time = 0 min) followed by PAA disinfection after 5 and 15 min for each strain.

Strain variation was clearly evident after an exposure time of 5 min. The ATCC strains differed significantly ($p < 0.05$) from environmental strains at a contact time of 5 min. The ATCC strains 25922 and 35218 displayed steeper inactivation curves than environmental isolates at 5 min, showing average reductions of 4.50 log (most sensitive) and 3.94 log, respectively. The environmental strains (F11.2, MJ58, M53 and MJ56) were more resistant to PAA disinfection at 5 min with reductions below 2.60 log (Fig. 3). Differences between environmental *E. coli* strains were also evident at a contact time of 5 min. There was no significant difference between MJ58 and M53 ($p > 0.05$), however, F11.2 and MJ56 differed significantly ($p < 0.05$) from each other at a contact time of 5 min. Of the environmental isolates investigated, MJ56 was the most sensitive strain at 5 min (2.51 log reduction) compared to F11.2 (most resistant strain) (1.54 log reduction), MJ58 (1.88 log reduction) and M53 (1.99 log reduction) (Fig. 3). An extended exposure time (15 min) resulted in a more effective *E. coli* disinfection in comparison to 5 min. No differences in strain sensitivity ($p > 0.05$) were observed as most *E. coli* strains (ATCC 25922, ATCC 35218, MJ56, F11.2 and MJ58) were totally inactivated after 15 min (Fig. 3).

Reference strains were much more sensitive to PAA treatment than environmental strains after an exposure time of 5 min. This result was expected as environmental strains may be better adapted to survival in adverse conditions than reference culture strains. Škaloud *et al*. (2003) compared the susceptibility of environmental *E. coli* STEC O157 and *E. coli* STEC O26 to *E. coli* ATCC 25922 against PAA disinfection (0.001 – 0.2 % ($v.v^{-1}$)). Results indicated that pathogenic *E. coli* (STEC) strains were generally more resistant to PAA disinfection compared to the ATCC 25922

reference strain. Wojcicka *et al*. (2007) investigated monochloramine sensitivity, of different environmental *E. coli* O157:H7 isolates against that of a reference *E. coli* O157:H7 strain. Results showed that environmental *E.coli* O157:H7 strains were in all cases more resistant to monochloramine disinfection than the reference O157:H7 strain. Also, Giddey *et al*. (2015) investigated strain variation during hydrogen peroxide treatment (250, 300 and 350 mg.L^{-1} and 120 min contact time) on eight environmental and three ATCC *E. coli* strains. Of the 11 *E. coli* strains investigated, environmental strains were generally more resistant to hydrogen peroxide disinfection (Giddey *et al.*, 2015). Peracetic acid functions in the same manner as peroxides and other chemical oxidants as it reacts with the sulfhydryl and sulfur bonds in proteins and enzymes (Chapman, 2003).

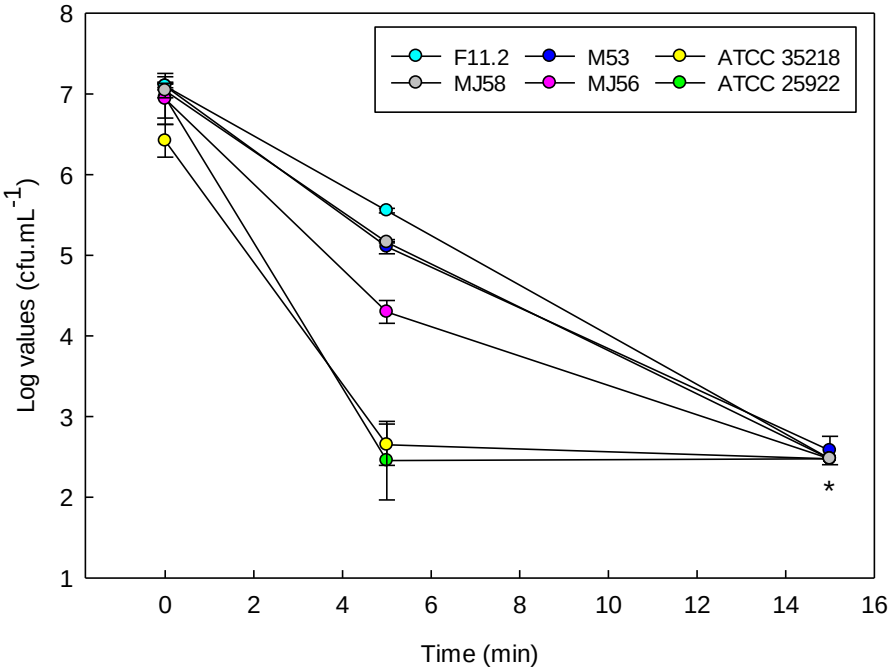

Figure 3 Inactivation curve for six *E. coli* strains against 6 mg.L^{-1} PAA at 5 and 15 min in saline. Error bars are calculated from standard deviation at a 95% confidence level. * - No growth detected at lowest dilution (10^0)

Upon exposure, microorganisms use radical defense mechanisms to protect themselves against an oxidant (Biswal *et al*., 2014). They undergo physiological changes that can protect them against environmental stressors. Resistance showed by environmental strains could have developed either through genetic phenotypic alteration, phenotypic adaptation or genetic acquisition (Chapman, 2003), decreasing the PAA disinfection efficiency. For example, genetic Sigma (σ) factors are involved in enhanced stress resistance (Davidson & Harrison, 2002). These factors are produced in response to stress, binding to core RNA polymerases which leads to the production of stress proteins that can protect them. For example, *Rpos* is a regulatory gene factor involved in gene activation for tolerance to environmental stresses (Davidson & Harrison, 2002).

The main advantages of using single reference strains for inactivation experiments include increased reproducibility of replicate tests (also between different laboratories), simplistic

interpretation of results and no interferences from the natural water environment, such as the presence of other bacteria. However, these strains are not necessarily representative of how an environmental microbial population would react to disinfection, especially if they are more sensitive than single environmental strains. The use of single environmental *E. coli* strains instead of reference *E. coli* strains during disinfection treatment optimisation might provide a more accurate indication of dosages and contact times required for effective river water treatment. Altogether, when considering the correct concentration and contact time combination for effective disinfection, also taking into account financial feasibility, the result of lower PAA concentrations (< 6mg.L^{-1}) and extended contact times need to be examined.

STUDY 3: Optimisation of PAA treatments in terms of concentration and contact time

Based on results from Study 2, the most resistant strain, environmental *E. coli* F11.2, was selected to test against PAA concentrations below 6 mg.L^{-1}. Figure 4 shows mean logarithmic reductions observed during treatments with 0.5, 1.5, 3.0, 4.5 and 6.0 mg.L^{-1} PAA at time intervals of 5, 15 and 25 min.

At a contact time of 5 min, doses of 0.5, 1.5 and 3.0 mg.L^{-1} PAA show average log reductions of 0.002, 0.01 and 0.07 (Fig. 4). The log reductions achieved during 4.5 and 6.0 mg.L^{-1} PAA treatment were, however, higher with average log inactivations of 1.44 and 1.20, respectively (Fig. 4). After a contact time of 15 min, *E. coli* inactivation increased significantly. At 3.0, 4.5 and 6.0 mg.L^{-1} the average log reductions after 15 min were 0.68, 4.51 and 5.44, respectively. However, the log inactivations obtained at 0.5 and 1.5 mg.L^{-1} PAA remained below 1 log ($p > 0.05$) for *E.coli* F11.2. After a disinfection period of 25 min, the log reductions remained more or less in the same range as observed at 15 min against 0.5, 1.5, 3.0, 4.5 and 6.0 mg.L^{-1}. At a dose 6 mg.L^{-1}, no *E. coli* was detected after 25 min (Fig.4).

Results indicated that the low PAA concentrations of 0.5, 1.5 and 3.0 mg.L^{-1} were ineffective (< 1.5 log reduction) in reducing *E. coli*, even after 25 min (Fig. 4). Reductions achieved at 4.5 and 6.0 mg.L^{-1} PAA were below 1.5 logs at a contact period of 5 min but were markedly increased to 5.06 log and 5.45 log after 15 min. No significant differences ($p > 0.05$) were observed between 15 and 25 min of disinfection (Fig. 4) as the log reductions went from 5.06 to 4.94 (4.5 mg.L^{-1}) and from 5.45 to 5.51 (6.0 mg.L^{-1}) after 25 min. It was clearly noted that for PAA dosages of 4.5 mg.L^{-1} and 6.0 mg.L^{-1}, longer contact times (15 – 25 min) were necessary to achieve the desired disinfection (3 log reduction) during this study.

Escherichia coli inactivation occurred rapidly between the 5 min and 15 min interval, after which inactivation stabilised in the same range after 25 min. This result was confirmed by other studies stating that most significant microbial reductions occurred within the first 10 – 15 min of contact time (Koivunen & Heinonen-Tanski, 2005a). Antonelli *et al*. (2013) also found that no significant increase in inactivation was observed at PAA doses exceeding 5 mg.L^{-1} and contact times over 18 min. Also, results correlated with another study conducted in wastewater indicating that

most *E. coli* were eliminated within the first 13 min of contact time (Koivunen & Heinonen-Tanksi, 2005b). This phenomenon is commonly experienced among many disinfectants. The slowing of microbial inactivation over an increased contact time is attributed to the persistence of viable microbes that are difficult to kill. This is called microbial clumping during which the microbes are protected from disinfection and they can be eliminated by longer contact times or increased concentrations (Koivunen & Heinonen-Tanksi, 2005b). It was concluded that effective PAA disinfection is dependent on both concentration and contact time.

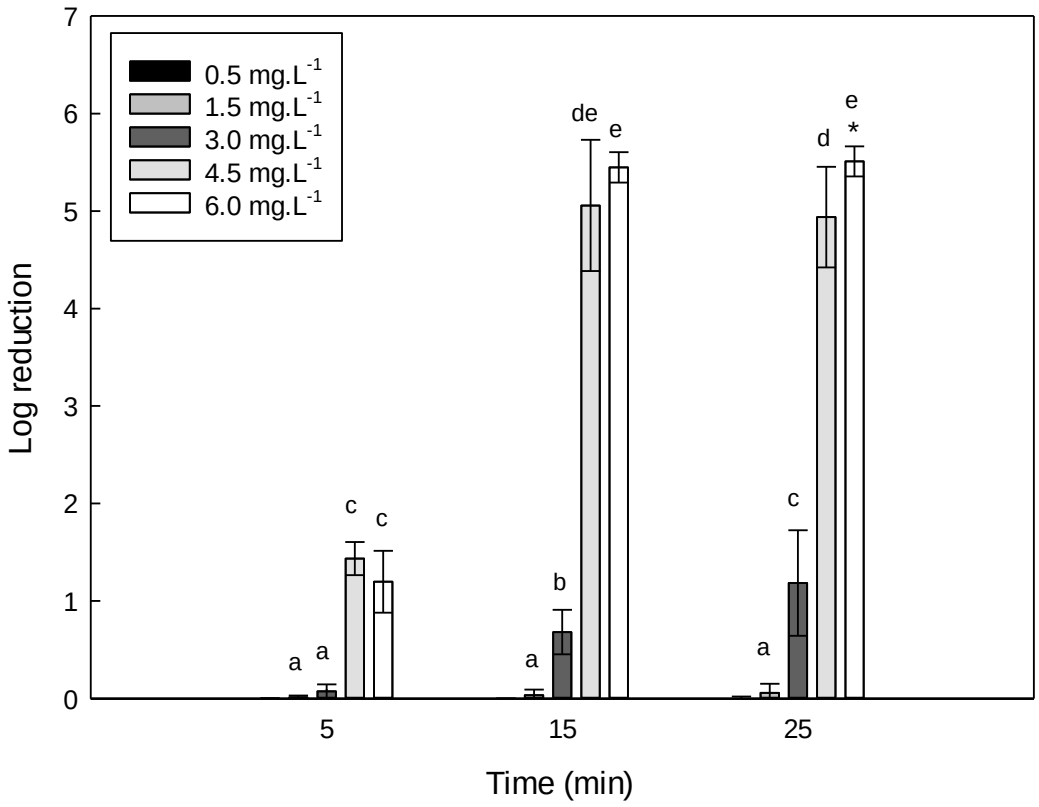

Figure 4 Log reductions observed for *E. coli* F11.2 at five PAA concentrations after 5, 15 and 25 min in saline. Error bars are calculated from standard deviation at a 95% confidence level. Statistical analysis was done using the mixed model repeated measures ANOVA and the Fisher LSD post hoc test. * - No growth detected at lowest dilution (10^0)

The greater sensitivity of *E. coli* to 4.5 – 6.0 mg.L^{-1} PAA compared to 0.5 – 3.0 mg.L^{-1} PAA indicated that the cells probably withstood the oxidative stress of PAA up to a certain maximum tolerance level. As the concentration increased to a level above this maximum, the cells became susceptible to PAA, consequently leading to increased disinfection. Microorganisms have developed many defence strategies against cellular oxidation. It could be possible that, in this study, as the PAA concentration was increased to 4.5 and 6.0 mg.L^{-1}, the bacterial cells' defence mechanisms may have been overwhelmed, resulting in significant surface, cell wall and intracellular damage.

From the results obtained in this study in saline, a suggestion could be made for river water disinfection. A PAA concentration ranging from 4.5 – 6.0 mg.L^{-1} and a contact time of 15 – 25 min could be recommended. However, this recommendation could be subject to change as the microbial population naturally present in river water together with environmental parameters (i.e. alkalinity, COD, TSS and turbidity) could influence the efficacy of PAA disinfection.

STUDY 4: Influence of the water quality on PAA treatment efficiency in river water

The effect water quality on PAA efficacy was investigated during two trials where autoclaved river water was inoculated with PAA resistant strain, *E. coli* F 11.2. Figure 5 (Trial 1) shows log reductions obtained for *E. coli* F11.2 during treatment with 4.5 mg.L^{-1} PAA for 25 min in SSS compared to untreated and flocculated river water. The water quality parameters measured for the river water sample are summarised in Table 3.

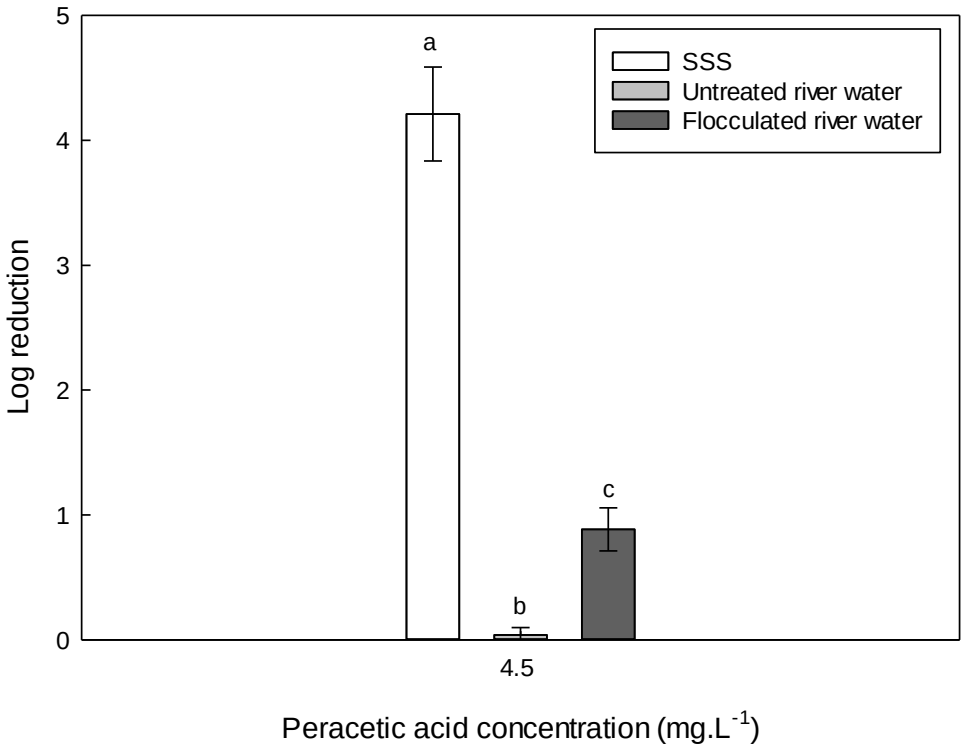

Figure 5 Log reductions observed for *E. coli* F11.2 in SSS compared to untreated and flocculated river water during disinfection Trial 1. The strain was exposed to 4.5 mg.L^{-1} peracetic acid for a contact time of 25 min. Error bars are calculated from standard deviation at a 95% confidence level. Statistical analysis was done using the one way ANOVA and the Fisher LSD post hoc test.

SSS – sterile saline solution; * - No growth detected at lowest dilution (10^0)

Significant differences ($p < 0.05$) in *E. coli* inactivation between SSS, untreated and flocculated river water were observed during Trial 1. *Escherichia coli* F11.2 was effectively reduced by 4.2 log in SSS compared to 0.04 log and 0.88 log in untreated and flocculated river water,

respectively (Fig. 5). Reductions observed in SSS were significantly higher ($p < 0.05$) than those obtained in untreated and flocculated river water. The pH of river water was 7.22 during this trial. DWAF (1996) classifies the pH of irrigation water into three different classes: Class 1 (pH < 6.5), Class 2 (pH 6.5 – 8.4) and Class 3 (pH > 8.4). Guidelines (DWAF, 1996) states that irrigation water with a pH ranging between 6.5 and 8.4 is harmless to plant crops. The river water in Trial 1 can be classified as Class 2 irrigation water and fall within the desired pH range. Furthermore, untreated river water showed a COD of 79 $mg.L^{-1}$ and was slightly lowered after flocculation to 74 $mg.L^{-1}$ during Trial 1 (Table 3). Also, the TSS content for untreated river water was 8.75 $mg.L^{-1}$ compared to 0.73 $mg.L^{-1}$ after flocculation (Table 3). The alkalinity of untreated and flocculated river water was 100 and 137.5 $mg.L^{-1}$ $CaCO_3$. Small differences were seen between electrical conductivities of 57 (untreated) and 60 $mS.m^{-1}$ (flocculated). The turbidity and UVT% of untreated river water were 16.82 NTU and 35.2% respectively, compared to 15.24 NTU and 36.5% after flocculation (Table 3). The electrical conductivity value recorded in Trial 1 (57 $mS.m^{-1}$) exceeded the guideline limit of less than 40 $mS.m^{-1}$ for irrigation water (DWAF, 1996). The electrical conductivity is an indication of the amount of dissolved solids present in the water. High amounts of dissolved solids can influence PAA disinfection efficacy (Luukkonen *et al.*, 2014) consequently resulting in insufficient microbial inactivation.

Table 3 Water quality parameters of river water before (untreated) and after flocculation (flocculated) during peracetic acid disinfection Trial 1 and Trial 2

	Trial 1		**Trial 2**	
	Untreated	**Flocculated**	**Untreated**	**Flocculated**
pH	7.22	ND	7.02	ND
COD ($mg.L^{-1}$)	79	74	18	18
TSS ($mg.L^{-1}$)	8.75	0.73	7.30	0.50
Turbidity (NTU)	16.82	15.24	6.73	1.00
Alkalinity ($mg.L^{-1}$ $CaCO_3$)	100.0	137.5	37.5	25
Conductivity ($mS.m^{-1}$)	57	60	88	89
UVT%	35.2	36.5	76	89

ND – Not determined

Firstly, it was clear that there were large differences in *E. coli* inactivation between the different solutions tested (SSS, untreated and flocculated river water) in Trial 1. Insufficient bacterial inactivation was noted in river water samples compared to effective *E. coli* inactivation in SSS. The alkalinity of river water in Trial 1 ranged between 100.0 – 137.5 $mg.L^{-1}$ $CaCO_3$. This was much higher than the river water alkalinity (25.0 – 37.5 $mg.L^{-1}$ $CaCO_3$) during Trial 2 (Table 3). The average

alkalinity of saline used in the laboratory was 12 mg.L^{-1} $CaCO_3$ and this value was much lower than the alkalinity of river water in Trial 1. The high alkalinity levels in river water may have affected PAA disinfection and explains the great differences in log reductions between saline and river water samples. Water alkalinity is an indication the water's ability to neutralise acids and at alkalinity levels determined in Trial 1, possible neutralisation of PAA could have occurred due to the water's high buffering capacity, consequently lowering PAA efficiency toward bacteria. Although the alkalinity was lowered from 137.5 to 100.0 mg.L^{-1} $CaCO_3$ after flocculation, PAA disinfection was still influenced reaching *E. coli* inactivations below 1 log.

With regards to the effect of flocculation, increased microbial inactivation and a slight improvement of the water quality were observed. Reductions in alkalinity, TSS and COD levels were observed. Although *E. coli* inactivation was slightly increased by flocculation, the overall log reduction was still low.

Increased COD can require an increase in the initial PAA dose and contact time needed for disinfection. The penetration through the cell membrane can be delayed due to the protection provided by these pollutants against the disinfectant (Wilson, 2014). The COD of saline solution under laboratory conditions was on average 100 mg.L^{-1}. This was higher than the COD detected in river water samples and could therefore not be the reason for low log reductions observed in river water. The TSS levels in river water may also have contributed to ineffective *E. coli* inactivation (Table 3). Suspended solids provide protection to microorganisms from PAA disinfection consequently consuming PAA through oxidative reactions (Koivunen & Heinonen-Tanksi, 2005b). The TSS content of untreated and flocculated river water during Trial 1 was fairly low. The irrigation guidelines, specified for drip irrigation systems, for suspended solids is 50 mg.L^{-1} (DWAF, 1996). Suspended solids below this guideline will cause no clogging problems during irrigation. Results of Trial 1 were in accordance with literature reports stating that PAA disinfection was not influenced at TSS levels of 100 mg.L^{-1} (Lefevre *et al*., 1992) and 10 – 40 mg.L^{-1} (Stampi *et al*., 2001).

American Public Health Association defines turbidity as the optical property of water that causes light to be scattered and absorbed and this measurement can be influenced by the interaction between light and the suspended particles (TSS) in water (Daphne *et al*., 2011). Therefore, water turbidity gives a good indication of the solids concentration in water, although it is not a direct measure of the TSS in water (Daphne *et al*., 2011). The turbidity and TSS levels of river water during Trial 1 could not have been the main reason for the large differences seen in reductions between saline and river water samples (< 3 log reduction), however, even a small influence on the disinfection efficiency of PAA should not be neglected.

Electrical conductivity values were above the recommended guideline values (< 40 mS.m^{-1}) (DWAF, 1996) for Trials 1 and 2. As mentioned, conductivity is an indication of the dissolved solids content and according to Luukkonen *et al*. (2014), high amounts can influence PAA disinfection efficacy. Values exceeding the guideline could probably not have been the only reason for ineffective removal of *E. coli* in Trial 1 as conductivity levels were even higher during Trial 2 where total *E. coli*

reduction was observed. Adding to this, the UVT% of river water was low and this could be correlated to the presence of organic material in river water as well as high levels of dissolved solids. Otherwise, UVT% correlated poorly with the low TSS and turbidity levels.

The water quality of river water samples in Trial 1 did affect *E. coli* inactivation. Water quality in this trial was beter than that reported in other research (Gehr *et al*., 2003), however, the influence thereof on disinfection efficiency was greater.

Figure 6 displays *E. coli* reductions observed in Trial 2 during disinfection with 6.0 $mg.L^{-1}$ PAA for 25 min in SSS, untreated and flocculated river water. The log reductions achieved for *E. coli* F11.2 in river water (untreated river water and flocculated) did not differ significantly ($p > 0.05$) from the log reductions observed in SSS (Fig. 6). The log reductions were all above 5 log in SSS, untreated and flocculated river water (Fig. 6), with no microbial growth detected after PAA disinfection in all cases (Fig. 6). The COD content remained the same after flocculation, however, TSS levels were lowered from 7.30 to 0.50 $mg.L^{-1}$ (Table 3). The alkalinity of water was slightly increased to 89 $mg.L^{-1}$ $CaCO_3$ by means of flocculation. The turbidity was lowered to 1.00 NTU and the UVT% was increased to a high of 89% after flocculation. There were differences in the water quality between Trials 1 and 2. Some of the water quality parameters measured (COD, TSS, alkalinity and turbidity) were lower than those found in Trial 1. The COD and turbidity levels, in particular, were much lower in Trial 2 in comparison to Trial 1. This result was directly reflected by the high UVT% values measured in the river water samples. The water in Trial 2 had a lower content of organic matter than in Trial 1, therefore it was expected that the inhibitive influence on PAA disinfection would be lower than observed in Trial 1. River water on this day displayed an overall better quality compared to Trial 1 and this was related to the overall lower concentrations of the water quality parameters observed during Trial 2. Also, the low UVT% of untreated and flocculated river reflected good river water quality.

Sufficient bacterial deactivation was observed in all three solutions (SSS, untreated and flocculated river water) as no *E. coli* F11.2 colonies were detected after treatment with 6.0 $mg.L^{-1}$ PAA for 25 min. The good correlation observed between disinfection in SSS compared to river water was attributed to the low alkalinity, COD, TSS, turbidity and electrical conductivity levels that reflected good water quality in Trial 2. The alkalinity of river water, in particular, was much lower than in Trial 1 (Table 3). It was concluded that PAA disinfection efficacy was not influenced at the low alkalinity levels observed during this trial. Also, the TSS content of water was very low and was further decreased by flocculation. Suspended solids are known to provide protection to microorganisms from chemical disinfection consequently lowering the inactivation efficiency of disinfectants (Van Haute *et al*., 2013). In this case, TSS levels were probably too low to impact PAA disinfection as high log reductions were observed in both untreated and flocculated river water. This result is supported by previous studies that reported good PAA disinfection over a wide concentration of TSS present in water (Lefevre *et al*., 1992; Stampi *et al*., 2001).

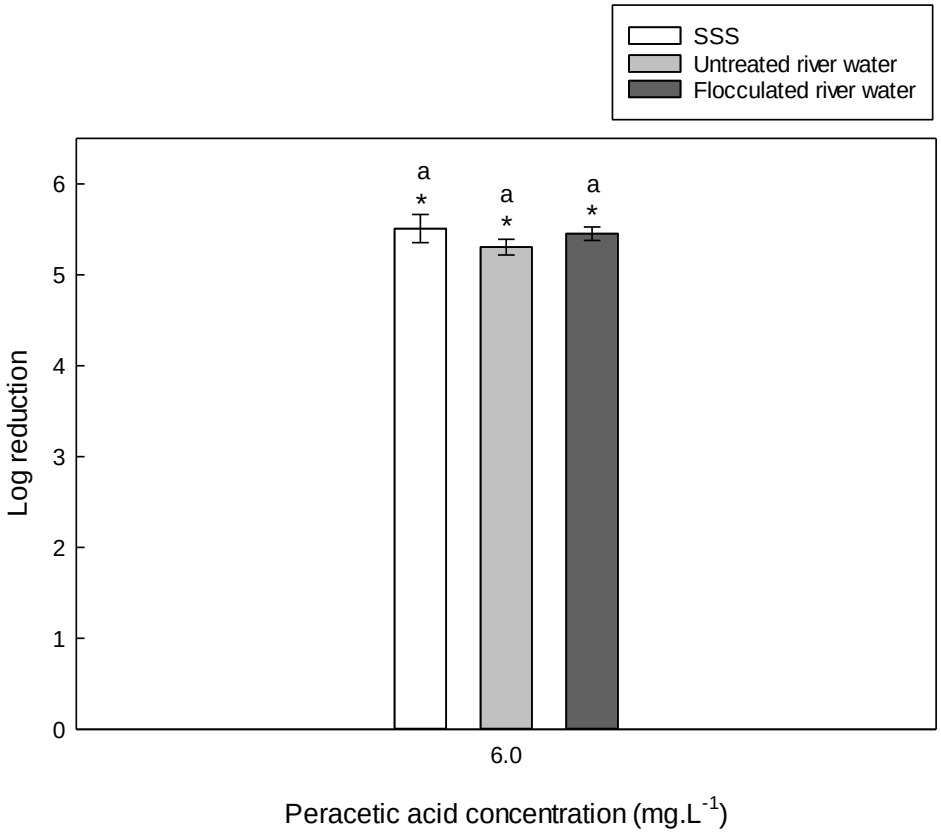

Figure 6 Log reductions observed for *E. coli* F11.2 in SSS compared to untreated and flocculated river water during disinfection Trial 2. The strain was exposed to 6.0 mg.L^{-1} peracetic acid for a contact time of 25 min. Error bars are calculated from standard deviation at a 95% confidence level. Statistical analysis was done using the one way ANOVA and the Fisher LSD post hoc test.

SSS – sterile saline solution; * - No growth detected at lowest dilution (10^0)

The differences seen between Trial 1 and 2 were attributed to varying water qualities. A lesser water quality was noted in Trial 1 in comparison to that of Trial 2. From the results it was concluded that the influence of certain water quality parameters on PAA disinfection can be significant. All water properties can influence PAA disinfection to a certain extent but the buffer capacity (alkalinity) of river water was considered the most influential factor in this study. It was also seen that flocculation had a greater effect on the improvement of water quality and increasing microbial disinfection efficacy in water with high levels of alkalinity, COD, TSS and turbidity than lower levels of these parameters. High concentrations of organic matter might consume the available PAA, lowering disinfection efficacy and limiting microbial inactivation. In cases where insufficient bacterial inactivation occurred, in the presence of low TSS levels, the presence of dissolved matter could have limited effective PAA disinfection. Large differences in the log reductions were seen between Trial 1 (4.5 mg.L^{-1}) and Trial 2 (6.0 mg.L^{-1}). Since the differences in log reductions (treated at 4.5 – 6.0 mg.L^{-1} PAA) obtained in Study 3 (saline studies) were small, the differences between Trial 1 and 2 could be attributed to the varying river water quality. Water quality varies over time due to environmental factors such as rainfall and pollution from different sources.

The variation in water quality is also accompanied with variation in microbial numbers and species (Britz *et al*., 2013). Therefore, the disinfectant concentration evaluated in this study should be adjusted depending on the characteristics of the river water. Since a single *E. coli* strain was used during Trial 1 and 2, it should also be considered that the microbial population of river water might react differently towards PAA doses evaluated in this study.

CONCLUSION

Results indicated that the investigated *E. coli* strains responded differently to PAA disinfection and the variability of strains within the same species was, thus, clearly evident. *Escherichia coli* isolates that served as reference strains were in all cases more sensitive to PAA disinfection than environmental *E. coli* strains. Bacteria are generally equipped with various defence mechanisms against chemical oxidative stresses. The development of these defence strategies is an adaptive response as a result of continual stresses posed by the environment and the resistance of environmental strains can be generated by multiple mechanisms. Thus, it is important to use environmental *E. coli* strains during disinfection optimisation rather than reference *E. coli* strains, as the latter may be very sensitive to biocides and not a good representation of the actual river water population.

A wide range of PAA dosages and contact times were effective against *E. coli* removal. The *E. coli* strains tested were inactivated at commercial concentrations and very short contact times. Therefore, the effect of lower PAA doses and longer contact times was investigated in an attempt to decrease the cost of dosing. Having said this, longer contact times may delay the production rate (limit the capacity of the plant) of treated water at commercial scale by increasing the size of the tanks or storage dams required for the longer contact times. At lower PAA doses and longer contact times, it was evident that the degree of *E. coli* disinfection was concurrently dependent on both the PAA concentration and contact time. Low PAA doses ranging from 0.5 – 3.0 $mg.L^{-1}$ were ineffective (< 1.18 log reduction) over a 25 min contact period. At these concentrations, a tolerance limit was exhibited by *E. coli*. This is explained by the fact that a slight increase in the concentration to 4.5 and 6.0 $mg.L^{-1}$, resulted in significant disinfection after 15 and 25 min (> 4 log). The disinfection efficiencies at a contact time of 5 min were much lower than reductions obtained after 15 and 25 min showing that a longer contact time can contribute to increased disinfection.

Variation in water quality was evident on different sampling days. The possible neutralisation of PAA possibly occurred in water with an alkalinity of 100 – 137.5 $mg.L^{-1}$ $CaCO_3$. If so, the germicidal capability of PAA was significantly decreased. *Escherichia coli* inactivation was, however, not limited in water representing alkalinity levels between 25.0 – 37.5 $mg.L^{-1}$ $CaCO_3$.

Overall, PAA can be suggested as an efficient and cost effective disinfection method for contaminated river water due to its high efficacy at low concentrations and contact times. The optimum PAA dosage and contact time suggested as a treatment option for contaminated river water

would be 4.5 – 6.0 mg.L^{-1} for a contact period of 25 min. This recommendation is subject to change as water quality plays a major role in the disinfection efficiency of PAA and also, the water quality varies over time. Therefore, water quality of any specific source will need to be considered individually before a treatment regime is implemented. The concentration of PAA can be increased easily since there are no concerns regarding environmental impacts. This may, however, increase costs. The microbial population in river water may react differently to PAA disinfection than the *E. coli* strains tested in this study. Therefore, further investigation into how effective PAA is against an unknown mixed microbial population in river water, taking into account varying water quality properties may give an indication of concentrations and contact times needed for irrigation water disinfection.

The effect of another well-known chemical disinfectant, chlorine, should be investigated in also considering its potential for river water disinfection. The effectiveness of this chemical disinfectant towards a wide range of microorganisms has been reported numerously.

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CHAPTER 4

THE INVESTIGATION ON THE EFFICACY OF SODIUM HYPOCHLORITE AND CALCIUM HYPOCHLORITE ON SELECTED *ESCHERICHIA COLI* STRAINS AT LABORATORY-SCALE

SUMMARY

Evidence on the deteriorating quality of irrigation water in South Africa is concerning. *Escherichia coli* (*E. coli*) is an international indicator of water quality and, if present in water systems, it could be transferred to crops during irrigation. *Escherichia coli* is an indicator of the possible presence of pathogenic isolates that can cause serious illnesses in humans if water is not disinfected prior to irrigation. Therefore, the disinfection efficiency of two chlorine sources, NaOCl and $Ca(OCl)_2$, against reference and environmental *E. coli* strains was investigated. All *E. coli* isolates were exposed to three chlorine concentrations (6, 9 and 12 $mg.L^{-1}$) for a contact period of 120 min. Reference *E. coli* strains were generally more sensitive to chlorine disinfection than environmental *E. coli* strains. Environmental *E. coli* MJ58 was the most resistant strain to chlorine disinfection during this study. Taking into account a target reduction of > 3 log, chlorine doses (NaOCl) up to 12 $mg.L^{-1}$ were considered insufficient for effective microbial inactivation as maximum reductions achieved for environmental *E. coli* were 2.48 log. Therefore, the effect of higher chlorine concentrations (14 and 24 $mg.L^{-1}$) for a contact period of 30 min on MJ58 was investigated. A concentration of 24 $mg.L^{-1}$ and 30 min inactivated *E. coli* MJ58 (> 4 log reduction). The difference between two chlorine sources, NaOCl and $Ca(OCl)_2$, against *E. coli* was studied at a chlorine dose of 12 $mg.L^{-1}$ for 120 min. The efficacy of the two disinfectants differed significantly as NaOCl resulted in lower log reductions (2.00 log reduction) than $Ca(OCl)_2$ (4.36 log reduction). The effect of water quality, in sterile river water inoculated with *E. coli* MJ58, on chlorine disinfection efficiency was investigated. Chlorine disinfection (12 $mg.L^{-1}$, 120 min) was evaluated in flocculated river water as well as untreated (not flocculated) river water and compared to SSS (sterile saline solution). Remarkable differences were seen in the reductions observed between SSS, untreated and flocculated river water. *Escherichia coli* MJ58 was totally inactivated (> 5 log reduction) after 120 min of disinfection compared to the 1.62 log observed in SSS. The chemical oxygen demand (COD) of river water ranged between 74 – 79 $mg.L^{-1}$ and did not influence chlorine disinfection. The residual chlorine levels obtained in river water samples after disinfection ranged between 2.11 – 2.34 $mg.L^{-1}$ and did not meet the chosen limit for this study (≤ 1 $mg.L^{-1}$). From this study, it was clear that chlorine was very effective in reducing *E. coli* in river water, therefore, it can be considered a potential treatment option for contaminated irrigation water. However, a varying water quality may inhibit effective microbial disinfection.

INTRODUCTION

Surface water resources are the main sources of irrigation in South Africa. Many South African rivers are unsuitable for irrigation due to high contamination levels of faecal microorganisms such as *E. coli* (Obi *et al*., 2002; Olaniran *et al*., 2009; Britz *et al*., 2013). The use of contaminated surface water resources can result in the contamination of irrigated fresh produce items. Compared to any other food category, contaminated fresh produce have been associated with the most foodborne disease outbreaks the past few years (Warriner & Namvar, 2010).

This emphasises the urgent need for on-farm water treatment options to reduce the high contaminant loads in water prior to irrigation. Chemical disinfectants that can be used to disinfect water include chlorine, peracetic acid, hydrogen peroxide, bromine and ozone. Of all the chemicals, chlorine is the most widely applied and its first application in water disinfection dates back to 1901 in Belgium (Schoenen, 2002). Since then, many studies reported its efficacy toward microorganisms such as bacteria and to a lesser extent against viruses and protozoa (Lazarova & Bahri, 2005). Some research conducted on coliform microorganisms has shown that they are easily inactivated by chlorine. Freese *et al*. (2003) showed that a chlorine concentration of 6 $mg.L^{-1}$ reduced faecal coliforms and *E. coli* with 2 – 3 logs over a contact period of 30 min. Similarly, another study showed that *E. coli* was reduced by 4.5 log units after 7.5 $mg.L^{-1}$ chlorine exposure for a contact period of 18 min (Antonelli *et al*., 2013). The chlorine concentration needed for effective disinfection is dependent on water quality as certain characteristics such as COD load and total suspended solids (TSS) may limit effective chlorine disinfection (Van Haute *et al*., 2013). The organic content of river water reacts with chlorine and lowers the amount of available chlorine for microorganisms. Van Haute *et al*. (2013) reported that the COD load in river water greatly affected *E. coli* disinfection.

Advantages regarding the use of chorine as an irrigation water disinfectant include its low cost and ease of dosing (Freese & Nozaic, 2004; Van Haute *et al*., 2013). Chlorine also provides a residual that can prevent pathogen recontamination (Voigt *et al.* 2013). Chlorine residuals can however, also react with organic and inorganic matter in the water and produce disinfection by-products (DBPs) (trihalomethanes) that may have carcinogenic and mutagenic properties (Sayyah & Mohamed, 2014). These components can affect crops and ultimately fresh produce consumers (Bouwer, 2002). Depending on the water quality, the use of chlorine in high concentrations should be limited in order to prevent high residual levels remaining in water.

Notwithstanding, there is variation in microorganisms' susceptibility toward chemical disinfectants and also, resistance between strains from the same species can also differ. Laboratory studies often make use of reference strains, however, their inactivation kinetics can differ from environmental strains (Cherchi & Gu, 2011; Li *et al*., 2013). Strains can also develop certain mechanisms over time to protect themselves against the oxidative stress conducted by chemicals.

During this research study, *E. coli* strain variation was evaluated against chlorine, using both i) calcium hypochlorite ($Ca(OCl)_2$) and ii) sodium hypochlorite (NaOCl), on different *E. coli* isolates. Disinfection trials were conducted at various chlorine concentrations and contact times for the

evaluation of chlorine as a potential disinfectant for contaminated river water. Lastly, iii) the influence of water quality on chlorine disinfection was investigated in considering its potential for contaminated river water disinfection.

MATERIALS AND METHODS

Experimental design

The efficacy of chlorine, using both High Test Hypochlorite (HTH) calcium hypochlorite ($Ca(OCl)_2$ and sodium hypochlorite (NaOCl)), was investigated on the survival of various *E. coli* strains. This was done by means of four independent studies (Study 1, 2, 3 & 4).

During Study 1, the effect of $Ca(OCl)_2$ on three *E. coli* strains (ATCC (American Type Culture Collection) 25922, M53, MJ58) was evaluated. Of the three strains, one served as a reference strain (ATCC 25922) and the other strains were environmental isolates (M53 and MJ58) that had previously been isolated from different sources (Table 1). A stock solution, representing 1 000 $mg.L^{-1}$ ($Ca(OCl)_2$) available chlorine, was prepared from granular HTH $Ca(OCl)_2$ prior to disinfection. Thereafter, each strain was dosed separately with chlorine concentrations of 6, 9 and 12 $mg.L^{-1}$ and the effect thereof was evaluated after contact periods of 30, 60, 90 and 120 min.

During Study 2, the effect of NaOCl on six *E. coli* strains (Table 1) was studied. Two of these strains (ATCC 25922 and ATCC 35218) served as reference strains and were compared to four environmental strains (M53, MJ56, F11.2 and MJ58). Each strain was treated individually with 6, 9 and 12 $mg.L^{-1}$ chlorine and *E. coli* growth was determined after four time intervals (30, 60, 90 and 120 min). Based on these results, the most resistant strain was selected to evaluate its performance against increased chlorine concentrations (14 and 24 $mg.L^{-1}$ for 30 min) in SSS.

In Study 3, the two chlorine sources, NaOCl and $Ca(OCl)_2$, were compared. The efficacy of these two disinfectants was compared using the most resistant strain from Study 2. The *E. coli* suspension was dosed with 12 $mg.L^{-1}$ chlorine (derived from both NaOCl and $Ca(OCl)_2$, respectively) and a contact time of 120 min was allowed. The residual chlorine concentration remaining after 120 min disinfection was measured using a Spectroquant cell test kit (Merck, Germany). The USEPA (2004) recommends a limit of ≤ 1 $mg.L^{-1}$ residual chlorine for reclaimed water intended for land irrigation. This was the residual limit chosen for this study as there are no residual levels specified for the irrigation of fresh produce. Also, to analyse the possible effect of pH, the pH (using a 320 pH meter (Merck, Germany)) was recorded after chlorine disinfection.

During Study 4, the effect of river water quality on chlorine disinfection was investigated. Sterile saline solution as well as sterilised (untreated and flocculated) river water were inoculated with *E. coli* MJ58 prior to chlorine disinfection. Each sample was exposed to 12 $mg.L^{-1}$ chlorine for 120 min. The chlorine residual was measured shortly after disinfection using a cell test kit (Merck, Germany). Physico-chemical analyses were performed on untreated and flocculated river water samples (pH, COD, TSS, electrical conductivity, alkalinity, turbidity and UVT%).

General materials and methods

Escherichia coli strains

Six *E. coli* strains were used for laboratory studies (Table 1). As described in Chapter 3 of this thesis, API 20E analysis (Biomérieux, South Africa) was performed on these *E. coli* strains to confirm their identity, before they were used in treatment evaluation studies. Strains were stored in 40% glycerol ($v.v^{-1}$) at freezer temperatures of -80°C. Prior to disinfection trials, each strain was removed from the freezer and 100 µL of the bacterial solution was pipetted into 5 mL nutrient broth (NB) (Biolab, South Africa). The nutrient solution was incubated for 8 h at 36°C. Thereafter, a loop full was streaked out on Levine eosin methylene blue agar (L-EMB) (Oxoid, South Africa) and incubated at 36°C for 24 h. *Escherichia coli* are generally displayed as greenish metallic colonies on L-EMB agar.

Escherichia coli enumeration

Escherichia coli enumeration was done according to standard methods (SANS 4832, 2007). After a dilution series was prepared, plates were poured in duplicate using Violet Red Bile Agar (VRBA) (Biolab, South Africa) that was prepared according to the manufacturer's instructions. *Escherichia coli* colonies were enumerated after a 24 h incubation period at 36˚C. Only purple-pink and reddish colonies (with a red halo) were counted (Merck, 2005).

Table 1 Reference and environmental *E. coli* strains used in this study as well as environmental strains' isolation sources

Strains	Source	% API Confidence levels
ATCC 25922	Reference (ATCC)	99.9 *E. coli*
ATCC 35218	Reference (ATCC)	99.5 *E. coli*
M53	River water	99.9 *E. coli*
MJ56	Parsley	99.5 *E. coli*
F11.2	River water	99.9 *E. coli*
MJ58	Parsley	99.9 *E. coli*

Solutions

Saline (SSS) (0.85% ($m.v^{-1}$)) (with average pH of 5.4) served as the test medium for most of the trials conducted, except in the case where river water was investigated. A sterile sodium thiosulfate ($Na_2S_2O_3$) (Merck, South Africa) stock solution (1%) ($m.v^{-1}$)) was used to stop the action of chlorine ensuring the exact contact time was reached. One millilitre of the stock solution was added to 8 mL of SSS and this was added only to the 10^{-1} dilution (Mazzola *et al*., 2006). Calcium hypochlorite and NaOCl representative of 70% ($m.m^{-1}$) and 15% ($m.v^{-1}$) available chlorine were supplied by Metsi Water Solutions (South Africa). Sodium hypochlorite was stored in a dark area away from sunlight as it decomposes easily and $Ca(OCl)_2$ was kept in a dry, cool area.

Statistical analysis

Data was analysed using Statistica 12.5 (Statsoft, 2014). Analyses were performed using one way or two way ANOVA as required. The two way ANOVA was used to analyse *E. coli* log reductions at respective chlorine dosages (Study 1 & 2). The one way ANOVA was used to investigate the log reductions obtained at increased chlorine concentrations (Study 2) and to analyse the differences in log reductions after NaOCl and $Ca(OCl)_2$ treatments (Study 3). The one way ANOVA was also used to analyse the log reductions obtained in SSS, untreated and flocculated river water (Study 4). Fisher LSD (least significance difference) post hoc tests were used. A 5% ($p < 0.05$) significance level was used as a guideline for significant results.

Chlorine disinfections

The general chlorine disinfection protocol is displayed by Figure 1. Prior to each disinfection experiment, a single typical *E. coli* colony from L-EMB agar was transferred to 5 mL NB followed by an incubation period for 20 h at 36°C. A bacterial inoculum was prepared with a cell density similar to 0.5 McFarland standard, in 50 mL of SSS (Fig. 1).

Firstly, initial counts (control plates) were determined in duplicate (time = 0 min) on VRBA (Biolab, South Africa) (Fig. 1). The bacterial solution was then dosed with chlorine at the particular concentration investigated and *E. coli* growth was investigated at different time intervals of 30, 60, 90 and 120 min (10^{-1} – 10^{-6}). A 1% ($m.v^{-1}$) $Na_2S_2O_3$ was used to quench chlorine activity at the specific contact time investigated. Triplicate tests were conducted for each disinfection treatment and 'no growth' was recorded as 300 $cfu.mL^{-1}$ (2.48 log $cfu.mL^{-1}$) at the lowest dilution investigated.

Water sampling (Study 4)

River water was sampled from the Plankenburg River (33°56'15.4''S, 18°50'53.0''E) in Stellenbosch. The river is located downstream from the informal Kayamandi settlement and small industrial area and is used by farmers for irrigation. Water sampling was done according to standard sampling procedures (SANS method 5667-6, 2006). Samples were transported in cooler boxes and analysed within an hour of sampling. River water was sample on one day in triplicate (three samples).

Flocculation (Study 4)

The flocculent (Zetafloc 553L, Zetachem, South Africa) was prepared in a stock solution representing a flocculent concentration of 700 $mg.L^{-1}$. This was added 5 L of river water to obtain a final flocculent concentration of 7 $mg.L^{-1}$. Subsequently, river water was rotated by a Heidolph mixer (Labotech, South Africa) for 2 min at 100 rpm followed by a decreased speed of 40 rpm for 15 minutes. For optimum flocculation, a settling time of 15 min was allowed after mixing and thereafter the sample was filtered through a Whatman No. 1 filter.

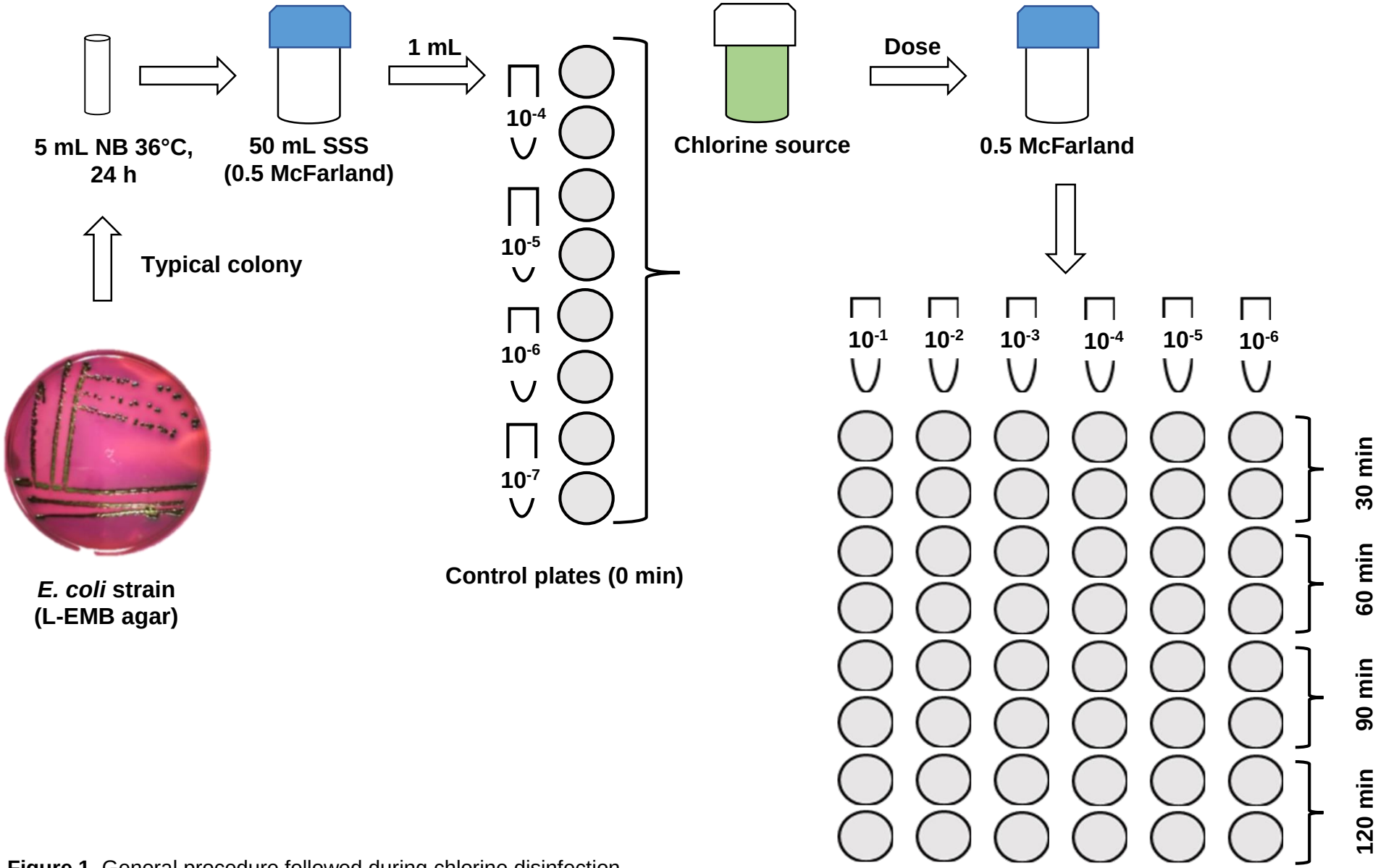

Figure 1 General procedure followed during chlorine disinfection.

NB – nutrient broth, SSS – sterile saline solution

Physico-chemical analyses (Study 4)

COD, TSS and alkalinity

The COD, TSS and alkalinity of river water were determined by standard procedures (APHA, 2005). A range of 10 – 150 $mg.L^{-1}$ was selected for the determination of COD. Analysis of COD content was based on a colorimetric method determined by a spectrophotometer set at 585 nm (DR 2000 HACH, Hach Co. Loveland, CO). The method of TSS determination is based on total water removal, only resulting in suspended solids. Water alkalinity is expressed as $mg.L^{-1}$ $CaCO_3$ and this parameter indicates the buffer capacity of solution to resist changes in pH.

pH, electrical conductivity, turbidity, ultraviolet transmittance percentage (UVT%)

The pH of river water was determined using a 320 pH meter (WTW, Germany). The electrical conductivity ($mS.m^{-1}$) was measured by a HI 8711 conductivity meter (Hanna Instruments, USA). This gives an indication of the amount of inorganic dissolved solids in water. Furthermore, water turbidity is defined as the ability of light to pass through water. A portable turbidity meter, Oreon AQ3010 (Thermo Scientific, USA) was used after it was calibrated with deionised water. The UVT% of river water was measured using the Sense™ UV-Transmittance Monitor (Berson, Germany) calibrated with deionised water.

RESULTS AND DISCUSSION

STUDY 1

The effect of $Ca(OCl)_2$ on *E. coli* inactivation

The effect of $Ca(OCl)_2$ on three *E. coli* strains was studied over a total contact period of 120 min. Figure 2 illustrates the effect of 6 $mg.L^{-1}$ chlorine on different *E. coli* strains (ATCC 25922, M53 and MJ58) over four different time intervals (30, 60, 90 and 120 min). Similarly, Figures 3 and 4 show the inactivation of these *E. coli* strains against 9 and 12 $mg.L^{-1}$ chlorine, respectively. The objective was to achieve a 3 log reduction based on previous research on the Plankenburg River, that reported high levels (250 000 – 1 000 000 $MPN.100mL^{-1}$) (Lamprecht *et al*., 2014) of faecal contamination (*E. coli*), in order to reduce microbial numbers below DWAF (1996) and WHO (1989) guidelines (≤ 1 000 faecal coliforms.100 mL^{-1}) for safe irrigation through disinfection.

The initial inoculum concentration before chlorine disinfection ranged between 6 – 7.4 log $cfu.mL^{-1}$ (Fig. 2 – 4). Differences were seen between reference ATCC 25922 and environmental *E. coli* M53 and MJ58 after a disinfection period of 120 min (Fig. 2 & 3). The reference strain (ATCC 25922) was highly sensitive to all chlorine treatments (6 – 12 $mg.L^{-1}$) and no growth was detected after 120 min (Fig. 2 – 4). At the lowest chlorine dose investigated (6 $mg.L^{-1}$), *E. coli* MJ58 showed a concentration of 6.88 log $cfu.mL^{-1}$ after 120 min of disinfection and did not vary much from the initial concentration prior to disinfection (Fig. 2). Similarly, M53 displayed a concentration of 6.20 log

cfu.mL^{-1} after disinfection which did not differ much from the initial concentration (Fig. 2). At an increased chlorine concentration of 9 mg.L^{-1} (Fig. 3), *E. coli* were reduced to lower levels after 120 min compared to 6 mg.L^{-1}. Environmental *E. coli* strains MJ58 and M53 were present in 4.52 and 3.34 log cfu.mL^{-1} after chlorine disinfection (Fig. 3). At 12 mg.L^{-1} chlorine, *E. coli* MJ58 and M53 was lowered to 2.72 log cfu.mL^{-1} and 2.60 cfu.mL^{-1} after 120 min of disinfection (Fig. 4) and increased sensitivity to chlorine was observed at this concentration. Of the two environmental strains, *E. coli* MJ58 was overall more resistant to chlorine after treatments of 6 and 9 mg.L^{-1} chlorine (< 3 log reduction).

Strain variation was clearly observed between the three chlorine concentrations investigated and this reflects the differences in their resistance levels (Fig. 2 – 4). The greatest strain variability was noted between 6 – 9 mg.L^{-1} chlorine and to a much lesser extent at 12 mg.L^{-1}. The ATCC strain showed overall great sensitivity towards 6, 9 and 12 mg.L^{-1} chlorine and was undoubtedly the most sensitive strain to chlorine disinfection. Environmental *E. coli* MJ58 and M53 showed increased sensitivity toward chlorine as the concentration increased from 6 to 12 mg.L^{-1}. This result was particularly indicated by the steeper inactivation lines displayed in Figures 3 and 4 compared to Figure 2. Contact time also played an essential role during chlorine disinfection. The most sensitive *E. coli* strain, ATCC 25922, was mainly reduced within the first 30 min of disinfection (Fig. 2 – 4). Strains that showed higher resistance to chlorine (M53 and MJ58) needed a longer contact time and their highest log reduction was mainly observed within the first 60 min of exposure (Fig. 2 & 3), however, this was also dependent on the chlorine dosage used.

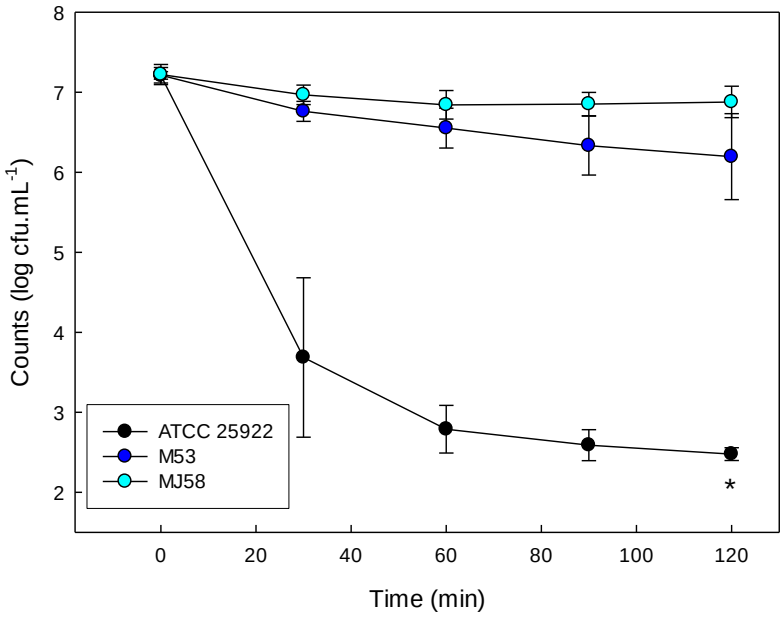

Figure 2 Inactivation curve for three *E. coli* isolates (ATCC 25922, M53 and MJ58) against 6 mg.L^{-1} chlorine ($Ca(OCl)_2$) over 30, 60, 90 and 120 min contact period in saline. Error bars were calculated from the standard deviation at a 95% confidence level. * - No growth detected at the lowest dilution (10^{-1})

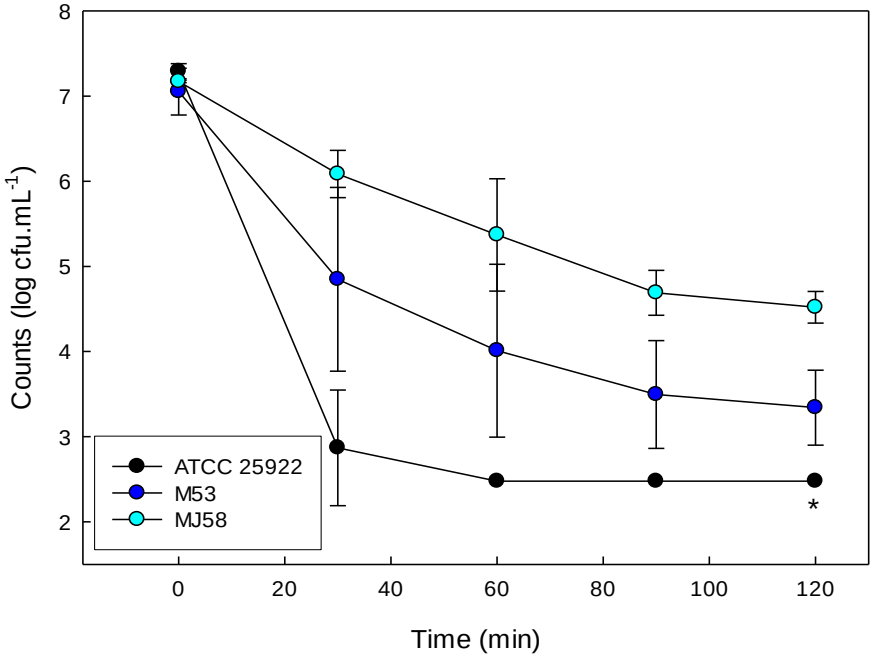

Figure 3 Inactivation curve for three *E. coli* isolates (ATCC 25922, M53 and MJ58) against 9 mg.L^{-1} chlorine ($Ca(OCl)_2$) over 30, 60, 90 and 120 min contact period in saline. Error bars were calculated from the standard deviation at a 95% confidence level. * - No growth detected at the lowest dilution (10^{-1})

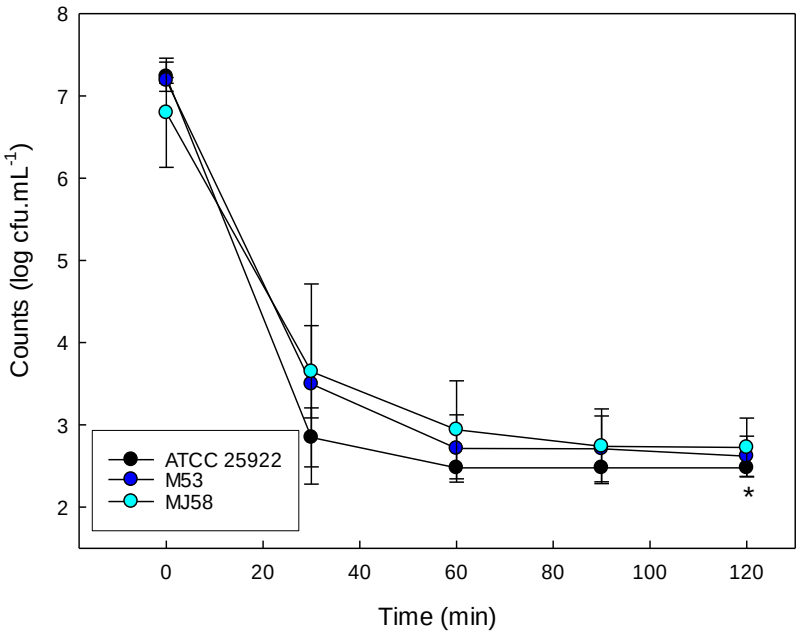

Figure 4 Inactivation curve for three *E. coli* isolates (ATCC 25922, M53 and MJ58) against 12 mg.L^{-1} chlorine ($Ca(OCl)_2$) over 30, 60, 90 and 120 min contact period in saline. Error bars were calculated from the standard deviation at a 95% confidence level. * - No growth detected at the lowest dilution for ATCC 25922 (10^{-1})

Differences were also seen between the two environmental strains. There was a greater difference at 9 $mg.L^{-1}$ between MJ58 and M53 than at 6 $mg.L^{-1}$ indicating that M53 was much more sensitive to 9 $mg.L^{-1}$ chlorine than MJ58. Of all the strains investigated, MJ58 showed the greatest resistance towards chlorine disinfection, except at 12 $mg.L^{-1}$. At 12 $mg.L^{-1}$ chlorine, little strain variation was observed indicating that this concentration was very effective in reducing all *E. coli* strains over a contact period of 120 min. Ultimately, the chlorine dosage had a greater influence on disinfection than different contact times. This is proved by the results: as the concentration increased, *E. coli* disinfection also increased.

The log reductions obtained after a contact period of 120 min (using $Ca(OCl)_2$ as a chlorine source) for ATCC 25922, M53 and MJ58 are displayed in Figure 5. This graph demonstrates the effect of free chlorine concentration in reducing *E. coli* over a 120 min disinfection period. The ATCC strain displayed a similar reduction trend, with no significant difference ($p > 0.05$) (average reduction of 4.77 log) at all concentrations as it was completely inactivated (Fig. 5). The inactivation of environmental strains (M53 & MJ58) increased as the chlorine concentration increased. There were significant differences ($p < 0.05$) in the log reductions observed between 6 and 9 $mg.L^{-1}$ chlorine for both environmental *E. coli* strains M53 and MJ58 (Fig. 5). At higher doses, more chlorine reacts with microorganisms, therefore increased log reductions were observed. *Escherichia coli* M53 showed average reductions of 1.02, 3.71 and 4.57 log at 6, 9 and 12 $mg.L^{-1}$ chlorine ($p < 0.05$) (Fig. 5). *Escherichia coli* MJ58 was the least sensitive to chlorine disinfection and was reduced by 0.34, 2.65 and 4.07 log after 6, 9 and 12 $mg.L^{-1}$, respectively (Fig.5). The log reduction for M53 at 9 $mg.L^{-1}$ chlorine compared well with log reduction achieved at 12 $mg.L^{-1}$ for MJ58 ($p = 0.16$) (Fig 5). Interestingly, at the highest chlorine dose investigated (12 $mg.L^{-1}$), there were no significant differences in the log reductions between ATCC 25922 and M53 ($p = 0.59$). There were, however, significant differences between ATCC 25922 and MJ58 at 12 $mg.L^{-1}$ ($p = 0.018$) (Fig. 5). At a chlorine dose of 6 $mg.L^{-1}$, environmental *E. coli* strains (M53 and MJ58) did not reach the 3 log reduction target level. However, at 9 $mg.L^{-1}$ (M53) and 12 $mg.L^{-1}$ chlorine (M53 & MJ58), the 3 log target reduction on environmental strains was exceeded. The main conclusion that can be made from Figure 5 is that increased *E. coli* inactivation was dose-dependent.

The ATCC reference strain was more sensitive to chlorine disinfection compared to the environmental *E. coli* isolates. Environmental strains could be using different resistance strategies that makes them more resistant to chlorine than reference strains. Also, environmental strains' inactivation kinetics may differ from that of control strains (Wojcicka *et al*., 2007). There is also a possibility that previous exposure of environmental *E. coli* isolates to chlorine may have occurred. Strains that had been previously exposed to low chlorine dosages could adapt due to a natural property of the microorganism that enables them to resist disinfection (intrinsic resistance), consequently decreasing their susceptibility toward chlorine (McDonnell & Russell, 1999). Calcium hypochlorite was effective against the three different *E. coli* isolates investigated. Effective

disinfection mostly depended on the chlorine concentration used. Notably, contact time also played a role as chlorine reacts with microorganisms over time, especially with resistant organisms.

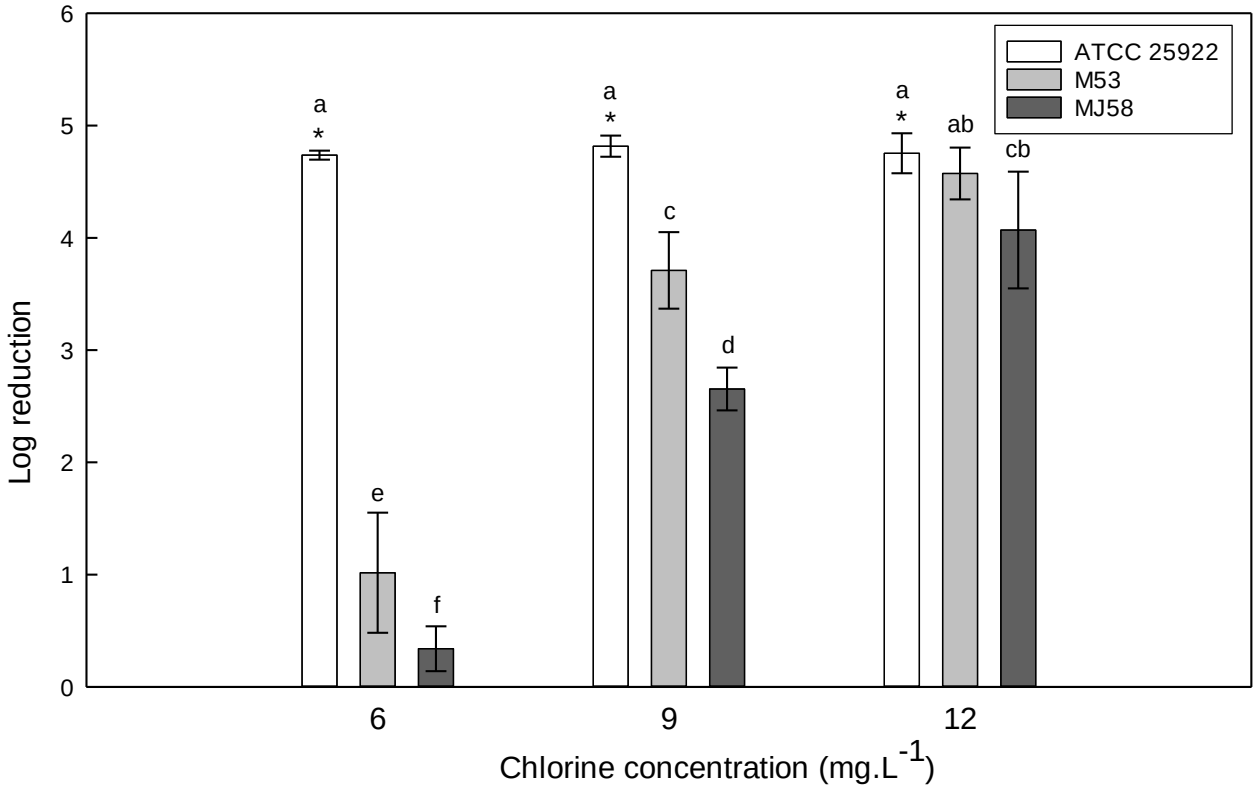

Figure 5 Log reductions achieved for ATCC 25922, M53 and MJ58 at different chlorine concentrations ($Ca(OCl)_2$) of 6, 9 and 12 $mg.L^{-1}$ over a contact period of 120 min in saline. Error bars were calculated from the standard deviation at a 95% confidence level. Statistical analysis was done using the two way ANOVA and the Fisher LSD post hoc test. * - No growth detected at lowest dilution investigated (10^{-1})

STUDY 2

The effect of NaOCl on *E. coli* inactivation

The effect of chlorine, using NaOCl, on different *E. coli* isolates was studied at various concentrations and contact times. Results display the inactivation curves for six *E. coli* strains (Table 1) at 6 (Fig. 6), 9 (Fig. 7) and 12 $mg.L^{-1}$ (Fig. 8) chlorine and four contact times (30, 60, 90 and 120 min). At 6 $mg.L^{-1}$ chlorine, the six *E. coli* strains showed very low inactivation over a contact period of 120 min. The amount of microorganisms that remained after 120 min were 7.18 (ATCC 25922), 6.81 (ATCC 35218), 7.13 (M53), 6.75 (MJ56), 7.23 (F11.2) and 7.06 log $cfu.mL^{-1}$ (MJ58) (Fig. 6). These results did not differ with more than 1 log from another. At an increased chlorine dose of 9 $mg.L^{-1}$, the disinfection of *E. coli* strains were more prominent. In this case, a difference was observed between reference and environmental *E. coli* isolates (Fig. 7). The ATCC strains were reduced to 5.85 (ATCC

25922) and 5.77 log cfu.mL^{-1} (ATCC 35218) after a contact period of 120 min (Fig. 7). This was 1.33 (ATCC 25922) and 1.04 (ATCC 35218) log units more than observed at 6 mg.L^{-1} chlorine. This was not the case for environmental strains as their inactivation after 9 mg.L^{-1} were highly comparable to results seen at 6 mg.L^{-1} after 120 min (Fig. 6 & 7). Final counts observed for environmental strains (M53 – 5.62, MJ56 – 6.05, F11.2 – 4.85 and MJ58 – 6.81 log cfu.mL^{-1}) in the presence of 9 mg.L^{-1} NaOCl only varied slightly from their initial inoculum concentrations (Fig. 7). At the highest concentration (12 mg.L^{-1}), increased variation was observed between all *E. coli* strains. Some of the lines on the graph, each representing an *E. coli* isolate, displayed steep reductions during chlorine disinfection (Fig. 8). The reference strains showed a final concentration much lower than compared to environmental strains after 120 min disinfection (Fig. 8). Note that differences were also visible between different environmental *E. coli* strains. *Escherichia coli* M53, MJ56, F11.2 and MJ58 were present in 5.62, 6.05, 4.85 and 6.81 log cfu.mL^{-1} after a total disinfection period of 120 min (Fig. 8). Of the strains tested, *E. coli* MJ58 was the least affected by chlorine disinfection as high microbial counts were determined after disinfection (Fig. 8). Environmental *E. coli* F11.2 was more sensitive to 12 mg.L^{-1} chlorine compared to 9 mg.L^{-1} (Fig. 7 & 8). Of all environmental strains evaluated, F11.2 was the most sensitive to 12 mg.L^{-1} chlorine (Fig. 8).

Although the disinfection after a total contact period of 120 min was discussed above, disinfection at different time intervals within the 120 min contact period should also be considered. The graphs indicate that longer exposure to chlorine resulted in increased *E. coli* inactivation. However, this was primarily dependent on the chlorine concentration. At 6 mg.L^{-1} (Fig. 6), the time of disinfection did not result in increased inactivation. It could be that the available chlorine concentration was too low to inactivate microorganisms with more than 1 log. As the chlorine concentration increased to 9 and 12 mg.L^{-1}, contact time did have an influence on disinfection. At a dose of 9 mg.L^{-1}, ATCC strains were affected the most within the first 60 min of disinfection (ATCC 25922 & ATCC 35218). However, the environmental strains showed low susceptibility in the presence of 9 mg.L^{-1} during the 120 min contact period. At the highest chlorine concentration investigated (12 mg.L^{-1}), the effect of contact time was clearly visible (Fig. 8). The ATCC strains (25922 & 35218) again showed the highest inactivation within the first 60 minutes of disinfection (Fig. 8). Similarly, environmental strains that were sensitive to chlorine at this concentration (M53 & F11.2) also showed the highest inactivation within this time bracket (Fig. 8).

The age of the population within a specific *E. coli* strain can differ. Young organisms are inactivated more easily than older ones as the latter develop a polysaccharide shell over their cell walls supporting their resistance to disinfectants (Sutherland, 2001). During this study, the inoculum was exposed to a constant incubation period prior to each disinfection experiment to limit age differences within strains.

Overall, MJ58 was the most resistant to chlorine disinfection. Very low inactivation of MJ58 was observed at the highest concentration investigated (12 mg.L^{-1} for 120 min). Other studies

reported the efficacy of NaOCl over a 30 min contact period on total coliforms, faecal coliforms and *E. coli* (Veschetti *et al*., 2003; Freese *et al*., 2003; Winward *et al*., 2008; Li *et al*., 2013).

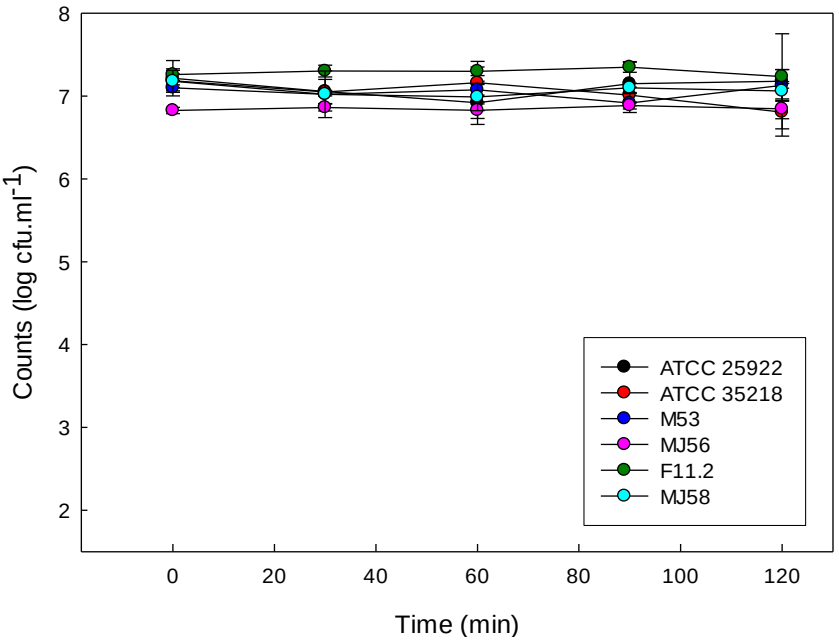

Figure 6 Inactivation curves of six *E. coli* strains (ATCC 25922, ATCC 35218, M53, MJ56, F11.2 and MJ58) against 6 mg.L^{-1} chlorine (NaOCl) at different time intervals (30, 60, 90 and 120 min) in saline. Error bars are calculated from standard deviation at a 95% confidence level.

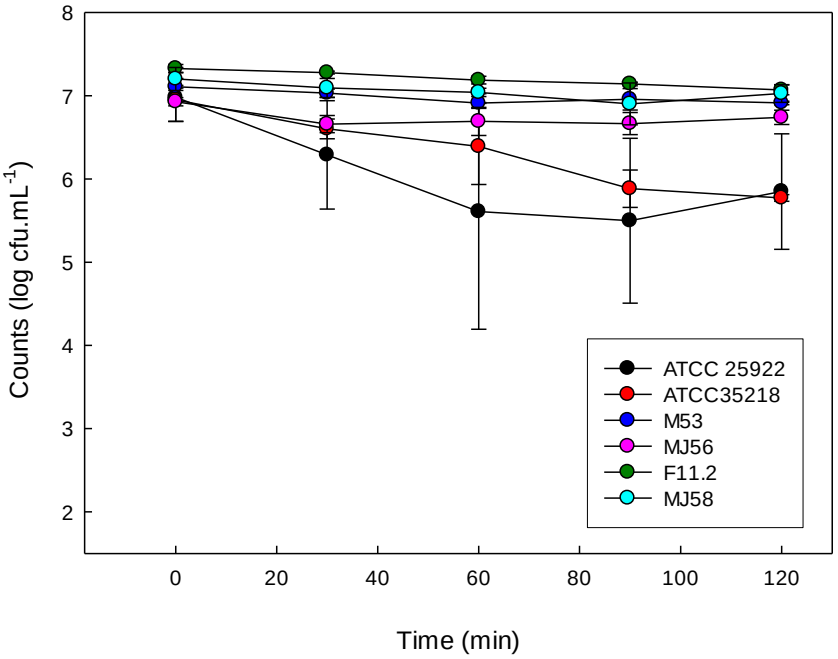

Figure 7 Inactivation curves of six *E. coli* strains (ATCC 25922, ATCC 35218, M53, MJ56, F11.2 and MJ58) against 9 mg.L^{-1} chlorine (NaOCl) at different time intervals (30, 60, 90 and 120 min) in saline. Error bars are calculated from standard deviation at a 95% confidence level.

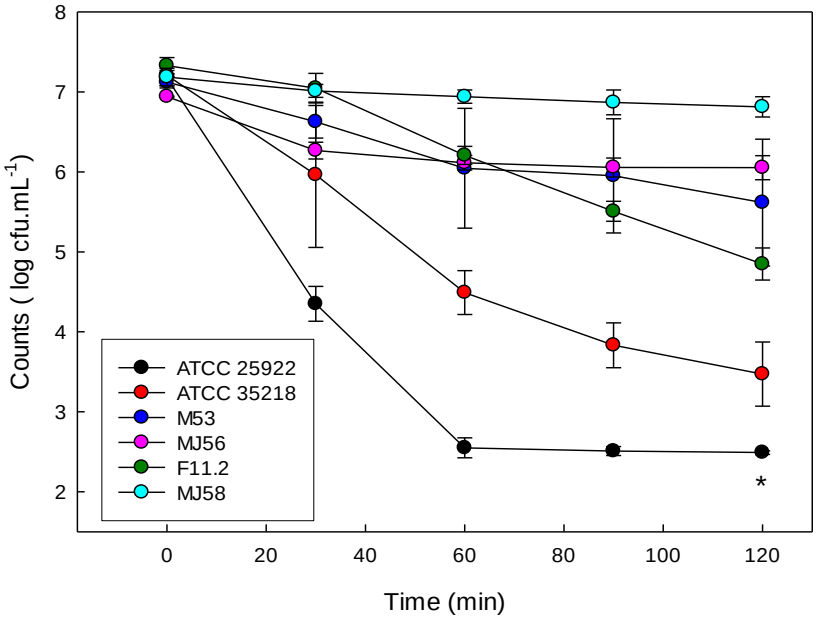

Figure 8 Inactivation curves of six *E. coli* strains (ATCC 25922, ATCC 35218, M53, MJ56, F11.2 and MJ58) against 12 mg.L^{-1} chlorine at different time intervals (30, 60, 90 and 120 min). Error bars are calculated from standard deviation at a 95% confidence level. * - No growth detected at lowest dilution (10^{-1})

In this study, a contact time exceeding 60 min did not result in significant disinfection most of the time. This can be attributed to the rapid reaction between the oxidising agent and microorganisms consequently resulting in less available chlorine for further disinfection. On the contrary, it is suggested that longer contact time is indeed needed for disinfection of possible persisting organisms expressing higher resistance toward chlorine. This was true in the case of environmental *E. coli* F11.2 (at 12 mg.L^{-1}) (Fig. 8) that was decreased significantly after an additional 60 min of disinfection. Results were in correlation with previous research by Winward *et al*. (2008) who illustrated that coliform survival decreased with longer contact times. They found that total coliforms were reduced from 2.74 to 0.85 log cfu.mL^{-1} as the contact time was extended from 10 to 120 min. Finally, contact time is an important parameter to consider during chemical disinfection as resistance within a certain population is unknown.

Figure 9 shows the log reductions of the six *E. coli* strains (ATCC 25922, ATCC 35218, M53, MJ56, F11.2 and MJ58) investigated at 6, 9 and 12 mg.L^{-1} after a total disinfection period of 120 min. Noticeable differences were observed between *E. coli* strains at each chlorine concentration (Fig. 9). At a concentration of 6 mg.L^{-1}, very low log reductions were observed and were not significantly different ($p < 0.05$) (Fig. 9). The two ATCC strains, 25922 and 35218, showed reductions of 0.36 and 0.43 log compared to environmental strains that resulted in 0.04 (M53), 0.02 (MJ56), 0.006 (F11.2), 0.12 (MJ58) log reductions (Fig. 9). Greater log inactivations were observed at increased chlorine concentrations. At 9 and 12 mg.L^{-1} chlorine treatment, significant differences ($p < 0.05$)

between reference ATCC strains and environmental strains can be observed (Fig. 9). For instance, at 9 mg.L^{-1} chlorine, reductions obtained were 1.14 (ATCC 25922), 1.18 (ATCC 35218), 0.50 (M53), 0.19 (MJ56), 0.53 (F11.2) and 0.17 log (MJ58) after 120 min of disinfection. The log reductions were the highest at 12 mg.L^{-1}, with inactivations of 3.74 (ATCC 35218), 1.51 (M53), 0.90 (MJ56), 2.48 (F11.2) and 0.37 log (MJ58) (Fig. 9). The ATCC 25922 strain was totally inactivated after 120 min in the presence of 12 mg.L^{-1} chlorine (Fig. 9).

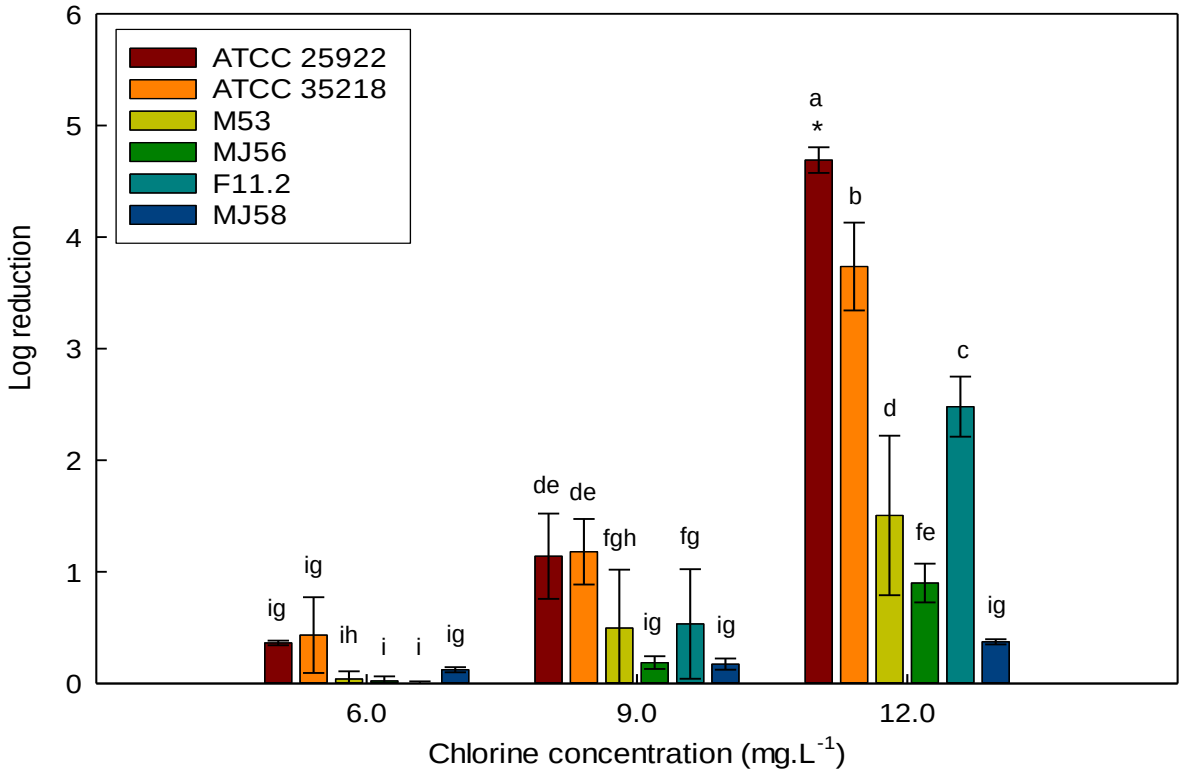

Figure 9 Log reductions obtained for six *E. coli* strains (ATCC 25922, ATCC 35218, M53, MJ56, F11.2 and MJ58) at different chlorine concentrations (NaOCl) over a contact period of 120 min in saline. Error bars are calculated from standard deviation at a 95% confidence level. Statistical analysis was done using the two way ANOVA and the Fisher LSD post hoc test. * - No growth detected at lowest dilution investigated (10^{-1})

No significant differences ($p > 0.05$) were noted between environmental *E. coli* strains at 9 mg.L^{-1} chlorine, however, this was not the case at 12 mg.L^{-1} chlorine ($p < 0.05$) (Fig. 9). As the chlorine concentration increased to 12 mg.L^{-1}, resistance levels of environmental *E. coli* strains were more prominent (Fig. 9). Significant increases ($p < 0.05$) in the log reductions of environmental strains were observed between 9 and 12 mg.L^{-1} chlorine, except for *E. coli* MJ58.

Variation in log reductions between different *E. coli* isolates were evident, especially between reference and environmental *E. coli* strains. At the lowest chlorine dose evaluated (6 mg.L^{-1}), the least strain variation was observed as the reductions of all *E. coli* isolates were lower than 1 log, displaying no significant differences ($p > 0.05$) between strains. Comparing this to 9 and 12 mg.L^{-1}, differences in log reductions between control and indigenous *E. coli* isolates became significant ($p <$

0.05). From the data, it can be concluded that ATCC 25922 and ATCC 35218 were the most sensitive to chlorine disinfection of all the strains studied. Environmental *E. coli* MJ58 was the most resistant to chlorine disinfection as can be seen by the low log reductions observed at all the chlorine dosages investigated. The log reductions (MJ58) at the different concentrations did not differ significantly ($p < 0.05$). This *E. coli* isolate was much more resistant than other environmental strains as it only showed a maximum reduction of 0.37 log at the highest chlorine concentration investigated. It was also noted that most strains became increasingly sensitive to chlorine when higher chlorine doses were used. Results are in correlation with previous research by Baker *et al*. (2002) indicating that differences between microorganisms increase as the chemical dose increases. The target reduction of 3 logs was not reached in most cases, except at 12 $mg.L^{-1}$ chlorine, where the inactivation of ATCC strains (25922 and 35218) exceeded this target (Fig. 9).

Strains that have been previously exposed to sub-lethal chlorine concentrations can develop resistance (Wojcicka *et al*., 2007). If any of the environmental strains tested in this study had been previously exposed to chlorine this could explain the high resistance displayed by some environmental *E. coli* isolates, especially *E. coli* MJ58. General resistance to biocides can either be identified as intrinsic or acquired resistance (Russell, 1998). Intrinsic resistance is commonly associated with gram-negative bacteria (Russell, 1998). For an oxidant to gain access to the inside of the cell, the outer layer (cell membrane) must be crossed (McDonnell & Russell, 1999). The cell membrane is considered the key target involved in bacterial inactivation by chlorine. Previous studies reported alterations in the permeability barrier (cell membrane) after chlorine disinfection (Venkobakar *et al*., 1999). The natural properties and composition of this barrier depend on the organism itself and may reduce uptake of chlorine compounds (McDonnell & Russell, 1999). A study by Gundlacht & Winter (2014) stated that resistance to hypochlorous acid stress in *E. coli* was accompanied by the alteration of outer membrane proteins together with the expressed OxyR regulon, the major factor conferring hypochlorous acid resistance. The development of certain proteins under stress conditions has been widely reported (Blom *et al*., 1992). When *E. coli* is exposed to chlorine, a certain set of proteins is synthesised similar to those that are frequently associated with heat shock and carbon starvation, ultimately lowering the microorganism's susceptibility towards chlorine (Cherchi & Gu, 2011). Gundlacht & Winter (2014) compared *E. coli* strains that showed maximum hypochlorous acid resistance (previously exposed to hypochlorous acid) to a control strain (no hypochlorous acid resistance). The resistant strains recovered fast from hypochlorous acid stress. The control strains responded by showing a long lag-phase of growth or did not recover and were not viable after hypochlorous acid stress (Gundlacht & Winter, 2014). It may be that chlorine resistant *E. coli* strains during this study recovered fast from hypochlorous acid stress. Environmental isolates (possibly previously exposed to chlorine) may be better adapted to survival in stressful conditions (high oxidative stress by chemicals) than reference strains (Wojcicka *et al*., 2007). Interestingly, the most resistant environmental strain (*E. coli* MJ58) was isolated from parsley (Table 1). Environmental *E. coli* MJ58 was resistant to the maximum chlorine concentration

investigated in this study (12 mg.L^{-1} chlorine). Therefore, if pathogenic *E. coli* strains in untreated irrigation water display similar level of resistance than MJ58, much higher chlorine dosages will be required for effective inactivation. This can increase the risk for foodborne outbreaks if *E. coli* is transferred to fresh produce items.

The 3 log target reduction was not reached for any of the environmental strains investigated. The use of higher chlorine concentrations will lead to high residual chlorine levels remaining in water, increasing the risk of possible by-product formation. By-product formation is commonly associated with the use of chlorine and is one of the main drawbacks of chlorination. It is however, unknown what the response of environmental *E. coli* would be towards chlorine in the natural river water environment. The character of saline solution used in this study vary from that of river water that will be used for irrigation of fresh produce items. Environmental factors play a role during chlorine disinfection, therefore, further research includes the influence of river water quality on chlorine efficacy.

The effect of increased chlorine concentrations on *E. coli* MJ58 survival

The effect of higher chlorine concentrations, using NaOCl, was studied on environmental *E. coli* MJ58 (most resistant strain). A contact time of 30 min was allowed. Figure 10 shows the log reductions in the presence of 6, 9, 12, 14 and 24 mg.L^{-1} chlorine.

Large differences were seen between the various concentrations investigated. The log reductions were 0.06, 0.12, 0.17, 1.31 and 4.58 at chlorine concentrations of 6, 9, 12, 14 and 24 mg.L^{-1}, respectively. As also seen by the results obtained in the previous section, chlorine doses that ranged between 6 – 12 mg.L^{-1} were ineffective in reducing *E. coli* MJ58 in saline after 30 min (< 1 log) (Fig. 10) and these log reductions did not differ significantly from each other ($p > 0.05$). At 14 mg.L^{-1}, an increase in *E. coli* inactivation was observed (1.31 log reduction) and this log reduction differed significantly ($p < 0.05$) from the reductions obtained at lower chlorine doses studied (6, 9 and 12 mg.L^{-1}) (Fig. 10). At 24 mg.L^{-1}, significant *E. coli* elimination was observed and a log reduction of 4.60 was noted after 30 min. This reduction exceeded 3 logs (target log reduction) and no *E. coli* growth was detected after 30 min. Results obtained from 24 mg.L^{-1} chlorine differed significantly ($p < 0.05$) from the lower chlorine concentrations evaluated over a 30 min contact period (6, 9, 12 &14 mg.L^{-1}) (Fig. 10).

From these results it was concluded that *E. coli* MJ58 was resistant to chlorine up to a certain concentration and was then inactivated at the highest concentration investigated (24 mg.L^{-1}). An increase of 10 mg.L^{-1} from 14 mg.L^{-1} led to a drastic reduction in microbial resistance. It is however unknown at which point *E. coli* became increasingly sensitive towards chlorine. Previous research investigated *E. coli*'s response to chlorine against concentrations ranging between 5 – 30 mg.L^{-1} and showed that the highest resistance to chlorine was observed up to 20 mg.L^{-1} (< 1 log inactivation) (Virto *et al*., 2005). Cell death was reported at chlorine doses exceeding 20 mg.L^{-1} (Virto *et al*., 2005). This supports results found in this study as the lethality by chlorine was seen at a dose of 24

mg.L^{-1}. It may be that the cells could not withstand the oxidative stresses provided by high chlorine concentrations as a result of the decreasing resistance provided by the permeability barrier (cell membrane). It is also possible that environmental *E. coli* MJ58 had been previously exposed to sub-lethal chlorine concentrations. This could have led to increased resistance observed at the lower dosages evaluated in this study. Again, these results indicate the possible presence of highly resistant *E. coli* strains in the environment.

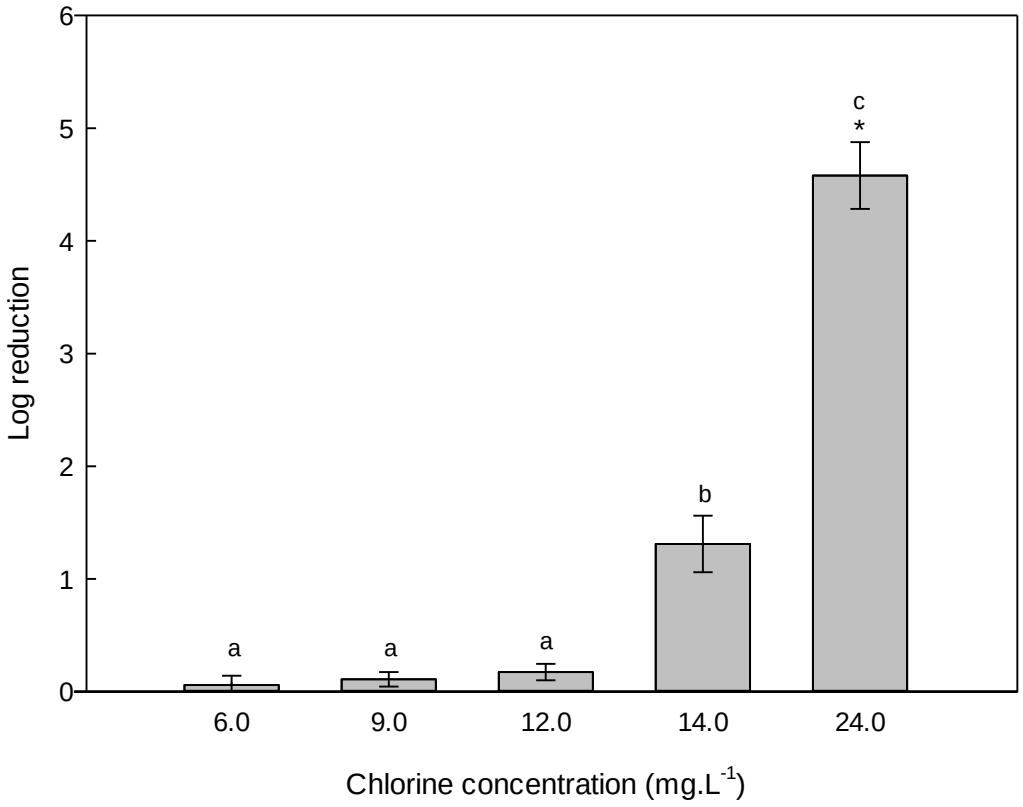

Figure 10 Log reductions after 30 min for *E. coli* MJ58 against the following chlorine concentrations (NaOCl) in saline: 6, 9, 12, 14 and 24 mg.L^{-1}. Error bars were calculated from the standard deviation at a confidence level of 95%. Statistical analysis was done using the one way ANOVA and the Fisher LSD post hoc test. * - No growth detected at lowest dilution investigated (10^0)

STUDY 3

Comparing disinfection capabilities of NaOCl and $Ca(OCl)_2$ on *E. coli* survival

Ultimately, the effect of two chorine sources, NaOCl and $Ca(OCl)_2$), was compared in SSS inoculated with *E. coli* MJ58. The selection of this strain was based on the results obtained in Study 1 and 2. Figure 11 indicates the individual log reductions for NaOCl and $Ca(OCl)_2$ after chlorine treatment (12 mg.L^{-1}, 120 min). Adding to this, Table 2 displays the residual chlorine levels after disinfection for both NaOCl and $Ca(OCl)_2$ at four different time intervals (30, 60, 90 and 120 min). Herewith, it also includes the pH of the solution after disinfection.

Distinct variation was seen in the log reductions observed between the two chlorine sources investigated (Fig. 11). The log reductions differed significantly ($p < 0.05$) as the log reduction using NaOCl was 2.00 compared to 4.36 when $Ca(OCl)_2$ was used. There was an average difference of 2.36 log between the two disinfectants used. On the other hand, the residual chlorine levels of NaOCl and $Ca(OCl)_2$ were comparable to one another. The residual levels were 0.86 $mg.L^{-1}$ (NaOCl) and 0.82 $mg.L^{-1}$ ($Ca(OCl)_2$) after 30 min disinfection and decreased gradually over time (Table 2). The residual levels after NaOCl disinfection (0.60 $mg.L^{-1}$) was comparable to those after $Ca(OCl)_2$ disinfection (0.57 $mg.L^{-1}$) after 120 min (Table 2). The big differences in log reductions observed between NaOCl and $Ca(OCl)_2$ disinfection in this study were not expected because residual levels correlated strongly between the two disinfectants used. Throughout the 120 min disinfection procedure, chlorine residuals were always below 1 $mg.L^{-1}$ and met the limit chosen for this study. Although log inactivation results varied significantly, the residual levels by NaOCl and $Ca(OCl)_2$ disinfection reflects a similar demand by *E. coli* microorganisms.

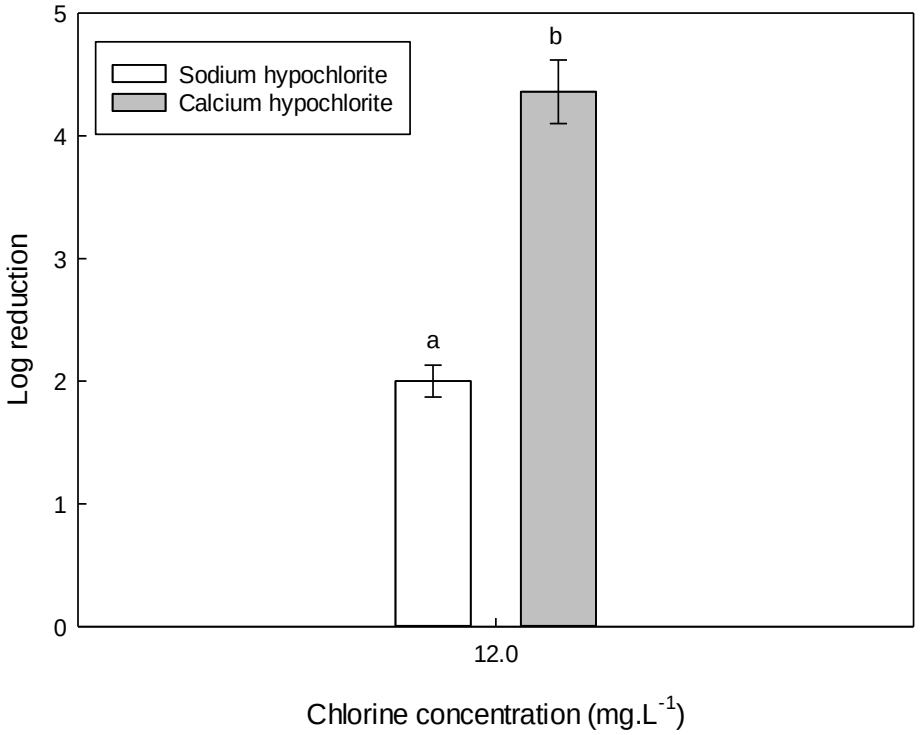

Figure 11 Log reductions obtained against 12 $mg.L^{-1}$ for 120 min on *E. coli* MJ58 for sodium and calcium hypochlorite in SSS. Error bars are calculated form standard deviation at a 95% confidence level. Statistical analysis was done using the one way ANOVA and the Fisher LSD post hoc test.

Results were in agreement with a study done by Lima & Ahmed (2015) who indicated that $Ca(OCl)_2$ had an overall greater bactericidal activity than NaOCl. This was a comparative study conducted on various surfaces such as wood, tin, formica and ceramic tiles, for the elimination of *E. coli* and *Staphylococcus aureus*. The germicidal value of $Ca(OCl)_2$ was better than NaOCl on wood towards both *E. coli* and *Staphylococcus aureus*. Due to the reduced surface tension of

$Ca(OCl)_2$, the disinfectant reached the pores and killed bacteria (Lima & Ahmed, 2015). The reduced surface tension of $Ca(OCl)_2$ might be applicable to solutions containing suspended solids during disinfection.

The pH of a solution plays a significant role in chlorine disinfection and can possibly be responsible for the differences seen in log reductions. As chlorine in any form reacts with water, it produces hypochlorous acid (HOCl) (Spellman, 2009). Hypochlorous acid is defined as a weak acid and dissociates into a chemical species known as hypochlorite ion (OCl^-) depending on the pH of the solution (Spellman, 2009). Note that HOCl has a much greater disinfection capability than OCl^- (Spellman, 2009). These two chemical species are in equilibrium based on the pH of the solution. Below a pH of 7.5, HOCl is the dominant species and above 7.5, OCl^- is predominant (Spellman, 2009). Although the pH after disinfection tests (120 min) was measured at 7.12 (Table 2), subsequent tests (involving only saline and chlorine solution and not bacteria) revealed that the initial pH, directly after the addition of the respective commercial chlorine solutions to saline, was much higher. For $Ca(OCl)_2$, it was about 8.26 and for NaOCl in the range of 8.65. High initial pH, which can probably be attributed to extremely low buffer capacity of saline (alkalinity tested as 12 $mg.L^{-1}$ $CaCO_3$) might have influenced HOCl/OCl^- proportions. It could be that the ratio of HOCl to OCl^- was initially higher in $Ca(OCl)_2$ solution than NaOCl due to different pH values directly at the start of the disinfection cycle. The ratio of the chemical species involved could have affected disinfection. A table displaying the percentage available HOCl as a function of the temperature and pH is presented by Randtke (2010). According to this table, the amount of hypochlorous acid present, assuming a temperature of approximately 20°C, at pH values of 8.26 ($Ca(OCl)_2$) and 8.65 (NaOCl), is 16.06% and 5.70%, respectively (Randtke, 2010). This is more than double the amount HOCl present when $Ca(OCl)_2$ was used compared to NaOCl. If this was the case, it can explain the differences seen in *E. coli* inactivation.

Table 2 Residual chlorine levels after chlorine disinfection including pH values after disinfection

	Residual chlorine ($mg.L^{-1}$)	
Time (min)	**NaOCl**	**$Ca(OCl)_2$**
30	0.86	0.82
60	0.86	0.84
90	0.60	0.62
120	0.66	0.57
pH		
After disinfection	7.12	7.12

The composition of NaOCl and $Ca(OCl)_2$ differ and some substances within the product could therefore influence the pH of a solution. Sodium hypochlorite (NaOCl) is a yellow green solution

compared to $Ca(OCl)_2$ that exists in granular form. The available chlorine content in hypochlorite solutions is critical. Sodium hypochlorite solutions are unstable and has been shown to deteriorate over time due to exposure to sunlight, heat, air and reaction with organic components. Due to its instability, OCl^- decomposes over time and produces products such as chlorates (ClO_3^-) and chlorites (Cl^-) (Frais *et al*., 2001). The decomposition rate depends on the hypochlorite ion and the pH (Frais *et al*., 2001). Results by Anon. (2007) have shown that sodium hypochlorite (15 %) ($v.v^{-1}$) storage at room temperature over a period of 6 months reduced the available chlorine content by more than 50%. It is therefore essential to confirm the amount of free chlorine present after dosing.

With regards to the practical application of chlorine within a farm setup for irrigation water disinfection, NaOCl is preferred due to its easier application. Liquid chlorine can be directly injected or added to contaminated water. This is not the case with $Ca(OCl)_2$ as it contains some inert material that is insoluble in water (WHO, 2015). These residues should be separated prior to disinfection as they may cause clogging in pipelines and lead to blockages (WHO, 2015). However, the practical implication thereof will possibly require an extra holding tank to remove the supernatant for disinfection. Rather, the use of NaOCl will simplify the disinfection procedure as it dissolves easily in water. The storage conditions and storage time should be carefully monitored to prevent decomposition.

STUDY 4

The effect of water quality on chlorine disinfection

The effect of water quality on chlorine disinfection was studied in autoclaved river water (untreated and flocculated) inoculated with *E. coli* MJ58. This was compared to the disinfection in SSS. Figure 12 compares the log reductions obtained in SSS, untreated and flocculated river water for *E. coli* MJ58 after being treated with 12 $mg.L^{-1}$ chlorine (NaOCl) for 120 min. Also, Table 3 includes the river water properties and the residual chlorine concentration that remained after disinfection.

Significant differences ($p < 0.05$) between SSS and river water samples (untreated and flocculated) were observed (Fig.12). The *E. coli* reduction obtained in SSS after chlorine disinfection was 1.62 log (Fig. 12). On the contrary, no *E. coli* growth was detected in any of the river water samples after 120 min disinfection (Fig. 12). Table 3 displays the river water characteristics before and after flocculation. The pH of untreated river water was 7.22 and after flocculation, river water had a pH of 7.30. It is clear that the flocculent caused a slight increase in the pH of river water. The COD of untreated river water was 79 $mg.L^{-1}$ and was lowered to 74 $mg.L^{-1}$ after flocculation. River water presented low TSS levels of 8.75 $mg.L^{-1}$ (untreated) and 0.73 $mg.L^{-1}$ (flocculated) (Table 3). Also, the turbidity was lowered from 16.8 NTU (untreated) to 15.2 NTU (flocculated) by flocculation. This decrease in COD, TSS and turbidity levels was attributed to the effect of flocculation and subsequent filtration resulting in the removal of some suspended particles and organic material in the water. Furthermore, the alkalinity, electrical conductivity and UVT% were 100 $mg.L^{-1}$ $CaCO_3$, 57

mS.m^{-1} and 35.2% before flocculation and increased to 137 mg.L^{-1} $CaCO_3$, 60 mS.m^{-1} and 36.5% after river water flocculation (Table 3). Also, great differences were seen between the residual chlorine levels in SSS and river water samples. The residual concentrations were 0.63 mg.L^{-1} in SSS compared to 2.11 mg.L^{-1} and 2.34 mg.L^{-1} in untreated and flocculate river water (Table 3).

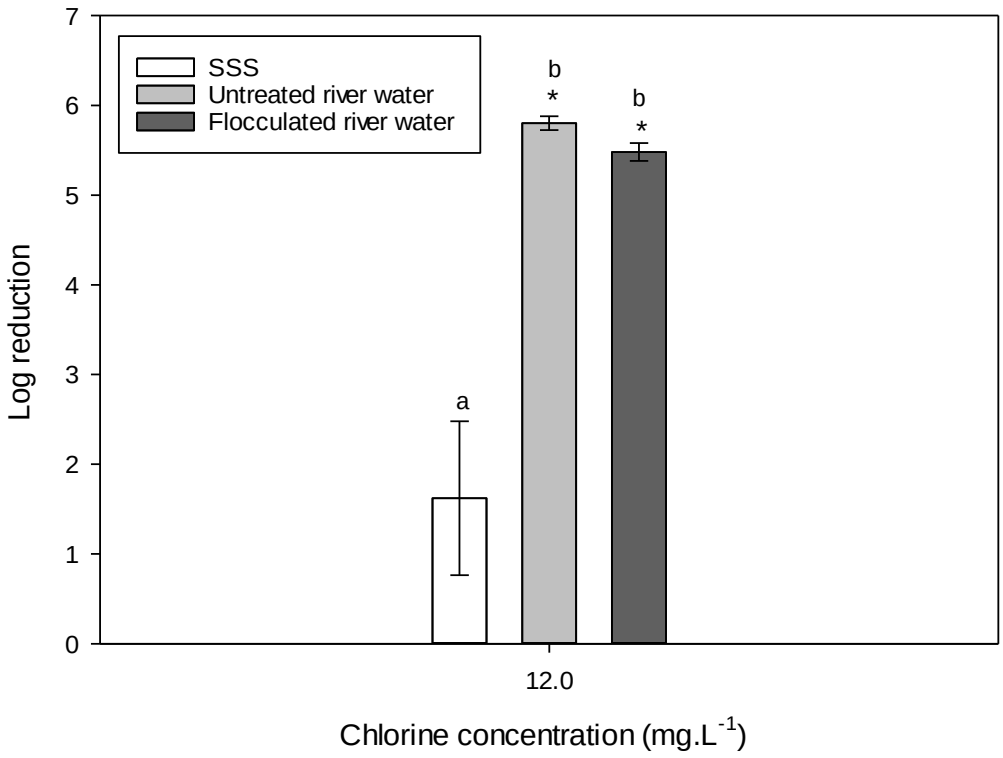

Figure 12 Comparison between chlorine disinfection in SSS, untreated and flocculated river water inoculated with *E. coli* MJ58 after chlorine exposure (12 mg.L^{-1}) for 120 min. Error bars were calculated from the standard deviation at a confidence level of 95%. Statistical analysis was done using the one way ANOVA and the Fisher LSD post hoc test.

SSS – Sterile saline solution; * - No growth detected at lowest dilution investigated (10^0)

Effective chlorine disinfection was observed in river water. Flocculation increased the pH of untreated river water slightly, however, river water samples from Trial 1 and Trial 2 were still in the range (pH 7.4 – 7.6) (Newman, 2004) for optimum chlorine disinfection. The effect of flocculation on *E. coli* removal did not differ significantly (p = 0.46) from *E. coli* disinfection in untreated river water because in both river water samples, no *E. coli* growth was observed after disinfection. Chlorine disinfection in river water exceeded the 3 log target reduction on *E. coli* organisms. This was not the case when SSS served as a disinfection medium (1.62 log reduction). The composition of the solutions used in this investigation differed from each other and could explain the variation in *E. coli* inactivation. Saline solution (SSS) is commonly used in microbiological laboratory studies as it provides an isotonic environment to microorganisms, preventing bacterial lysis due to osmotic pressure balance in the cell. The average COD of SSS used in disinfection studies was 100 mg.L^{-}

[1]. This was higher compared to the COD (74 – 79 $mg.L^{-1}$) of river water. Also, the average alkalinity of SSS used during disinfection trials was exceptionally low (12 $mg.L^{-1}$ $CaCO_3$) compared to the alkalinity of river water samples (100 – 137.5 $mg.L^{-1}$ $CaCO_3$). The river water thus represented a higher buffer capacity which could have maintained the pH at optimum levels (a very important factor for chlorine disinfection). This could explain the big differences in log reductions between saline and river water samples. The residual concentration obtained in SSS (0.63 $mg.L^{-1}$) was lower compared to river water samples (untreated – 2.34 $mg.L^{-1}$, flocculated – 2.11 $mg.L^{-1}$) and relates to the differences in the solutions' properties such as the COD. Solutions with higher COD levels will consume more chlorine, consequently resulting in lower residuals.

Table 3 River water properties and chlorine residual before (untreated) and after flocculation (flocculated)

		Untreated	**Flocculated**
pH		7.22	7.30
COD ($mg.L^{-1}$)		79	74
TSS ($mg.L^{-1}$)		8.75	0.73
Turbidity (NTU)		16.8	15.2
Alkalinity ($mg.L^{-1}$ $CaCO_3$)		100.0	137.5
Conductivity ($mS.m^{-1}$)		57	60
UVT%		35.2	36.5
	SSS	**Untreated**	**Flocculated**
Residual chlorine ($mg.L^{-1}$)	0.63	2.34	2.11

SSS –Sterile saline solution

Regarding the effect of water quality on chlorine disinfection, water properties including COD, TSS and turbidity did not influence chlorine disinfection on *E. coli* inactivation. Research reported by Ayyildiz *et al*. (2009) showed that as the COD in secondary wastewater decreased from 50 $mg.L^{-1}$ to 12.5 $mg.L^{-1}$, the total coliform and *E. coli* removal was increased by a factor of 1.5 – 2 when low chlorine dioxide dosages (1 – 2 $mg.L^{-1}$) were used. However, they have also obtained high *E. coli* reductions at high COD levels (75 $mg.L^{-1}$) when increased chlorine dosages (3 $mg.L^{-1}$) were applied (Ayyildiz *et al*., 2009). Thus, the effect of COD on bacterial inactivation was more obvious at low chlorine dosages and higher COD loads (Ayyildiz *et al*., 2009). It was clear that bacterial inactivation is dose-dependent. Therefore, the COD range of river water reported in this study (74 – 79 $mg.L^{-1}$) did not influence *E. coli* inactivation. The available chlorine (12 $mg.L^{-1}$) was more than enough to meet the demand by organic particles and *E. coli* suspended in river water. Moreover, electrical conductivity is an indication of the dissolved solids content and the South African Water

Quality Guidelines (DWAF, 1996) suggest a limit for irrigation water to less than 40 $mS.m^{-1}$. Results (Table 3) exceeded this guideline, however, chlorine disinfection was not influenced in this study. It can be suggested that the amount (and size) of dissolved solids present was not enough to provide protection to microorganisms from chlorine disinfection also taking into consideration that the reaction between chlorine and substances in the water is target specific (Van Haute *et al*., 2013).

With regards to the residual chlorine concentration, differences were seen between SSS and river water samples. The residual remaining in river water indicated a lower demand by other constituents such as organic and inorganic material than compared to the demand presented by saline solution. In both river water samples, the residual limit chosen for this study (≤ 1 $mg.L^{-1}$) was exceeded.

The disinfection of *E. coli* MJ58 in river water was notably better compared to that obtained in studies using saline as disinfectant medium (Study 2) (at the same chlorine concentration used). This might be due to differences (COD and alkalinity) between the solutions investigated (saline and river water) that resulted in variation in *E. coli* inactivation. Chlorine disinfection resulted in total *E. coli* inactivation at the water quality reported in this study. Since a single *E. coli* strain displaying high resistance to chlorine disinfection was used in this study and it is unknown what the resistance of a mixed *E. coli* population in river water would be. Studies should be done on river water to investigate the effect of lower chlorine concentrations on both microbial loads as well as on the resulting residual chlorine concentrations to determine if effective disinfection can coincide with residual levels lower than 1 $mg.L^{-1}$ (as suggested by USEPA, 2004).

CONCLUSION

Strain variation was prominent during chlorine studies. Reference strains were always more sensitive than environmental strains as reference strains showed higher log reductions. The reference strain ATCC 25922 was the most sensitive strain and environmental *E. coli* MJ58 showed the lowest reduction trend throughout chlorine disinfection in saline. Greater resistance by environmental strains indicates the variability in susceptibility towards chlorine. From this study it was evident that strains from the same species may differ in their response to chlorine and implies the development of various resistance mechanisms to withstand oxidative stress. The most resistant strain (MJ58) was completely inactivated in saline at a chlorine dosage of 24 $mg.L^{-1}$ (NaOCl) and 30 min. Chlorine doses that ranged from 6 – 12 $mg.L^{-1}$ (NaOCl) were inadequate to effectively reduce *E. coli* strains in saline.

Of the two chlorine sources investigated during SSS studies, $Ca(OCl)_2$ was much more effective disinfectant on *E.coli* than NaOCl. Generally, the NaOCl solutions can degrade over time. Therefore, it is very important to ensure the use fresh NaOCl solutions and confirm the actual free chlorine concentration prior to the application towards contaminated river water.

Chlorine disinfection is pH-dependent. In this study, different chlorine sources resulted in slightly different initial pH levels, directly after the addition NaOCl and $Ca(OCl)_2$ to saline. The

exceptionally low alkalinity (buffer capacity) of SSS was probably responsible for this phenomenon. Higher pH levels in saline solutions containing NaOCl resulted in significant differences in *E. coli* disinfection between NaOCl and $Ca(OCl)_2$. The addition of chlorine to well-buffered systems is of utmost importance since the optimum pH range for chlorine disinfection range between 7.2 – 7.4. Fortunately, the pH of river water detected during this study (7.22 – 7.30) falls within the range for optimal chlorine functioning during chlorine disinfection. Of the two chlorine sources evaluated, NaOCl is preferred for commercial-scale applications. Calcium hypochlorite requires additional installations to filter the insoluble material before disinfection. Therefore, it is suggested that further investigation into chlorine disinfection on river water be based on the use of NaOCl as a chlorine source.

The influence of water quality on chlorine disinfection was investigated. River water displaying a COD load between 74 and 79 $mg.L^{-1}$ did not influence chlorine disinfection (12 $mg.L^{-1}$ for 120 min) and no *E. coli* growth was detected (> 5 log reduction). The chlorine concentration of 12 $mg.L^{-1}$ (NaOCl) met the demand posed by organic particles as well as microorganisms in river water. Note that at lower chlorine dosages, the effect of COD would have been more prominent. Adding to this, river water was well-buffered and contributed to effective *E. coli* disinfection compared to ineffective *E. coli* removal in saline solution (low buffer capacity). The residual chlorine levels were > 2 $mg.L^{-1}$ in river water samples treated with 12 $mg.L^{-1}$ chlorine. Maintaining a low residual concentration is of great importance. Therefore, the application of lower dosages, depending on the water quality, will probably result in lower residual levels consequently limiting the risk posed to the environment (by-product formation) and ultimately fresh produce items.

The resistance of the heterogenic population in river water to chlorine may differ from the isolates investigated in this study and therefore, may vary in their reactions to the chlorine doses evaluated in this study. Therefore, further research investigating the efficacy of chlorine on river water disinfection is necessary. Effective *E. coli* inactivation was achieved by chlorine at the water quality reported in this study. Therefore, chlorine can be considered a potential disinfectant for contaminated river water. The residual chlorine concentration is a limiting factor when choosing an optimum chlorine concentration for river water disinfection. A predetermined chlorine concentration could not be suggested for river water disinfection as the selected chlorine dosage should rather be based on river water quality and subsequent chlorine demand.

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CHAPTER 5

THE COMPARISON BETWEEN CHLORINE AND PERACETIC ACID DISINFECTION OF RIVER WATER CONSIDERING THE INFLUENCE OF WATER QUALITY AT LABORATORY-SCALE

SUMMARY

Previous research shows South African rivers are extensively polluted with faecal organisms and in particular, *Escherichia coli (E. coli)* that can cause serious diseases in humans. Therefore, the agricultural industry is searching for the most suitable irrigation water treatment option taking into account water of varying quality. This study compares the efficacy of chlorine (3.0 – 6.0 $mg.L^{-1}$, 120 min) and peracetic acid (PAA) (4.5 – 6.0 $mg.L^{-1}$, 25 min) on river water disinfection. The effect of these treatments were analysed against heterotrophic bacteria, total coliforms and *E. coli*. *Escherichia coli* numbers up to 5.40 x 10^5 cfu.100 mL^{-1} were detected in the Plankenburg River. Chlorine and PAA inactivated *E. coli* in river water and these results complied with irrigation guidelines limiting water to ≤ 1 000 faecal coliforms.100 mL^{-1} (WHO, 1989; DWAF, 1996). Chlorine disinfection resulted in overall higher reductions in heterotrophic microorganisms than compared to PAA. Maximum heterotrophic reductions achieved by 3.0 – 6.0 $mg.L^{-1}$ chlorine were 3.15 log in comparison to the 2.41 log reduction achieved by 4.5 – 6.0 $mg.L^{-1}$ PAA disinfection. Complete total coliform reduction was observed in all cases through chlorine disinfection, however, this was not the case with PAA disinfection. Moreover, the heterotrophic organisms were generally more resistant to chlorine and PAA disinfection than total coliforms and *E. coli*. This implies the presence of other microorganisms in river water with greater resistance to chlorine and PAA than those evaluated in this study. Large variations in the chemical oxygen demand (COD) of river water was observed (14 – 1 094 $mg.L^{-1}$). It was concluded that high COD inhibited PAA disinfection more than chlorine disinfection. The total suspended solids (TSS) content of river water (3.3 – 8.0 $mg.L^{-1}$) was too low to inhibit the disinfection efficacy of chemicals. In conclusion, chlorine was the better overall disinfectant in this study. Residual levels depended on the water quality and when used at concentration of 3.0 $mg.L^{-1}$ for 120 min, resulted in irrigation water of acceptable water quality. The chlorine residuals ranged between 0.30 – 2.3 $mg.L^{-1}$ and in most cases this result exceeded the chosen residual limit for this study (≤ 1 $mg.L^{-1}$). It is recommended that the chlorine demand of irrigation sources is carefully considered in future before treatment commence. The effect of water quality on chemical disinfection efficiency was evaluated. Water quality influenced PAA efficiency to a greater extent in comparison to the effect thereof on chlorine. Rather, PAA dosages higher than 4.5 $mg.L^{-1}$ (25 min contact time) are suggested for river water disinfection prior to irrigation.

INTRODUCTION

One out of nine persons on the planet does not have access to safe drinking water (UN-Water, 2013). The increasing lack of water resources results in a growing demand for good water quality

by the agricultural industry to irrigate fresh produce items (Falkenmark & Molden, 2008; UN-Water, 2013). An estimated 62% of all water withdrawals in South Africa are related to agricultural irrigation and water is mainly extracted from rivers and dams (DEAT, 2006). South African rivers are extensively polluted with pathogenic microorganisms as reported by multiple research studies on microbial water quality (Britz *et al*., 2013). Research done on the Plankenburg River in Stellenbosch reported high levels of *E. coli* with up to 3 500 000 colony forming units per 100 mL (Paulse *et al*., 2009). Also, Britz *et al.* (2013) showed that *E. coli* numbers from the Eerste River, also passing through Stellenbosch surroundings, were 78 886 MPN (most probable number) per 100 mL. *Escherichia coli* is considered to be from faecal origin and guidelines for irrigation water suggests a limit of ≤ 1 000 faecal coliforms per 100 mL (WHO, 1989; DWAF, 1996). The use of contaminated water for fresh produce irrigation is concerning as foodborne outbreaks related to fresh fruits and vegetables have increased over the past few years. Pathogenic microorganisms of greatest concern related to fresh produce foodborne illnesses typically are *E. coli* O157:H7 and *Salmonella* spp. (CDC, 2014).

There are many available options to reduce the prevalence of pathogenic bacteria in water distribution systems. The examination for possible on-farm irrigation water treatment options is focussed on the most suitable, in terms of microbial effectiveness and cost effectiveness, treatment method available. Chemical disinfection of contaminated water is widely applied and examples include chlorine, peracetic acid, hydrogen peroxide, ozone and bromine. Chlorine is the most widely applied disinfectant for treating contaminated water and wastewater. This is due to the ease of application, the low costs involved and the effectiveness towards a variety of microorganisms (USEPA, 1999; De Souza *et al*., *in press*; Luukkonen *et al*., 2014). However, the use of chlorine comes with the possible risk of disinfection by-product (DBP) formation that may be carcinogenic or mutagenic for human health (USPEA, 1999; Sayyah & Mohamed, 2014). This raises concerns with regard to its application in irrigation water treatment. The formation of these harmful products depends on the residual chlorine concentration present after disinfection. There are international residual guidelines in place to assist in lowering the risk of DBP formation. No South African guidelines, with regards to DBP or residual chlorine levels, have been established specifically for irrigation water. The United States Environmental Protection Agency recommends a limit of ≤ 1 $mg.L^{-1}$ residual chlorine for reclaimed water intended for land irrigation (USEPA, 2004). This was the residual limit chosen for this study as there are no residual levels specified for the irrigation of fresh produce.

The development of chlorine resistant bacteria has led to the consideration of other disinfectants (Luukkonen *et al*., 2014). Peracetic acid is an emerging water disinfectant and has been studied comprehensively since the 1980s (Luukkonen *et al*., 2014; Wilson, 2014). The advantage linked to the use of this disinfectant for water decontamination includes: ease of application, broad antimicrobial effectiveness and a low contact time required (Kitis, 2004). Up to

date, there is no information about PAA's toxicity and the greatest advantage includes no DBP formation (De Souza *et al*., *in press*).

A very important factor to consider is the effect of water quality as this could play a crucial role in the effective reduction of microorganisms. The actual dose and contact time required for effective disinfection is dependent on water quality (Luukkonen, 2013). Water properties such as pH, COD and TSS concentration have been reported to influence effective reduction of indicator microbes by disinfectants in water systems (Gehr *et al*., 2003; Newman, 2004; Falsanisi *et al*., 2008; Bitton, 2011; Van Haute *et al*., 2013; Julio *et al*., 2014). Rivers are natural flowing resources that display remarkable variation over time (Labajo-Villantes & Nuñeza, 2014). The ecological imbalance, commonly due to extensive pollution, continuous activities and natural phenomena, are the main causes of a varying water quality (Carr & Neary, 2008; Vorosmarty *et al*., 2010). Therefore, the susceptibility of the microbial population in river water to chemical disinfectants may differ from the pure *E. coli* strains investigated in Chapter 3 and 4.

The identification of a suitable disinfection method for contaminated irrigation water in South Africa should be examined. Therefore, a comparative study was conducted between PAA and chlorine disinfection on water from the Plankenburg River in Stellenbosch. The outcome was compared against the water quality represented by the river. Based on the results on specific dosages and contact times evaluated, the most suitable treatment option was recommended for contaminated irrigation water disinfection on commercial-scale.

MATERIALS AND METHODS

Experimental research design

River water disinfection was evaluated in two separate studies, Study 1 and 2. During Study 1, the chlorine demand of river water was analysed over a six week period prior to disinfection trials. The COD levels were recorded weekly from the 20th of January to the 17th of February. River water was treated with 1.5, 3.0, 6.0 and 9.0 mg.L^{-1} chlorine for 30 min. Thereafter, the residual chlorine concentration (mg.L^{-1}) was measured using a cell test kit (Merck, Germany). The results, accompanied with the data reported in Chapter 3, were used to select a suitable chlorine concentration for river water disinfection studies.

During Study 2, river water was disinfected with PAA and chlorine on five different days. Liquid sodium hypochlorite (NaOCl) served as the chlorine source during this study. Table 1 displays the dosages, contact times and microorganisms investigated during each trial. After chlorine disinfection (120 min), the residual concentration (mg.L^{-1}) was measured using a cell test kit (Merck, Germany). Note that water quality analysis was performed on river water prior to disinfection. This included the evaluation of temperature, pH, COD, alkalinity, TSS, electrical conductivity, turbidity and UVT%.

Table 1 Information regarding concentration, contact time and microorganisms investigated during five disinfection trials using chlorine and PAA

	Concentration ($mg.L^{-1}$)	Contact time (min)	Microorganisms studied
Trial 1 – 3	PAA – 4.5 Chlorine – 6.0	PAA – 15, 25 Chlorine – 30, 60, 90, 120	Heterotrophic microorganisms Total coliforms *Escherichia coli*
Trial 4 – 5	PAA – 3.0 Chlorine – 3.0	PAA – 15, 25 Chlorine – 30, 60, 90, 120	

PAA – Peracetic acid

General materials and methods

Site selection and water sampling

River water was sampled from the Plankenburg River, passing the Bergkelder in Stellenbosch. Previous studies done on this river by Britz *et al*. (2013) identified sampling sites as Plank-1 and Plank-2. River water used during this study was sampled between these two sites (33°56'15.4''S, 18°50'53.0''E) situated before the confluence point of the Plankenburg and Eerste Rivers. Water was always sampled between 07:30 and 08:30 in the morning. Sampling was done according to the sampling method of the South African National Standards (SANS 4832, 2007). The sampling site was equipped with a sand filter that river water was passed through before the sample was drawn. River water was stored in 1L sterile bottles and transported in cooler boxes. Water samples were kept cool until used for disinfection trials in the laboratory.

Solutions

A commercial PAA solution, Tsunami 100 (Ecolab, South Africa), comprising of 31% acetic acid, 15% peroxyacetic acid and 11% hydrogen peroxide was used for PAA disinfection tests. Sodium hypochlorite solution (NaOCl) (Metsi Water Solutions, South Africa) with a strength of 15% ($v.v^{-1}$) available chlorine was used during chlorine disinfection studies. Dilution series' were prepared using 0.85% ($m.v^{-1}$) sterile saline solution (SSS). The bactericidal activity of both PAA and chlorine were quenched using sodium thiosulfate ($Na_2S_2O_3$) (Merck, Germany). This was done by transferring one millilitre of a 1% ($m.v^{-1}$) $Na_2S_2O_3$ stock solution to 8 mL SSS prior to preparing the first dilution (10^{-1}) of every dilution series according to the method used by Mazzola *et al*. (2006).

Peracetic acid and chlorine disinfection

Figure 1 displays the general procedure followed during chlorine and PAA disinfection of river water samples. Eighty millilitres of river water was used for treatment studies at laboratory-scale. Firstly, control plates were prepared (10^{-1} – 10^{-4}) to determine the initial microbial load present in river water (Fig. 1). Thereafter, the river water was dosed with the disinfectant (chlorine or PAA) at the particular concentration investigated (Fig. 1). A dilution series (10^{-1} – 10^{-4}) was prepared after disinfection in

SSS. Each dilution (as well as an undiluted sample) was transferred in duplicate to petri dishes after which the appropriate agar was added (Fig. 1). This was followed by duplicate plating and the bactericidal effect on microorganisms was determined at different time intervals for each disinfectant evaluated. Contact times of 15 and 25 min were used during PAA disinfection compared to the four time intervals evaluated during chlorine disinfection (30, 60, 90 and 120 min) (Fig. 1). Note that only total coliforms were studied at different time intervals. The levels of *E. coli* and heterotrophic microorganisms were only determined before (control) and after a total time of 120 min (chlorine) and 25 min (PAA). For each water sample, disinfectant trials were conducted in triplicate.

Heterotrophic plate count (HPC)

The total heterotrophic microorganisms were detected using plate count agar (PCA) (Biolab, South Africa) and duplicate plating was done according to standard methods (SANS 4833, 2007a). Inverted plates were incubated for 48 h at 30°C and the number of colonies was recorded as cfu.mL^{-1}.

Total coliform (TC) and *Escherichia coli* (EC) enumeration

Coliform (TC) and *E. coli* (EC) microorganisms were identified following the instructions of the method proposed by SANS 4832 (2007b). Total coliforms were cultivated on Violet Red Bile Agar (VRBA) (Biolab, South Africa) that was prepared according to the manufacturer's instructions. Coliform organisms express themselves in a purple-pink colour. These were counted as total coliforms (Merck, 2005). *Escherichia coli* growth were identified using Chromocult® Coliform Agar Enhanced Selectivity (CES) (Merck, South Africa) and blue colonies characteristic to EC were recorded. Enumerated TC and EC were visible after 24 h of incubation at 36˚C. Total coliforms and EC were recorded as cfu.mL^{-1}.

Water quality analysis

COD, TSS and alkalinity

These water parameters were determined following standard methods (APHA, 2005). A COD range of 10 – 150 mg.L^{-1} was used and samples were transferred to a digesting unit. The COD was measured after 2 h of digestion using a DR 2000 HACH spectrophotometer (Hach Co. Loveland, CO) at 585 nm. The unit recorded from the spectrophotometer was multiplied by a factor of 1 800 (Merck, Germany) and recorded as mg.L^{-1}. The TSS content was recorded as mg.L^{-1} (sample size: 200 mL river water). The alkalinity of water is an indication of a solution's buffer capacity and relates to its ability to neutralise acids. The determination of alkalinity is solely based on the titration against sulphuric acid (H_2SO_4) to a pH of 4.2. Alkalinity results were recorded as mg.L^{-1} $CaCO_3$.

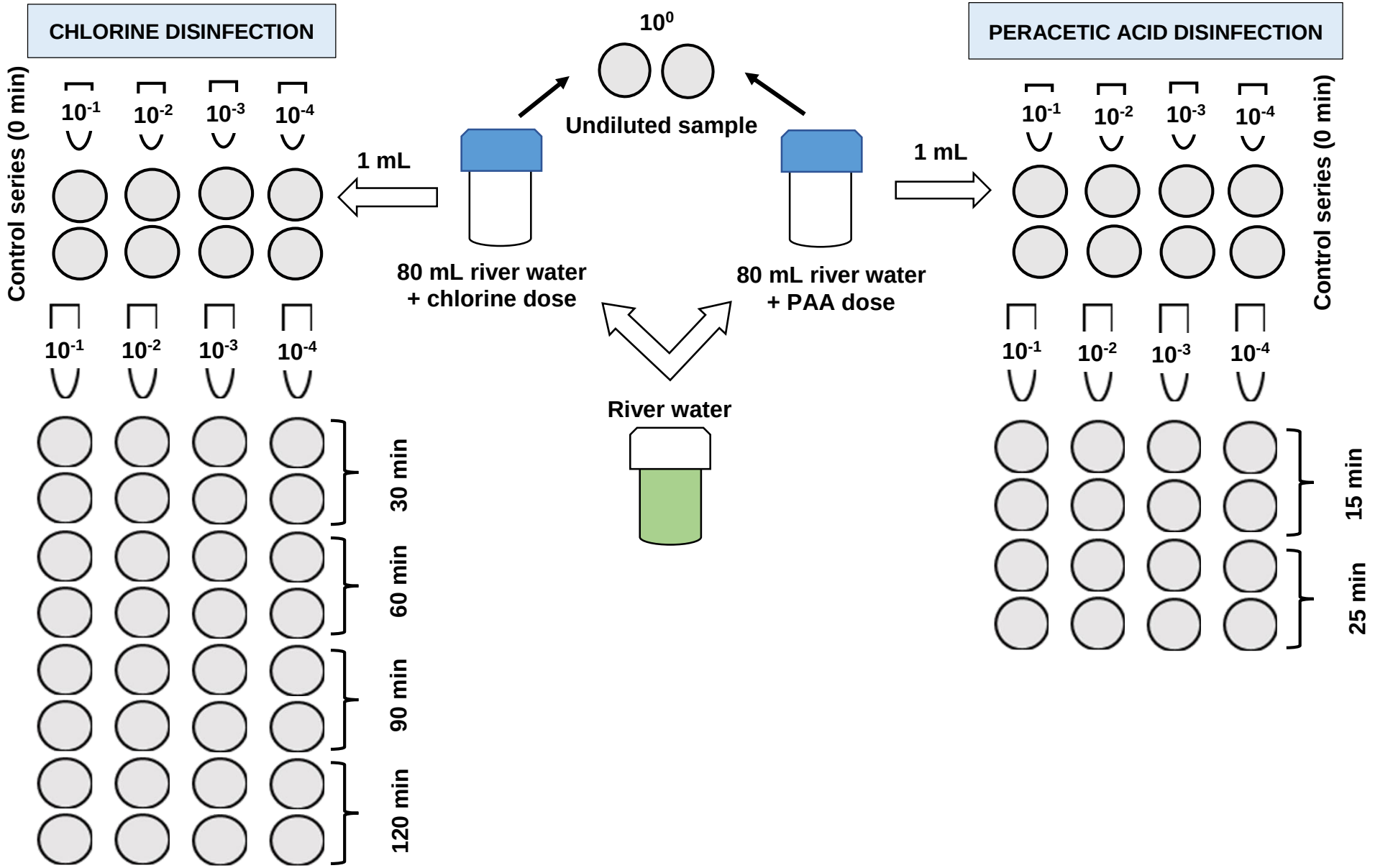

Figure 1 General protocol for chlorine and peracetic acid disinfection in river water.

Turbidity, electrical conductivity, pH and temperature

Water turbidity, expressed as nephelometric turbidity units (NTU), was determined by a portable Oreon AQ3010 turbidity meter (Thermo Scientific, USA) that was calibrated with deionised water. Electrical conductivity was measured using a HI 8711 meter (Hanna Instruments, USA). The meter consisted of a probe that was submerged in the river water sample. Electrical conductivity ($mS.m^{-1}$) is an indication of the amount of inorganic dissolved solids (salinity) present in the sample. The measurement is based on an electrical current that passes through the water. The pH and temperature of the river water were always measured before disinfection using a pH 320 meter (WTW, Germany). The South African guidelines for water intended for the irrigation of fresh produce are summarised in Table 2.

Table 2 South African guidelines for irrigation water quality (DWAF, 1996)

Water Quality Parameter	
pH	6.5 – 8.4
TSS	< 50 $mg.L^{-1}$
Electrical conductivity	< 40 $mS.m^{-1}$
Faecal coliforms	≤ 1 000 $cfu.100\ mL^{-1}$

RESULTS AND DISCUSSION

STUDY 1

Residual chlorine results

Table 3 displays the residual chlorine levels that remained in river water after 30 min of disinfection at various chlorine concentrations. Results include the COD load of river water detected on each day (Table 3). Clear differences were seen between the residual levels at the various chlorine concentrations investigated (Table 3). As the chlorine concentration increased, higher residual levels were observed. At the lowest chlorine concentration investigated (1.5 $mg.L^{-1}$), the residual levels ranged between 0.22 – 1.46 $mg.L^{-1}$ (Table 3). As the chlorine concentration increased to 3.0 $mg.L^{-1}$, the maximum chlorine residual obtained was 2.72 $mg.L^{-1}$. This was much higher compared to the maximum achieved at 1.5 $mg.L^{-1}$ chlorine (Table 3). At 6.0 and 9.0 $mg.L^{-1}$ chlorine, the maximum residual concentrations achieved were 4.51 and 4.90 $mg.L^{-1}$ (Table 3).

Results differed between sampling days. For instance, a big difference in the residual concentration at 6.0 $mg.L^{-1}$ was seen between 5 February (1.11 $mg.L^{-1}$), 11 February (2.71 $mg.L^{-1}$) and 17 February (4.51 $mg.L^{-1}$) (Table 3). The fluctuating COD levels between these three days can explain the differences in residual levels detected. The COD load of river water ranged between 30 – 96 $mg.L^{-1}$ over a period of six weeks (Table 3). On the 5th of February, the highest COD was

recorded (96 mg.L^{-1}) and this coincided with the lowest residual concentration (at 6.0 mg.L^{-1} chlorine treatment) compared to the 11th and 17th of February. The COD value represents the presence of organic particles in water (Huisamen, 2012). Organic matter is oxidised by chlorine which will then result in a lower residual concentration after disinfection. Thus, on January the 27th, the COD (31 mg.L^{-1}) as well as the residual chlorine levels (0.22 – 0.31 mg.L^{-1}) were low. It is suggested that additional environmental parameters (TSS, water alkalinity and conductivity) may have had an influence on the lower residual concentrations remaining in the water on this day.

The COD load of river water did not always correlate with the residual chlorine concentration. On the 2nd and 11th of February, the COD was 61 mg.L^{-1} on both days, but resulted in different residual concentrations in water treated with 6.0 mg.L^{-1} chlorine. This result is explained by the fact that the composition of river water may have differed between those two days and therefore river water may have displayed a different chlorine demand. Ultimately, the results from Table 3 show that the COD and residual chlorine levels are slightly related and also depend on the physico-chemical character of river water.

Table 3 Residual chlorine levels after 30 min exposure to various chlorine concentrations over a period of six weeks.

		Chlorine concentration (mg.L^{-1})			
Date	**COD (mg.L^{-1})**	**1.5**	**3.0**	**6.0**	**9.0**
20 January 2015	30	ND	1.30	3.19	3.85
27 January 2015	31	0.22	0.27	0.31	-
2 February 2015	61	1.46	1.22	3.12	2.57
5 February 2015	96	1.12	2.01	1.11	2.51
11 February 2015	47	1.02	2.72	2.71	3.71
17 February 2015	61	1.30	2.21	4.51	4.90

ND – not determined

The reason for monitoring the chlorine demand of river water was to identify a chlorine concentration suitable for river water disinfection, simultaneously limiting high residual chlorine levels. The chosen limit for residual chlorine levels in this study was ≤ 1 mg.L^{-1}. This limit was not met 86.4% of the time during this study. Residual chlorine guidelines are specifically in place to reduce the possible risk of DBP formation in water resources as these substances can be detrimental towards human health (Sayyah & Mohamed, 2014). From the results seen in this study, together with the findings in Chapter 3, a chlorine concentration of at least 3.0 – 6.0 mg.L^{-1} was chosen for river water disinfection assays. Although residual levels were exceeded at these chlorine concentrations, adequate disinfection, despite the possible health effects caused by DBPs, was

considered the most important factor in ensuring safe water (Pieterse, 1988). Chlorine resistant organisms have developed over the years (Lisle *et al*., 1998) and may also contribute to residual chlorine levels exceeding the chosen limit by restricting chlorine access to the cell. During this study it was noted that the COD of river water is ever changing and this may cause fluctuation in the chlorine dosages used for river water disinfection. This research further investigates the treatment of river water with 3.0 – 6.0 $mg.L^{-1}$ chlorine for the inactivation of microorganisms such as heterotrophic bacteria, total coliforms and *E. coli*. This also includes the evaluation of PAA disinfection of river water as it does not leave a residual and breaks down into biodegradable by-products that are harmless to the environment (Zanetti *et al*., 2007).

STUDY 2

Peracetic acid and chlorine disinfection in river water

Results of water quality analysis (Trials 1 – 5)

Table 4 displays the physico-chemical as well as the microbiological characteristics of river water sampled on different days (Trials 1 – 5). Variation in the river water quality was seen during these five trials. The temperature and pH of river water fluctuated between 15 and 18.5°C and 6.73 – 7.43, respectively (Table 4). DWAF (1996) classifies the pH of irrigation water into different categories: Class 1 (pH < 6.5), 2 (pH 6.5 – 8.4) and 3 (pH > 8.4). A neutral pH is recommended for optimum chlorine disinfection. The river water used in Trials 1 – 5 was classified as Class 2 irrigation water according to their pH, which is considered to be acceptable for optimal chlorine disinfection. At these levels, irrigation water will also not have detrimental effects on crop yield or quality (DWAF, 1996). The poorest water quality was detected during Trial 2 as it showed high levels of COD (1 094 $mg.L^{-1}$), alkalinity (95 $mg.L^{-1}$ $CaCO_3$), electrical conductivity (64.4 $mS.m^{-1}$), turbidity (13.34 NTU) and the lowest UVT% (15%) of all trials (Table 4). The COD load, in particular, varied the most between Trial 2 and the other trials. The organic content in Trial 2 was unexpectedly high (COD of 1 094 $mg.L^{-1}$) (Table 4) and indicated high levels of organic pollution. On the other hand, much lower COD levels were seen in Trials 1 (30 $mg.L^{-1}$), 3 (21 $mg.L^{-1}$), 4 (14 $mg.L^{-1}$) and 5 (108 $mg.L^{-1}$). These results correlated with the results from Table 3 in Study 1, showing that the COD load of river water fluctuated frequently between different days.

The lowest COD was detected in Trial 4 (14 $mg.L^{-1}$) and this result correlated with the low levels of other water quality parameters measured during this trial such as TSS and turbidity. The same trend was observed for Trial 5. The TSS guideline for irrigation water (< 50 $mg.L^{-1}$) (DWAF, 1996) was met during all trials in this study. Trials 4 and 5, however, displayed the lowest levels in terms of TSS (Trial 4 – 3.3 $mg.L^{-1}$, Trial 5 – 4.5 $mg.L^{-1}$) and turbidity (Trial 4 – 5.76 NTU, Trial 5 – 4.11 NTU). These results indicated low concentrations of suspended matter in river water. Based

on these results it was concluded that the river water analysed during Trial 4 and 5 represented an overall better water quality compared to Trial 2.

Table 4 Physico-chemical and microbiological parameters of river water before disinfection trials 1 – 5.

Parameters	Trial 1	Trail 2	Trail 3	Trial 4	Trial 5
Temperature (°C)	18	18.5	16	15	17
pH	7.32	6.73	7.43	7.12	6.90
COD ($mg.L^{-1}$)	30	1 094	21	14	108
Alkalinity ($g.L^{-1}$ $CaCO_3$)	119	95	88	75	87.5
TSS ($mg.L^{-1}$)	8.0	8.0	8.0	3.3	4.5
ECO ($mS.m^{-1}$)	52.9	64.6	35.7	41.5	49.6
Turbidity (NTU)	12.05	13.34	12.51	5.76	4.11
UVT (%)	49.9	15.1	62.3	36.9	53.3
HPC ($cfu.100\ mL^{-1}$)	18.32×10^6	18.58×10^6	13.47×10^6	7.60×10^5	9.63×10^5
TC ($cfu.100\ mL^{-1}$)	1.51×10^6	1.69×10^6	1.66×10^5	1.61×10^4	1.66×10^4
EC ($cfu.100\ mL^{-1}$)	1.97×10^5	5.40×10^5	6.38×10^4	2.85×10^3	3.52×10^3

HPC – Heterotrophic plate count; ECO – Electrical conductivity; TC – Total coliforms; EC – *Escherichia coli*

The irrigation water guideline for electrical conductivity (< 40 $mS.m^{-1}$) (DWAF, 1996) was exceeded in most of the trials except in Trial 3 (35.7 $mS.m^{-1}$). Electrical conductivity is an indication of the amount of dissolved solids in water. River water in Trial 2 displayed the highest electrical conductivity (64.6 $mS.m^{-1}$) and therefore represented the highest level of dissolved solids in river water compared to the other trials.

The microbial quality of river water was also determined and these levels differed immensely between Trials 1 – 5. *Escherichia coli* is a well-known indicator of faecal pollution in water sources and therefore also gives an indication of the possible presence of pathogenic microorganisms (Huisamen, 2012). River water showed high levels of faecal pollution as EC numbers ranged between 2 850 and 540 000 $cfu.100\ mL^{-1}$ during this study (Table 4). Another study by Lamprecht *et al*. (2014) reported EC loads between 250 000 and 1 000 000 MPN (most probable number) per 100 mL^{-1} in the Plankenburg River. From these results, it was clear that this river is regarded unsuitable for irrigation as levels exceeded the guideline limit for irrigation water of 1 000 faecal coliforms.100 mL^{-1} (WHO, 1989; DWAF, 1996). Untreated sewage pollution from informal settlements located upstream from the Plankenburg River has been previously reported to be the

possible cause of this problem (Barnes, 2003). The Department of Water Affairs groups certain levels of *E. coli* and associates them with the risk involved in irrigation water: below 1 *E. coli*.100 mL^{-1} is associated with 'no risk', 1 – 1 999 *E. coli*.100 mL^{-1} indicates 'low risk' and 1 000 – 3 900 *E. coli*.100 mL^{-1} is associated with 'high risk' (DWAF, 1996). Therefore, there is a high probability that microbial transfer from river water to the surface of fresh produce may occur. Lamprecht *et al.* (2014) identified five pathogenic *E. coli* strains out of 81 *E. coli* organisms isolated from the Plankenburg River and these may have low infective doses. For instance, the infective dose of pathogenic *E. coli* O157:H7 is 10 $cfu.mL^{-1}$ (Jaeger & Acheson, 2000) and may lead to serious foodborne illnesses if transferred to fresh produce and subsequently ingested by humans.

The heterotrophs (18 580 000 $cfu.100\ mL^{-1}$), TC (1 690 000 $cfu.100\ mL^{-1}$) and EC (540 000 $cfu.100\ mL^{-1}$) (Table 4) were the highest during Trial 2 compared to the microbial levels detected in the other trials. This result correlated with the high levels of COD, alkalinity, electrical conductivity and turbidity detected during Trial 2. The microbial load detected in Trial 4 and 5 were notably lower than that observed in Trials 1 – 3 (Table 4). Environmental factors such as rainfall and lower temperatures which were experienced during Trial 4 and 5 can explain this result, as it could have had a diluting effect on the pollution levels of the river.

A correlation could thus be made between the physico-chemical and microbiological status of river water. Of all the trials, Trial 4 showed overall best water quality and Trial 2 the lowest. Results from this study agree with previous research reporting the deteriorating water quality of the Plankenburg River in the Western Cape (Lötter, 2010; Kikine, 2011; Huisamen, 2012, Britz *et al*., 2013). Informal settlements as well as local industries may have been the main cause of pollution levels in the Plankenbrug River. This is also the main reason for the deteriorating quality of many South African rivers. This further emphasise the great need for river water disinfection prior to irrigation of fresh produce items to limit the transfer of pathogenic microorganisms to humans.

Effect of chlorine and PAA on microbial inactivation in river water (Trials 1 – 3)

The following tables show the log cell values recorded for heterotrophic organisms (HPC), total coliforms (TC) and *E. coli* (EC) before and after chlorine and PAA treatment for three disinfection trials (Tables 5, 6 & 7). Similar disinfectant concentrations and contact times were tested in Trials 1 – 3. Based on the microbial levels detected in river water during this study as well as in previous studies, the reduction target for *E. coli* was set at 3 – 4 logs.

Table 5 indicates disinfection results obtained in Trial 1. A chlorine concentration of 6.0 $mg.L^{-1}$ was effective toward TC and EC organisms, resulting in total inactivation after a contact time of 120 min (Table 5). Similarly, a PAA dose of 4.5 $mg.L^{-1}$ and a contact time of 25 min caused total reduction of EC organisms (Table 5). This was not the case with TC bacteria as they were reduced with 2.63 log after PAA disinfection (Table 5). The HPC were higher after chlorine disinfection (3.24 log $cfu.mL^{-1}$) compared to that after PAA disinfection (2.85 log $cfu.mL^{-1}$). It was clear that the total heterotrophic bacteria in river water was more sensitive towards PAA than to

chlorine. The limits for EC bacteria (≤ 1 000 faecal coliforms.100 mL^{-1}) (WHO, 1989; DWAF, 1996) were reached during this trial. So, water could be considered safe for irrigational purposes.

Table 5 Log cell values (cfu.mL^{-1}) before and after chlorine and PAA disinfection Trial 1

	HPC	**TC**	**EC**
Chlorine (6.0 mg.L^{-1})			
Before treatment	5.26±0.04	4.00±0.05	3.30±0.0046
After treatment (120 min)	3.24 ±0.095	None	None
Peracetic acid (4.5 mg.L^{-1})			
Before treatment	5.26±0.04	4.28±0.21	3.30±0.0046
After treatment (25 min)	2.85±0.022	1.65±0.40	None

Table 6 shows the microbial counts (log cfu.mL^{-1}) after chlorine and PAA disinfection for Trial 2. The TC and EC results obtained after chlorine disinfection in Trial 1 were comparable to that of Trial 2. Again, total inactivation of TC and EC microorganisms took place after 120 min chlorine disinfection during Trial 2 (Table 6). This result complied with faecal coliform guidelines for irrigation water (WHO, 1989; DWAF, 1996). Chlorine reduced the total heterotrophic bacteria with 2.06 log and it was evident that some microorganisms in this group were more resistant to chlorine than TC and EC. In contrast to these findings, an overall increase in microbial numbers was observed after PAA disinfection. These results were not expected as the heterotrophic organisms, TC and EC increased with 0.06, 0.39, and 0.32 log after 25 min exposure to PAA (Table 6). It was clear that the bactericidal effect of PAA was reduced, consequently allowing TC and EC growth to occur during the incubation period.

Table 7 indicates the microbial levels in river water before and after chlorine and PAA disinfection for Trial 3. As observed in Trial 1 and 2, a chlorine dose of 6.0 $mg.L^{-1}$ and 120 min again resulted in total inactivation of TC and EC organisms. Similar results were noted after PAA disinfection. The heterotrophic population in river water decreased with 1.40 and 1.39 log after chlorine disinfection and PAA disinfection, respectively (Table 7). This result was in contrast to the PAA results seen in Trial 2 where PAA had no effect on heterotrophic bacteria. It was clear that the water had a higher quality during this trial compared to Trial 2 as PAA disinfection was inhibited as noted in Trial 2.

The residual chlorine concentration that remained at 6.0 $mg.L^{-1}$ after chlorine disinfection in Trials 1 – 3 is displayed in Table 8. In Trials 1 and 3, residual levels were above the chosen limit for this study (≤ 1 $mg.L^{-1}$). Contrasting to these results, the residual concentration detected in Trial 2 was below this limit.

Table 6 Log cell values (cfu.mL^{-1}) before and after chlorine and PAA disinfection Trial 2

	HPC	TC	EC
Chlorine (6.0 mg.L^{-1})			
Before treatment	5.19±0.13	4.19±0.045	3.74±0.0061
After treatment (120 min)	3.13±0.043	None	None
Peracetic acid (4.5 mg.L^{-1})			
Before treatment	5.19±0.13	4.22±0.17	3.74±0.0061
After treatment (25 min)	5.25±0.14	4.61±0.33	4.06±0.13

Table 7 Log cell values (cfu.mL^{-1}) before and after chlorine and PAA disinfection Trial 3

	HPC	TC	EC
Chlorine (6.0 mg.L^{-1})			
Before treatment	5.12±0.090	3.00±0.16	2.80±0.071
After treatment (120 min)	3.72±0.023	None	None
Peracetic acid (4.5 mg.L^{-1})			
Before treatment	5.12±0.090	3.34±0.14	2.80±0.071
After treatment (25 min)	3.73±0.11	None	None

Table 8 Residual chlorine levels after chlorine disinfection trials 1 – 3. Chlorine residuals were recorded after a contact period of 120 min.

	Trial 1	Trail 2	Trail 3
Residual chlorine (mg.L^{-1})	2.30	0.30	2.10

Figures 2 and 3 illustrate the inactivation curves of TC at different time intervals for chlorine and PAA disinfection during Trials 1 – 3. Figure 2 shows the inactivation of TC by chlorine over four time intervals (30, 60, 90 and 120 min) and from 30 min onwards, no TC growth was detected (Fig. 2).

Figure 3 indicates that the most PAA disinfection took place within the first 15 min with an average reduction of 2.21 log during Trial 1. Contrasting results were, however, observed during PAA inactivation in Trial 2 after 15 – 25 min exposure as TC increased with 0.32 log (Fig. 3). During Trial 3, TC bacteria were completely inactivated within the first 15 min of disinfection (Fig. 3). This was not the case with Trial 1 and 2 as TC growth was still visible after 25 min exposure to PAA.

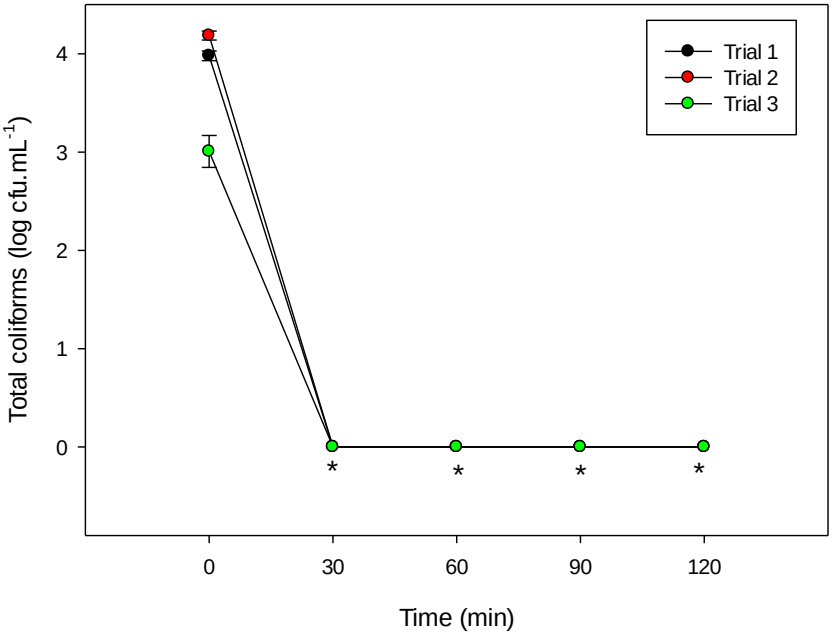

Figure 2 Inactivation curves for total coliform organisms determined at different time intervals (30, 60, 90 & 120 min) during chlorine disinfection Trials 1, 2 and 3. * - No growth observed at lowest dilution investigated (10^0)

Differences in the efficacy of microbial disinfection of river water were noted between Trials 1 – 3. This variability was attributed to different water qualities and fluctuating microbial levels in river water between different days. A chlorine dose of 6.0 mg.L^{-1} resulted in total inactivation of TC (after 30 min) and EC (after 120 min) for the three trials. Also, PAA (4.5 mg.L^{-1}, 25 min) was generally effective against TC and EC (Trials 1 & 3), however, the bactericidal activity of this disinfectant was more influenced by the fluctuating water quality (Trial 1 – 3) than chlorine. Unexpected results were obtained during PAA disinfection in Trial 2. A surprisingly high COD (1 094 mg.L^{-1}) (Table 4) was detected in river water during Trial 2. This indicated a high amount of organic material that could have influenced PAA disinfection. An unexpected increase in microbial numbers, particularly in TC (0.39 log increase) and EC (0.32 log increase), were observed (Fig. 3).

These results were in agreement with Stampi *et al*. (2001) and Gehr *et al*. (2003) who reported reduced PAA efficiency due to high organic matter present in wastewater. Gehr *et al.* (2003) reported that high COD levels, ranging between 123 – 240 mg.L^{-1} were responsible for the poor effectiveness of PAA (4.5 – 6.0 mg.L^{-1}) on faecal coliforms. Inactivation of PAA by organic matter may have occurred, consequently lowering its efficacy towards microorganisms. It is suggested that the high levels of organic matter reacted with the available PAA, consequently reducing its availability for microbial disinfection. As high organic loads influence PAA efficiency, increased dosages and contact times are required for water with high organic loads (Wilson, 2014). The turbidity of water is directly related to the organic and inorganic dissolved particles present in water (Ayyildiz *et al*., 2009).

The lowest chlorine residual (0.30 mg.L^{-1}) was detected during Trial 2 and was correlated to the highest COD level (1 094 mg.L^{-1}) also detected on this day. This indicated a very high demand by the river water. The high COD in Trial 2 was accompanied with the highest turbidity of the three trials (13.34 NTU) (Table 4). In contrast to PAA results in Trial 2, chlorine disinfection was not influenced by the high COD of the river water.

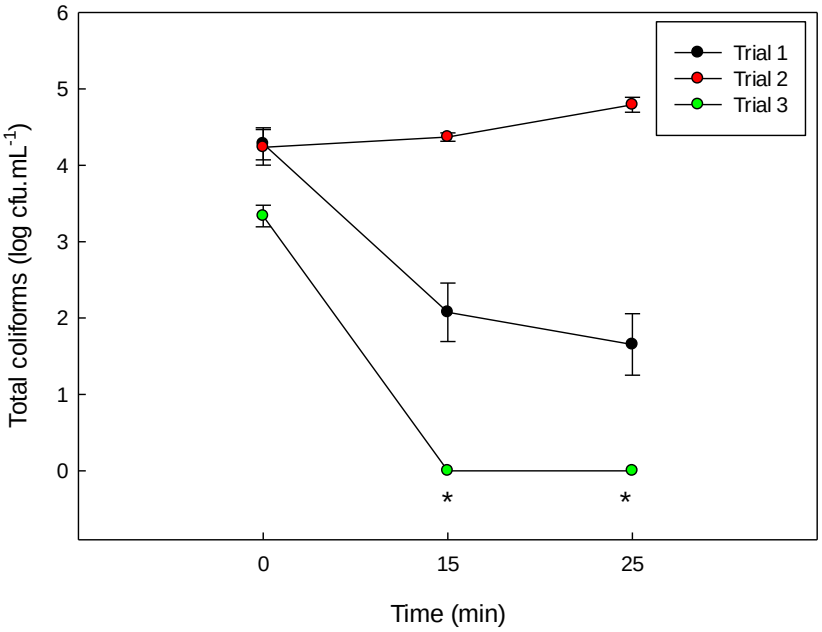

Figure 3 Inactivation curves for total coliform organisms at 15 and 25 min PAA disinfection for Trial 1, 2 and 3. * - No growth observed at lowest dilution investigated (10^0)

Of Trials 1 – 3, the highest water quality was detected in Trial 3. The COD (21 mg.L^{-1}), alkalinity (88 mg.L^{-1} $CaCO_3$) and electrical conductivity (35.7 mS.m^{-1}) as well as initial microbial levels in river water represented the lowest values compared to Trial 1 and 2 (Table 4). This explains the high disinfection efficiency of both chlorine and PAA in Trial 3.

Escherichia coli was totally inactivated during the three trials and the faecal coliform guideline for irrigation water was always met (DWAF, 1996). Also, total coliform bacteria were eliminated in most cases. On the other hand, heterotrophic organisms responded differently to chlorine and PAA disinfection during each trial. This indicates a changing heterogenic population in river water, displaying different resistances to chemical disinfection. Considering the effect of contact time, 25 – 30 min is the minimum required for a target of 1 000 faecal coliforms.100 mL^{-1} during PAA and chlorine disinfection. High residual chlorine levels exceeded the chosen residual limit for this study (≤ 1 mg.L^{-1}). In order to ensure effective river water disinfection, consequently limiting the persistence of high residual chlorine levels, the effect of lower dosages (≤ 3.0 mg.L^{-1}) can be considered in future.

Effect of chlorine and PAA on microbial inactivation in river water (Trial 4 & 5)

The effect of lower chlorine and PAA dosages on both microbial inactivation and residual chlorine levels was considered in Trials 4 and 5. Table 9 indicates the log microbial values before and after chlorine and PAA disinfection during Trial 4. Table 10 indicates the residual chlorine levels that remained after 120 min chlorine disinfection. Also, the results for Trial 5 are included in Table 11. River water disinfection was evaluated using chlorine and PAA concentrations of 3.0 $mg.L^{-1}$ for exposure times of 120 min (chlorine) and 25 min (PAA).

During Trial 4, no EC growth was detected after PAA and chlorine disinfection (Table 10). River water met the faecal coliform guideline (≤ 1 000 faecal coliforms.100 mL^{-1}) for irrigation water (WHO, 1989; DWAF, 1996). A chlorine concentration of 3.0 $mg.L^{-1}$ eliminated the presence of TC bacteria in river water. Figure 4 indicates that complete reduction of TC bacteria by chlorine took place during the first 30 min of disinfection. The heterotrophic microorganisms were sensitive to chlorine disinfection and were reduced by 3.15 log (Table 9). The residual chlorine concentration was 1.47 $mg.L^{-1}$ (Table 10) and this result did not meet the chosen limit for this study (≤ 1 $mg.L^{-1}$).

Peracetic acid showed a lower disinfection efficiency compared to chlorine. Organisms were more resistant to PAA than to chlorine. The HPC was 2.55 log $cfu.mL^{-1}$ and TC was 0.40 log $cfu.mL^{-1}$after PAA disinfection compared to 0.73 $log.cfu.mL^{-1}$ (HPC) and no TC growth after chlorine disinfection. Most TC inactivation (1.45 log reduction) took place within the first 15 min of PAA disinfection as indicated by the steep inactivation curve displayed in Figure 5.

Table 9 Log cell values ($cfu.mL^{-1}$) before and after chlorine and PAA disinfection Trial 4

	HPC	**TC**	**EC**
Chlorine (3.0 $mg.L^{-1}$)			
Before treatment	3.88±0.066	2.12±0.11	1.45±0.059
After treatment (120 min)	0.73±0.30	None	None
Peracetic acid (3.0 $mg.L^{-1}$)			
Before treatment	3.88±0.066	2.27±0.052	1.47±0.067
After treatment (25 min)	2.55±0.068	0.40±0.63	None

Table 10 Residual chlorine concentrations detected after 120 min chlorine disinfection for Trial 4 and 5

	Trial 4	**Trial 5**
Residual chlorine ($mg.L^{-1}$)	1.47	0.79

Table 11 shows the disinfection efficiency of chlorine and PAA in river water during Trial 5. Similarly to Trial 4, total inactivation of EC bacteria was noted after both PAA and chlorine disinfection and this complied with the irrigation guidelines for faecal coliforms (WHO, 1989; DWAF, 1996) (≤ 1 000 faecal coliforms.100 mL^{-1}). Total coliforms were completely inactivated by chlorine after 120 min of disinfection (Table 11). In fact, predominant inactivation took place in the first 30 min, similarly than to Trail 4 (Fig. 4). This was not the case with PAA where total coliforms were only reduced by 1.0 log after PAA disinfection and again, most disinfection took place within the first 15 min of disinfection (Fig. 5). The heterotrophic population was more resistant to PAA (0.62 log reduction) compared to chlorine (1.25 log reduction) (Table 11). During this trial, a low residual chlorine concentration (0.79 $mg.L^{-1}$) (Table 10) was obtained after chlorine disinfection and met the chosen limit for residual levels in this study (≤ 1 $mg.L^{-1}$).

Differences in disinfection results were noted between Trial 4 and 5. The initial microbial load of the river water was lower in Trial 4 compared to that of Trial 5 and together with this, river water showed an overall better water quality during Trial 4. The COD load detected in Trial 5 (108 $mg.L^{-1}$) was much higher than in Trial 4 (14 $mg.L^{-1}$). This represented higher levels of organic matter in river water during Trial 5 that could have influenced microbial disinfection resulting in lower microbial reductions, especially of the heterotrophic microorganisms and TC bacteria. The available disinfectant concentration may have been oxidised by organic matter consequently restricting microbial inactivation in Trial 5. This result is explained by the low residual chlorine concentration observed during Trial 5 (0.79 $mg.L^{-1}$). It was lower than the residual chlorine concentration measured in Trail 4 (1.47 $mg.L^{-1}$). It is clear that the degree of disinfection is predominantly dependent on the water quality, in particular on parameters such as the COD. Importantly, however, the degree of microbial resistance may vary on different days as the character of the microbial population may be inconsistent. This suggests that a specific chemical disinfectant concentration for use with river water is challenging as the quality of the river water continually changes over time.

Table 11 Log cell values ($cfu.mL^{-1}$) before and after chlorine and PAA disinfection Trial 5

	HPC	**TC**	**EC**
Chlorine (3.0 $mg.L^{-1}$)			
Before treatment	3.96±0.16	2.17±0.037	1.53±0.12
After treatment (120 min)	2.71±0.034	None	None
Peracetic acid (3.0 $mg.L^{-1}$)			
Before treatment	3.96±0.16	2.26±0.11	1.53±0.12
After treatment (25 min)	3.34±0.038	1.26±0.31	None

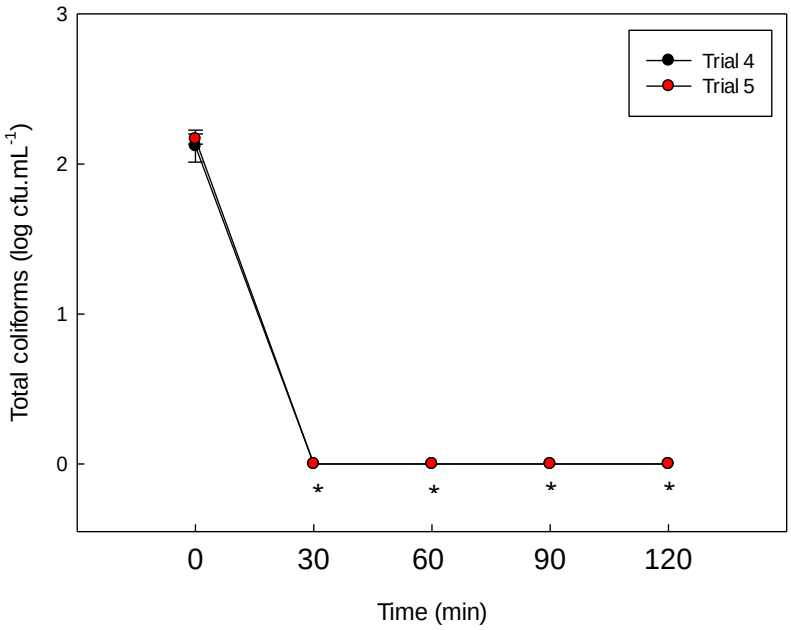

Figure 4 Inactivation curves for total coliform organisms determined at different time intervals (30, 60, 90 & 120 min) during chlorine disinfection Trials 4 and 5. * - no growth detected at lowest dilution investigated (10^0)

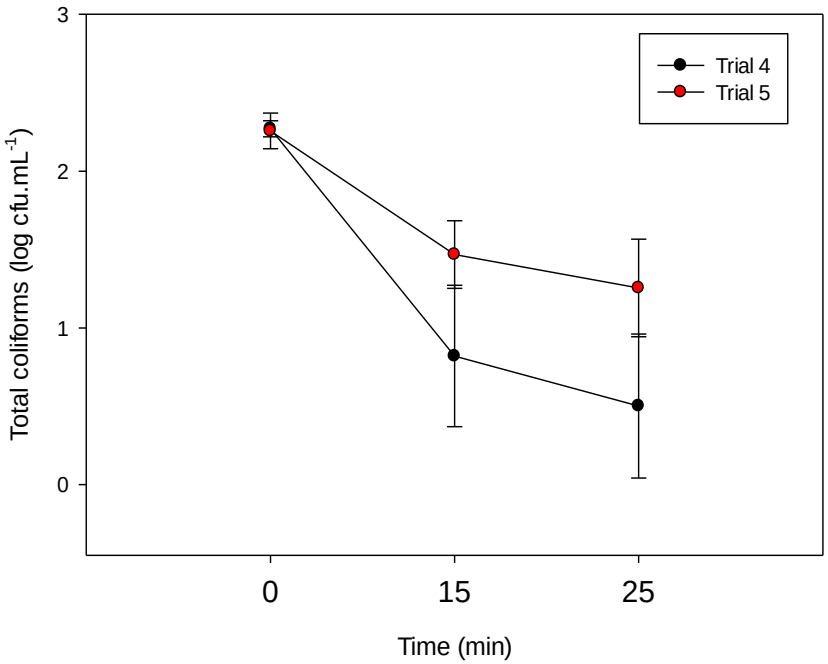

Figure 5 Inactivation curves for total coliform organisms at 15 and 25 min PAA treatment for Trial 4 and 5.

Chlorine disinfection always resulted in better reductions of heterotrophic microorganisms and TC bacteria than PAA disinfection in Trial 4 and 5. A chlorine and PAA dose of 3.0 $mg.L^{-1}$ caused total inactivation of EC during Trial 4 and 5 met the faecal coliform guideline (≤ 1 000 faecal

coliforms.100 mL^{-1}) for irrigation water (WHO, 1989; DWAF, 1996). Moreover, an exposure time of 30 min was sufficient for total TC inactivation by chlorine. This was not the case with PAA disinfection. At the maximum contact time of 25 min, total inactivation of TC did not occur. Heterotrophic organisms and TC organisms were more resistant to PAA at a dose of 3.0 $mg.L^{-1}$ and a contact time of 25 min than to chlorine (3.0 $mg.L^{-1}$, 120 min). Higher log reductions were obtained at a PAA dosage of 4.5 $mg.L^{-1}$ compared to a concentration of 3.0 $mg.L^{-1}$ in previous section. The effect was more pronounced against HPC and TC than EC. This was not the case in chlorine studies where chlorine was equally effective at the concentration range (3.0 – 6.0 $mg.L^{-1}$) investigated in this study. This is because the varying water quality had a larger effect on PAA efficiency compared to chlorine.

Altogether, different microorganisms reacted differently toward chlorine and PAA disinfection during Trials 1 - 5. Chlorine and PAA disinfection efficiency was generally lower against heterotrophic organisms than against TC and EC. This observation was also reported in previous research studies done on the effect of PAA on TC, EC and heterotrophs (Mezzanotte *et al.*, 2003; Kitis, 2004; Mezzanotte *et al*., 2007). The heterotrophic population tested in this study included organisms as bacteria which require an external source of carbon for growth (WHO, 2003). No legal limit exists for the presence heterotrophs in irrigation water but this can be used as a direct indication of the hygienic condition of water (Mezzanotte *et al*., 2003). The heterotrophic population includes a variety of bacterial species that may have been more resistant to chlorine and PAA disinfection than coliform organisms (e.g. *Bacillus*) (Stampi *et al*., 2001). Gram-negative and gram-positive bacteria are included in the heterotrophic population and research identified variation in their susceptibility toward disinfectants (Van Haute *et al*., 2013). Van Haute *et al*. (2013) found that *Listeria monocytogenes* (gram-positive) was more susceptible to chlorine disinfection than *E. coli* O157:H7 (gram-negative). They reasoned that there were differences in the susceptibility toward chlorine in gram-negative and gram-positive membranes. Differences in microbial resistances may have contributed to differences in gram-negative and gram-positive cell surface layers (Van Haute *et al*., 2013). Great differences in the inactivation of TC and EC organisms were seen between Trials 1 – 5. The effect of PAA and chlorine were higher on EC than on TC. These results agree with previous research reported on the differences between TC and EC inactivation (Mezzanotte *et al*., 2007). This situation can be explained by the differences in the starting concentrations between TC and EC and also the various species and strains present within a wide group such as TC (Mezzanotte *et al*., 2007). Furthermore, the heterotrophic population and TC bacteria showed overall greater resistance toward PAA disinfection than to chlorine. It may be that these organisms could withstand certain acid conditions as a result of previous exposure to acids in the Plankenburg River. Previous research showed that some *E. coli* strains isolated from untreated surface water and soil can produce amino acid lysine decarboxylase which helps them to adapt to more acidic conditions within the environment (Kanjee *et al*., 2011).

From this study, chlorine was the most effective disinfectant during river water disinfection. Thus, higher doses and/or longer contact times are recommended for effective PAA disinfection. In contrast to chlorine, the increase of PAA concentrations is not a concern as it decomposes fast and poses a low risk to the environment.

CONCLUSION

In this study it was evident that water quality played an essential role during chemical disinfection of river water. The level of chlorine residual remaining after river water disinfection was mainly dependent on the initial chlorine dose as well as the COD of river water. The COD accounts for chlorine consumption, therefore at the highest COD detected in Study 1 (96 $mg.L^{-1}$), a low residual chlorine concentration of 1.11 $mg.L^{-1}$ remained. The direct correlation between the COD and residual chlorine concentration could not always be made due to other water parameters that also may have played a role during disinfection. The COD level of river water varied over a period of six weeks and probably reflected different activities in the surrounding areas of the river as well as the influence of different rainfall patterns.

Results form Study 2 showed that the Plankenburg River is extensively polluted and displayed EC levels up to 540 000 $cfu.100\ mL^{-1}$. This river is regarded unsuitable for irrigation as results exceeded the faecal coliform guideline for irrigation water (WHO, 1989; DWAF, 1996). This raises the concern of disease outbreaks as the Plankenburg River is frequently used for irrigation by farmers.

From the results it was observed that the microbiological quality of river varied vastly between different days. Together with this, fluctuation in the physico-chemical parameters also occurred. Of all the physico-chemical characteristics evaluated, the COD had the greatest influence on chemical disinfection. The COD load of the Plankenburg River ranged between 14 – 1 094 $mg.L^{-1}$. The disinfection efficiency of PAA was greatly influenced at high COD levels (1 094 $mg.L^{-1}$), to such an extent that an increase in microbial growth occurred during the treatment period. This was not the case with chlorine and this chemical was effective over the range of COD levels recorded in this study.

The EC population in river water was always eliminated in this study and conformed to the faecal coliform guideline for irrigation water in South Africa (DWAF, 1996) and also the target reduction target of 3 – 4 logs. The heterotrophic microorganisms were more resistant to chemical disinfection. Note that this group is a heterogeneous population that involves various strains and species that may show different levels of resistance to chlorine and PAA. It is suggested that chemical disinfection of other pathogens, possibly present in water distribution systems, should also be investigated to ensure the safety of water prior to crop irrigation.

With regards to chlorine disinfection, the residual limit chosen for this study ($\leq 1\ mg.L^{-1}$) was only met 40% of the time in this study. This result was related to high COD levels detected on these

days. Residual levels that exceeded this limit can pose a risk if discarded into the environment due to the risk of possible by-product formation. Lower chlorine concentrations could not be suggested as the microbial safety of irrigation water is of utmost importance.

Chlorine and PAA results were slightly comparable in some cases, although chlorine was the better disinfectant in this study. A chlorine concentration of ≤ 3.0 $mg.L^{-1}$ for a contact time of at least 30 min is suggested for river water disinfection, depending on the water quality on the particular day. Together with its low cost and high availability, chlorine will be a feasible option for irrigation water disinfection at commercial-scale (in terms of microbiological quality). On the other hand, PAA doses exceeding 4.5 $mg.L^{-1}$ are recommended for river water disinfection. The efficiency of this chemical is influenced by a high COD concentration in water. Increased PAA dosages negate these inferences during microbial disinfection. Higher PAA dosages imply higher costs, however, its use poses a lower risk to the environment than chlorine. In conclusion, water quality is ever changing, therefore required chemical dosages would also be subject to change. River water resources displaying a different character composition than the Plankenburg River could also react differently to chemicals and this should be considered in future studies.

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CHAPTER 6
GENERAL DISCUSSION AND CONCLUSIONS

Water scarcity is a global reality. Shortages are experienced in multiple regions around the world and more than one billion people do not have access to safe drinking water. Moreover, demands on global food supply have increased and threaten biodiversity as well as the availability of fresh water for the production of food. This scenario implicates an increased requirement for industrial and municipal water of good quality and will also increase the demand for high-quality irrigation water. Unfortunately, due to the high volumes used (70% of global water withdrawals) irrigation water quality will be impacted to the largest extent.

South Africa is a water scarce country in which 58% of the population lives in urban areas and 11.5% of the population in rural settlements where access to basic water services are limited (DEAT, 2006). A lack of clean water and sanitation facilities in both rural and informal urban settlements force inhabitants to make use of nearby rivers for their daily needs, which inevitably results in extensive pollution of the water. Local farmers are forced to draw water from these rivers for the purpose of irrigating their crops. Raw produce irrigated with untreated river water could carry a risk of bacterial contamination. This is concerning as extensive microbial contamination of some South African rivers (Western Cape) has recently been detected (Britz *et al*., 2013). Studies have reported faecal coliform (*E. coli*) levels in rivers above the standard guideline of less than 1 000 faecal coliforms per 100 mL (WHO, 1989; DWAF, 1996; Britz *et al*., 2013). Incidentally, pathogenic strains of *E. coli* and *Salmonella* spp. are often associated with a large number of fresh produce-related outbreaks.

These reports emphasise the urgent need for river water disinfection prior to fresh crop irrigation. Chemical disinfectants, in particular chlorine, have been applied in water disinfection for more than a century. Chlorine is still used today in the water disinfection industry due to its effectiveness against a broad range of microorganisms and its ease of application. On the other hand, the efficiency of peracetic acid (PAA) for water decontamination is also under investigation. Peracetic acid is highly effective against several microorganisms and its greatest advantage over chlorine is its ability to biodegrade into harmless by-products. This chemical has only been introduced recently to the fresh produce industry. This study set out to investigate the effect of chlorine and PAA on environmental *E. coli* strains in order to evaluate their potential as treatment options for microbiologically contaminated irrigation water.

The first phase of this research focussed on the effect of PAA on different *E. coli* strains. Commercially recommended PAA dosages were very effective towards environmental *E. coli* strains as no bacterial growth was detected following any of the treatments. At a lower PAA dose of 6 $mg.L^{-1}$, strain variation was clearly observed. Again, ATCC reference strains were more susceptible to PAA compared to environmental *E. coli* strains. The strain most sensitive to PAA was ATCC 25922 and the most resistant was *E. coli* F11.2. Previously developed resistance mechanisms in environmental *E. coli* strains could enable them to adapt to severe environmental

conditions. Supposing that the *E. coli* population present in river water has similar resistance levels than *E. coli* F11.2, higher dosages would be necessary for effective *E. coli* removal.

Based on previous results indicative of strain variation, the effects of lower PAA dosages and longer contact times were investigated on the most PAA resistant *E. coli* strain (F11.2). Some PAA dosages were too low to have any inhibitory effect. Increases in concentration from 3.0 to 4.5 – 6.0 $mg.L^{-1}$ PAA resulted in large increases in *E. coli* log reductions after 25 min of disinfection (> 3 log reduction). *Escherichia coli* F11.2 resisted the oxidative stress of PAA up to a certain limit after which its ability to resist high PAA doses decreased. Adding to this, contact time also played an important role during inactivation. The majority of *E. coli* inactivation occurred in the first 15 min of disinfection. Disinfection continued up to a total contact time of 25 min, but reductions at this point were in the same range as those observed after 15 min.

The quality of river water influenced PAA disinfection. The extent thereof was, however, dose-dependent. At 4.5 $mg.L^{-1}$ PAA, water quality had a large effect on PAA efficacy as *E. coli* log reductions achieved in SSS were much higher than noted in sterile river water. At 6.0 $mg.L^{-1}$ PAA, the *E. coli* log reductions achieved in different test solutions were highly comparable. The PAA dosages were however each investigated on separate days in different river water samples. Therefore, it was concluded that the varying water quality played a role in differences observed between log reductions obtained at respective concentrations. Farmers should thus apply an adequate PAA concentration to river water with high chemical oxygen demand (COD) levels to account for PAA consumption by organic matter and the possible influence of other physico-chemical parameters such as the neutralising effect of alkalinity. The effect of water quality on PAA efficacy was only tested on one *E.coli* strain (highly resistant) and not a heterogeneous *E. coli* population that resides in river water systems. Within a river water matrix, it is uncertain to what extent the indigenous microbial population will react to chlorine or PAA treatment.

The second phase of this research was based on the investigation of the effects of chlorine (using sodium hypochlorite (NaOCl) and calcium hypochlorite ($Ca(OCl)_2$) (6, 9 & 12 $mg.L^{-1}$ for 30, 60, 90 and 120 min) on ATCC and environmental *E. coli* strains. Firstly, strain variation was evident as *E. coli* strains responded differently to various chlorine treatments. In particular, the ATCC strains were more susceptible to chlorine than environmental isolates. A dose of 12 $mg.L^{-1}$ chlorine (NaOCl) applied for 120 min in saline resulted in a reduction of less than 1 log for the environmental *E. coli* strain MJ58. Under the same conditions, complete inactivation of reference *E. coli* strain ATCC 25922 was observed. Of all the strains evaluated, ATCC 25922 was the most sensitive to chlorine disinfection and environmental *E. coli* MJ58 (previously isolated from parsley) was the most resistant. Comparing the most resistant strains observed during chlorine and PAA disinfection, the level of resistance expressed by environmental strains was clearly disinfectant-specific. This is a concern as insufficient removal of resistant pathogens in river water can possibly lead to carry-over to fresh produce.

The effect of concentration and contact time were evident during chlorine studies. As the chlorine concentration was increased, greater *E. coli* inactivation was observed. A chlorine concentration (prepared from NaOCl) of 6 $mg.L^{-1}$ was ineffective in reducing *E. coli* over a contact period of 120 min (< 1 log reduction) in saline. As the concentration was increased to 9 and 12 $mg.L^{-1}$, the inactivation rate of *E. coli* increased significantly. Also, contact time played an important role during disinfection. The majority of disinfection occurred within the first 60 min of disinfection, however, disinfection continued for the remaining 60 min. The importance of contact time was emphasised as chlorine reacts with microorganisms over time. Longer contact times support successful inactivation of persisting microorganisms that display higher resistance to chlorine. A treatment of 24 $mg.L^{-1}$ chlorine (NaOCl) and 30 min resulted in total removal of the resistant *E. coli* strain MJ58. Nevertheless, the use of higher chlorine concentrations could lead to high residual levels remaining in water post-disinfection, consequently increasing the risk of possible by-product formation. Monitoring residual chlorine levels is, therefore, important to farmers as this will limit the environmental risks involved.

Subsequently, the influence of water quality on chlorine efficacy was evaluated in SSS and sterile river water (untreated and flocculated) inoculated with a resistant *E. coli* strain (MJ58). *Escherichia coli* disinfection in river water was not affected by water quality under the reported test conditions as complete inactivation was observed (> 3 log target reduction). Disinfection in SSS did not result in total *E. coli* removal. The natural composition of river water and SSS differed markedly as the COD of the saline solution used here was higher than the reported COD value of river water. It was further observed that the saline solution had a very low buffer capacity that can initially cause an uneven equilibrium between chlorine species (HOCl and OCl^-) in diluted solutions as a result of pH fluctuation. Chlorine disinfection is strictly pH-dependent. It is strongly recommended that chlorine disinfection should be conducted in well-buffered solutions to maintain desired pH for disinfection.

Lastly, differences between $Ca(OCl)_2$ and NaOCl were evident in this research. Calcium hypochlorite was much more effective towards *E. coli* than NaOCl in saline. This was attributed to pH differences that resulted from low buffer capacity of saline. Degradation of the NaOCl solution over time could have a significant effect on the efficacy of this chlorine source in practice. Environmental factors such as heat, exposure to UV light and air contributes to such degradation and should therefore, be carefully controlled by farmers. Farmers must ensure the application of fresh chlorine solutions to contaminated river water and should avoid extended storage of the chemical. Of the two chlorine sources investigated, the use of NaOCl is recommended for commercial-scale application. The use of $Ca(OCl)_2$ (granular chlorine source) in practice would have some operational drawbacks, since it has to be dissolved, and filtered, before addition to prevent irrigation pipe clogging.

The last phase of this research was thus conducted to compare chlorine with PAA for the purpose of river water disinfection. The Plankenburg River (Stellenbosch) has been reported to be

polluted with faecal coliforms to a degree exceeding standard guidelines (≤ 1 000 faecal coliforms.100 mL^{-1}) (WHO, 1989; DWAF, 1996). Chlorine (3.0 – 6.0 $mg.L^{-1}$ for 120 min) and PAA (3.0 – 4.5 $mg.L^{-1}$ for 25 min) disinfection of river water resulted in *E. coli* levels conforming to these standards. Of the two chemicals, greater microbial inactivation was achieved using chlorine. River water quality affected the disinfection capacity of the respective chemicals. Some water quality characteristics were detrimental to PAA disinfection in that it lowered its efficacy against microorganisms. Peracetic acid disinfection was impaired in river water expressing high COD and alkalinity. On the other hand, chlorine was effective at the range of physico-chemical water parameters reported in this study. Although chlorine disinfection was more effective than PAA in most cases, high residual chlorine (0.30 – 2.30 $mg.L^{-1}$) levels were always detected after disinfection. If water containing these high residual levels would be used for irrigation of fresh produce items, consumers may be exposed to harmful disinfection by-products that could be transferred during irrigation. Therefore, determining the chlorine demand of a specific river water site prior to disinfection is important.

Lastly, river water disinfection assessments showed differences in the final levels of heterotrophic organisms, total coilforms and *E. coli* after chemical disinfection. Each microbial group responded differently to the chlorine and PAA treatments. The total heterotrophic population showed the highest degree of resistance to chemical disinfectants. *Escherichia coli* bacteria were the most sensitive to chemical disinfection and were completely eliminated in most cases. No correlation between the overall physico-chemical characteristics and the microbial quality of river water was found. However, COD levels might provide a slight indication of the extent of microbial pollution occurring in river water.

Some recommendations and shortcomings should be highlighted from the results presented in this research. Environmental *E. coli* strains were always more resistant to chlorine and PAA compared to reference ATCC *E. coli* strains. Therefore, when considering a potential water treatment option for irrigation water disinfection, investigating the behaviour of environmental *E. coli* strains towards chemical disinfectants is preferred over reference *E. coli* strains. Environmental strains are a better representation of the actual river water population. However, the response of *E. coli* strains residing in river water may differ from the environmental *E. coli* strains investigated under laboratory conditions due to differences in growth phase as well as their unknown resistance levels.

Some resistant strains could also have a better chance of survival on crops due to their ability to ferment saccharose. Previously isolated environmental *E. coli* MJ56 (ability to ferment saccharose) survived in water after chlorine and PAA disinfection. There is, however, a greater chance that indigenous organisms having the same ability will survive on plants compared to those not having the ability to ferment saccharose.

This research also demonstrated that the water from the Plankenburg River is unsuitable for the purpose of irrigation and is a potential source of fresh produce contamination. This emphasises the importance of the need for properly implemented water treatment facilities on farms. Water

quality (physico-chemical as well as microbiological) plays an important part in chemical disinfection and should be evaluated prior to disinfection as this may give an indication of the required chemical concentration required for proper disinfection. However, the frequency of monitoring the character of river water is limited within a farm setup. Adding to this, a predetermined disinfectant concentration cannot be recommended due to the ever-changing physico-chemical character of river water. It is suggested that the river water site should be characterised in terms of its physico-chemical character prior to disinfection.

This study focused only on the reaction of *E. coli* strains to chlorine and PAA treatments. River water, however, encompass a heterogenic microbial population that might contain additional pathogens which may be also transferred to irrigated fresh produce items. Different microorganisms have unique structural compositions that will influence their susceptibility toward chemical disinfectants. Therefore, the effect of such treatments towards other potential pathogens (*Salmonella* spp. and *Cryptosporidium*) should be investigated in future studies as limited research is currently available.

River water studies was based only on the characterisation and treatment of river water from a single source. Future research should look into the investigation of rivers located in several regions of the Western Cape in order to better determine the role that physico-chemical parameters play during disinfection. River water from different regions may contain substances not generally found in the Plankenburg River which can influence chemical disinfection in a different manner than found in this research.

Peracetic acid eliminated *E. coli* organisms in river water studies. Peracetic acid efficiency, however, was more influenced by water quality compared to chlorine. The use of higher PAA dosages is not a health concern as this disinfectant degrades into biodegradable by-products that are harmless to the environment. Therefore, higher concentrations than evaluated in this study can be used for effective river water disinfection. However, increased costs will be involved.

Chlorine is considered a very effective disinfectant. The main drawback associated with its use is the high residual levels remaining after disinfection. Farmers can test treated water by applying an on-site rapid test using a test strip prior to irrigation of fresh produce items. Low residual chlorine levels should degrade and evaporate when water is exposed to air and sunlight. As mentioned by various researchers, the versatility of chlorine was proved again in this study as it functioned effectively over a wide range of water quality factors known to influence disinfection. It is, however, important for farmers to determine the chlorine demand of a specific river site as this will facilitate the choice of concentration required for disinfection.

Combination treatments, incorporating disinfection in a photochemical manner (UV light disinfection) in conjunction with chlorine or PAA, are also an option that should be considered in future water disinfection research. It must be taken into account that chemical disinfection, on commercial-scale, can be less effective than laboratory-scale studies due to larger volumes of water being treated. Water disinfection studies at pilot-scale will address this issue. In conclusion, this

research demonstrated that chlorine and PAA can be regarded as potentially effective treatment options for contaminated river water disinfection prior to irrigation, admittedly with some drawbacks.

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