THE QUEST FOR SAFE IRRIGATION WATER: INVESTIGATING UV IRRADIATION TREATMENT OF RIVER WATER TO REDUCE MICROBIAL LOADS

By

Marco Oosthuizen

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Supervisor:

Professor G. O. Sigge

Co-supervisor:

Dr C. Lamprecht

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DECLARATION

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ABSTRACT

Several studies have investigated the microbiological and physico-chemical characteristics of some Western Cape rivers used as sources of irrigation water for fresh produce. During this study, four Western Cape rivers were studied. The findings have shown that some of the rivers may pose a public health risk for consumers and jeopardise fresh produce safety, as tests indicated that *Escherichia coli* (*E. coli*) counts often exceeded the recommended irrigation water guidelines. As a water disinfection treatment, ultraviolet (UV) irradiation has proven to be effective and environmentally friendly, however, the application is still relatively novel in South Africa. Therefore, the aim of this study was to investigate UV irradiation treatment of river water to reduce microbial loads, for improved fresh produce safety.

In the first research chapter (chapter 3), the variation in microbial and physico-chemical characteristics of a Western Cape river system over a longer distance (including sites that were after confluence points with other rivers) was investigated. The results showed that the water quality of one river system varies at different sampling sites, often exceeding guideline limits. Ultraviolet transmission (UVT %) and *E. coli* counts ranged from 28.00 to 90.40% and 2.322 to 3.913 log CFU.mL^{-1}, respectively. Several point and non-point pollution sources along the river could have resulted in the variations observed. Shiga toxin-producing *Escherichia coli* (STEC) and Extended spectrum beta-lactamase (ESBL) – producing *Enterobacteriaceae* were detected at certain water sites. These results suggested that, left untreated, water from this river could affect fresh produce safety as a result of microbial transfer that can occur during irrigation.

The second research chapter investigated the effect of low-pressure, lab-scale UV doses (20, 40 and 60 mJ.cm^{-2}) on the Heterotrophic Plate count (HPC) and Total Psychrotrophic Aerobic Bacteria Count (TPAC) populations. Results indicated that these populations showed UV resistance, and certain pathogens such as *Bacillus cereus* and *Aeromonas hydrophila* were identified from the surviving populations. It was also observed that UV irradiation eliminated most STEC and ESBL-producing strains, which is an important observation considering the advantages UV disinfection can have for fresh produce safety.

In the third research chapter larger volumes of river water (1 000L) were treated in a medium-pressure UV disinfection pilot plant. Four different bag filters (5, 20, 50 & 100 μm) were evaluated as a pre-treatment step prior to UV disinfection, with the purpose of improving the water quality. Results showed slight improvements in suspended solids, with minimal reductions in dissolved and microbial content. However, bag filters with the smallest pore size of 5 μm showed best results.

Medium-pressure UV treatment at pilot-scale was tested on large volumes (1 000 L) of water from three rivers. Results showed that the efficacy of the UV system is highly dependent on the water

quality of the river. A single 20 mJ.cm^{-2} UV dose was applied, followed by a second UV dose of 20 mJ.cm^{-2} for each of the three rivers. The results indicated that *E. coli*, coliforms, STEC and ESBL-producing *Enterobacteriaceae* were inactivated with some HPC colonies showing UV resistance. In addition, other important pathogens such as *Listeria monocytogenes* that was detected in some the rivers, did not survive the lowest UV dose of 20 mJ.cm^{-2}.

Overall, it was established in this study that the water quality varies in river systems, where untreated river water often exceeded irrigation water limits. The efficacy of both the low-pressure laboratory-scale, and medium-pressure pilot-scale UV systems are highly dependent on the initial physicochemical water quality of the river treated. The UV dose response of microorganisms differed, as some survived the UV radiation applied, which should be monitored for pathogenic bacteria. However, with proper pre-treatment and UV dose optimisation, UV irradiation can effectively reduce pathogenic microbial loads to acceptable levels. This method shows potential for upscaling to on-farm UV disinfection of irrigation water.

UITTREKSEL

Verskeie studies het al die mikrobiologiese en fisies-chemiese kenmerke van sommige Wes-Kaapse riviere, wat as bronne van besproeiingswater vir vars produkte gebruik word, ondersoek. Die bevindinge van hierdie studies het gewys dat sommige van die riviere 'n gesondheidsrisiko vir verbruikers kan inhou, asook die veiligheid van vars produkte in gevaar stel. Die toetse het aangedui dat *Escherichia coli* (*E. coli*)-tellings dikwels die voorgestelde besproeiingswaterriglyne oorskry. Ultraviolet (UV)-bestraling, as waterontsmettingsbehandeling, is bewys as 'n doeltreffende en omgewingsvriendelike metode. In Suid-Afrika is the toepassing van UV-bestraling nog nuut, daarom was die doel van hierdie studie om UV-bestralingsbehandeling van rivierwater te ondersoek om sodoende mikrobiese tellings te verminder en verbeterde varsprodukveiligheid te verseker.

Die eerste navorsingshoofstuk (Hoofstuk 3) is die variasie in mikrobiese en fisies-chemiese kenmerke van 'n Wes-Kaapse-rivierstelsel oor 'n langer afstand ondersoek. Resultate het getoon dat die waterkwaliteit van een rivierstelsel by verskillende plekke verskil en dat die riglynlimiete dikwels oorskry word. Ultraviolet-transmissie (UVT%) en *E. coli*-tellings het gewissel van 28.00 tot 90.40 % en 2.322 tot 3.913 log CFU.mL^{-1}, onderskeidelik. Verskeie punt- en nie-punt- besoedelingsbronne langs die rivier kon gelei het tot die variasie wat waargeneem is. Shiga-toksienproduserende *Escherichia coli* (STEC) en uitgebreide spektrum beta-laktamase (ESBL)-produserende *Enterobacteriaceae* is by sekere punte van die rivierstelsel opgespoor. Hierdie resultate dui daarop dat onbehandelde water uit hierdie rivier varsprodukveiligheid kan beïnvloed, as gevolg van mikrobiese oordrag wat moontlik tydens besproeiing kan voorkom.

Die tweede navorsingshoofstuk (Hoofstuk 4) het die effek van laedruk, laboratoriumskaal UV-dosisse (20, 40 en 60 mJ.cm^{-2}) op die Heterotrofiese Plaattelling (HPC) en Totale Psigotrofiese Aerobiese Bakterietelling (TPAC)-populasies ondersoek. Resultate het aangedui dat hierdie populasies UV-weerstand toon waar sekere patogene uit die oorlewende populasies geïdentifiseer is. Daar is ook waargeneem dat UV-bestraling die STEC en ESBL-produserende organismes van hierdie studie uitgeskakel het.

In die derde navorsingshoofstuk (Hoofstuk 5) is groter volumes rivierwater (1 000L) in 'n mediumdruk UV-ontsmettingsloodsaanleg behandel. Vier verskillende sakfilters (5, 20, 50 en 100 μm) is geëvalueer as 'n voorbehandelingstap voor UV-ontsmetting, met die doel om die waterkwaliteit te verbeter. Resultate het getoon dat gesuspendeerde vastestowwe effektief deur die sakfilters verminder word, met minimale verlagings in opgeloste en mikrobiese inhoud. Sakfilters met die kleinste poriegrootte van 5 μm het egter die beste resultate getoon.

Mediumdruk UV-behandeling op proefskaal is op groter volumes water uit die drie riviere getoets. Resultate het getoon dat die doeltreffendheid van die UV-stelsel hoogs afhanklik is van die rivier se

waterkwaliteit. 'n Enkele 20 mJ.cm^{-2} UV-dosis is toegedien, gevolg deur 'n tweede UV-dosis van 20 mJ.cm^{-2} vir elk van die drie riviere. Die resultate het aangedui dat *E. coli,* kolivorme, STEC en ESBL-produserende *Enterobacteriaceae* geïnaktiveer is met sommige HPC-kolonies wat UV-weerstand toon. Verder het ander belangrike patogene soos Listeria monocytogenes wat in sommige van die riviere opgespoor is, nie die laagste UV-dosis van 20 mJ.cm^{-2} oorleef nie.

Ten slotte is daar in hierdie studie vasgestel dat die waterkwaliteit in rivierstelsels, waar onbehandelde rivierwater dikwels besproeiingswaterlimiete oorskry, verskil. Die doeltreffenheid van beide die laedruk laboratorium-skaal en mediumdruk proefskaal UV-stelsel is hoogs afhanklik van die aanvanklike fisies-chemiese waterkwaliteit van die rivier wat behandel word. Die UV-dosisreaksie van mikroörganismes het verskil, aangesien sommige die toegediende UV-bestraling oorleef het. Met behoorlike voorbehandeling en UV-dosisoptimisering kan UV-bestraling egter effektief patogeniese tellings tot aanvaarbare vlakke verminder. Hierdie metode toon potensiaal vir die opgradering na plaas UV-ontsmettingstelsels van besproeiingswater.

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

Contents

ABBREVIATIONS

ACFBW	Activated carbon filter backwash water
ARB	Antibiotic resistant bacteria
ARG	Antibiotic resistant gene
ATP	Adenosine triphosphate
BOD	Biochemical Oxygen Demand
BPW	Buffered Peptone Water
COD	Chemical Oxygen Demand
CDC	Centre of Disease Control
CFU	Colony Forming Units
CV	Clavulanic Acid
CP	Cefepime
CPD	Cyclobutene Pyrimidine Dimer
CTX	Cefotaxime
CAZ	Ceftazidime
DBPs	Disinfection By-products
DNA	Deoxyribonucleic acid
DWAF	Department of Water Affairs and Forestry
E. coli	*Escherichia coli*
EC	Electrical Conductivity
EC broth	*Escherichia coli* broth
EE broth	*Enterobacteriaceae* broth
Eerste	Eerste river
EHEC	Enterohemorrhagic *Escherichia coli*
ESBL	Extended-Spectrum Beta-Lactamase
EUCAST	European Committee on Antimicrobial Susceptibility Testing
FAD	Flavin Adenine Dinucleotide
FAO	Food and Agricultural Organisation
FC	Faecal coliforms
FDA-BAM	Food and Drug Administration Bacteriological Analytical Manual
FSMA	Food Safety Modernization Act
HC	Haemorrhagic colitis
HPC	Heterotrophic Plate Count
HUS	Haemolytic-uremic Syndrome
ISO	International Organization for Standardization
Jonkers	Jonkershoek river
L-EMB	Levine Eosin-Methylene Blue Agar
LP	Low-pressure
MALDI-TOF	Matrix-assisted Laser Desorption/Ionization Time-of-Flight
MP	Medium-pressure
mRNA	Messenger RNA

NA	Nutrient Agar
NER	Nucleotide Excision Repair
NTU	Nephelometric Turbidity Units
OD	Optical Density
OLR	Organic Load Rate
O&M	Operation & Maintenance
PAA	Peracetic acid
PAC	Powdered Activated Carbon
PCA	Plate Count Agar
PCR	Polymerase Chain Reaction
Plank	Plankenburg river
PPCP	Pharmaceuticals and personal care products
PSR	Produce Safety Rule
RNA	Ribonucleic acid
RTE	Ready-to-Eat
SABS	South African Bureau of Standards
spp.	Species
SANS	South African National Standards
STEC	Shiga-toxin producing *Escherichia coli*
TC	Total coliforms
TCM	Turbidity Causing Material
TDS	Total Dissolved Solids
TOC	Total organic carbon
TSB	Tryptic Soy Broth
TSS	Total Suspended Solids
UF	Ultrafiltration
USA	United States of America
USDA	United States Department of Agriculture
USEPA	United States Environmental Protection Agency
UV	Ultraviolet
UVT %	Ultraviolet Transmission Percentage
VRBGA	Violet Red Bile Glucose Agar
V-UV	Vacuum-UV
WHO	World Health Organization
WRC	Water Research Commission
WWTP	Wastewater treatment plant
XLD	Xylose Lysine Deoxycholate agar

CHAPTER 1

INTRODUCTION

Access to clean, safe water is critically important for human health, social development, and agriculture (Ao *et al*., 2019). As the global population growths exponentially, rapid urbanization, pollution, and water shortages have been a serious concern for global sustainability (Gunda et al., 2018). Climate change has also become a critical factor affecting agricultural water use, especially in semi-arid countries (Lu *et al*., 2019), such as South Africa (Schulze, 2008). The exponential growth of the global population coupled with the increased demand for water, has led to diminishing water sources, which are often contaminated with concerning levels of pollutants (Hanjra & Qureshi, 2010).

Furthermore, as water sources diminish, the change in water quality have been significant, causing future problems for the water dependant sectors such as the agricultural industry (Ao *et al*., 2019). Ziervogel & Ericksen (2010) reported that the agricultural sector is under strain due to various factors such as the growing populations and the effects of climate change. This results in agricultural systems not being able to meet agricultural demands and address malnutrition, ultimately causing food and nutrition insecurity (Ziervogel & Ericksen, 2010). Surface water, groundwater, and municipal water are frequently used sources for the irrigation of fresh produce and crops (Jongman *et al*., 2017).

As for South Africa, there is a safety concern regarding river water quality as industrial, agricultural, and domestic sectors frequently use the same river sources, ultimately contributing to severe river contamination (Olivier *et al*., 2015; Paulse *et al*., 2009). The lack of acceptable sanitation, poor management and waste and sewage removal in South Africa causes the deposition of human and animal waste to flow into river systems (Gemmell & Schmidt, 2013), ultimately increasing the public health risks of our rivers.

The U.S. Food and Drug Administration (FDA) defines 'fresh produce' as fresh, raw food that undergoes zero to minimal processing before being sold to consumers (FDA, 2011). The increased emphasis on a healthier lifestyle has led to significant amounts of fresh fruits and vegetables being consumed daily (Callejon *et al*., 2015). As of 2016, the U.S. Dept. of Agriculture (USDA) announced that fresh fruit and vegetable produce are worth more the 42 billion dollars, compared to 1996 when the value was only 19.6 billion strong (USDA-National Agricultural Statistics Service, 2017; USDA-Economic Research Service, 2018).

However, there are concerns, as a positive linear relationship exists between the increased consumption of fresh fruit and vegetables and foodborne illnesses related to fresh produce (Yeni *et al*., 2016). As of 2013, 51% of all foodborne infection outbreaks reported in the United States were associated with plants, where fruit and vegetables caused 45.9% of the outbreaks in the plant category

(Painter *et al*., 2013). Various factors may contribute to these outbreaks, but certain factors have been shown to be more dominant than others. Farm operations and preharvest environments have been shown to contribute to microbial contamination of fresh produce and crops (Chhetri *et al.,* 2014). However, agricultural water has been identified as the principal source of biological contamination on fresh produce (Warriner *et al*., 2009). Agricultural water should be regularly monitored if intended to be used in fresh produce production and processing (Qiu *et al*., 2019). With regards to the monitoring of irrigation water quality in South Africa, microbial limits have been specified by the Department of Water Affairs and Forestry (DWAF) along with limits for physico-chemical characteristics in irrigation (DWAF, 1996), which were consulted during this study.

Apart from fears regarding water availability in South Africa, concerns related to the quality of water have increased (Britz *et al*., 2013). Several researchers have indicated that microbial loads in some Western Cape rivers frequently exceed the irrigation water guidelines (Barnes & Taylor, 2004; Paulse *et al*., 2009; Lamprecht *et al*., 2014; Olivier, 2015, Sivhute, 2019), as stated by the Department of Water Affairs and Forestry (DWAF, 1996)

As for the microbial monitoring of agricultural water, detection methods for *Escherichia coli (E. coli)* can be used. The microorganism's recognisable characteristics makes it an important indicator of the potential contamination of water sources with human pathogens (Gekenidis *et al*., 2018). The presence of *E. coli* growth indicates that faecal contamination or ineffective treatment took place at some point of the farm-to-fork chain (Gekenidis *et al*., 2018).

With regards to fresh produce safety, universal food washing processes have shown to reduce microbial contamination on fresh produce, but would not eliminate all pathogens (FDA, 2011). To make things more complicated, another major concern is the occurrence of cross-contamination between fresh produce and wash water, as pathogens are transferred from fruits and vegetables to clean process water and vice versa (Luo *et al*., 2011). The contamination with pathogens can occur at any stage of the farm-to-fork pathway (Uyttendaele *et al*., 2015), however, preventing the transfer of pathogenic microorganisms to fresh produce during primary production by controlling the irrigation water quality more effectively, could make a significant difference.

To solve this problem, various methods have been developed for irrigation water treatment, which can be divided into three main categories, namely physical, chemical, and photochemical treatment options. Filtration (de Oliveira & Schneider, 2019), ozone (Martinez *et al*., 2011) and the use of chlorination (Chhetri *et al*., 2019) are commonly used for water disinfection. However, the effectiveness of filtration methods has shown to not be ideal for all surface water sources, due to the variability and complexity (Adhikari *et al*., 2019). The efficacy of filtration methods is also normally determined by the turbidity, microorganism type, filtration pore size and filter material, suggesting

these techniques may be sensitive to change (LeChavallier & Au, 2004). Another limitation associated with filtration methods is the fact that regular maintenance should be done in order to maintain the required flow rate and treatment efficiency (Burch & Thomas, 1998).

Furthermore, chemical treatments such as chlorine, hydrogen peroxide, Ferrate (IV) and peracetic acid have been shown to produce dangerous disinfection by-products (DBPs), which is not a sustainable and environmentally friendly option for irrigation water scenarios (Galvez & Rodrigues, 2010). Another disadvantage is the risk that crop and soil quality may be affected by certain chemical treatments (Hua & Reckhow, 2007).

One method that shows potential to overcome these limitations, is the use of UV-C light (Hijnen *et al*., 2006). The mode of action for UV disinfection is based on the formation of pyrimidine dimer compounds in the microorganism's DNA, where these compounds hinder processes such as replication, transcription and translation which ultimately result in cell death (Koutchma *et al*., 2009). Even though chlorine treatment is the most used method in most countries, the use of UV-C treatment has shown increasing interest (Hijnen *et al*., 2006). The increased popularity is due to UV being environmentally friendly with no by-product production, as this is not the case with chlorine treatment (Hijnen *et al*., 2006).

Current research in South Africa has shown that UV disinfection, using lab-scale collimated beam systems, can be very effective for the treatment of irrigation water, ultimately minimising the risk of contamination of fresh produce (Jones *et al*., 2014; Sivhute, 2019; Bursey, 2020). The research demonstrates a knowledge gap, as most UV treatment of irrigation water in South Africa is performed at lab-scale. Furthermore, various factors such as physico-chemical characteristics of the water, optimal UV dosage and targeted microorganisms should be considered to ensure constant UV disinfection and treatment optimisation.

Studies performed by Sivhute (2019) and Bursey (2020) suggested that physico-chemical characteristics of river water may influence the overall efficiency of the UV disinfection treatment. In addition, Topalcengiz et al. (2017) noted that the turbidity levels of surface water and groundwater were significantly higher compared to municipal water, ultimately affecting the UV-C efficacy. Considering the findings of Topalcengiz et al. (2017) and the suggestions made by Sivhute (2019) and Bursey (2020), it was recommended to further investigate the impact of fluctuating physico-chemical profiles of South African rivers on UV disinfection efficacy. Determining the physico-chemical variation within one river system and across different river water sources would be essential to contribute to the knowledge gap.

Furthermore, important pathogens such as Shiga toxin-producing *Escherichia coli* (STEC) and Extended spectrum beta-lactamase (ESBL) - producing *Enterobacteriaceae* should also be screened in

our river waters, as limited research is available regarding the detection in South African river water. Shiga toxin-producing *Escherichia coli* has been responsible for several outbreaks around the world (Parsons e*t al*., 2016). These pathogens are important with regards to public health, as STEC could cause a severe life-threatening condition known as haemolytic uraemic syndrome (HUS) and haemorrhagic colitis (HC) (Byrne *et al*., 2015). In addition, literature can support that STEC can be transferred from polluted river water, through irrigation networks to fresh produce (Isik *et al*., 2020; Visharm *et al*., 2021).

Furthermore, the detection of ESBL-producing *Enterobacteriaceae*, in river water in South Africa is a public health concern, as the rise of antibiotic resistant microorganisms are increasing globally (Almakki *et al*., 2019). If ESBL-producing *Enterobacteriaceae* is found in river water used as irrigation water, it could increase the public health risk for consumers of fresh produce. These organisms produce enzymes, called beta-lactamases, which provide multi-resistance to β-lactam antibiotics, ultimately making antibiotic treatment challenging for ill patients (Kar *et al*., 2015).

Jones et al. (2014) stressed that a study simulating a water treatment process in a real agricultural setting, with the use of significantly larger water volumes is of great importance to the test the UV-C efficiency. Previous studies performed by Sivhute (2019) and Bursey (2020) were conducted by using smaller volumes of river water and a laboratory scale collimated beam UV device (low-pressure UV). These findings suggest that the effect of a medium-pressure UV system, with similar doses to the lab-scale studies, should be performed at pilot-scale. The pilot-scale system will make use of larger river water volume. This could contribute to the previous studies performed, by filling knowledge gaps regarding the upscaling of the UV system, along with verifying UV dose responses of microorganisms between UV systems. Therefore, it would be suggested to study the effect of a medium-pressure UV system at pilot-scale, using larger volumes of river water, where UV dose responses and microbial counts could be compared and verified to laboratory studies performed on South African rivers. In addition, Bursey (2020) also suggested implementing a pre-treatment step, such as bag filtration, prior to UV disinfection.

Considering all the knowledge gaps and factors involved, this study aims to further investigate the ability of UV disinfection on microorganisms, contributing to the microbial safety of water. In addition, the optimisation of a UV system in an irrigation water setting. In addition, the bigger goal of the study was to take certain upscaling factors into account, which may contribute to the successful implementation of a UV treatment system at farm-scale, producing safe water for the agricultural sector in South Africa and worldwide.

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CHAPTER 2

LITERATURE REVIEW

2.1 State of Global water resources

Fresh water scarcity is one of the major environmental and global challenges we currently face in the 21st century (Jasim *et al*., 2016). It is estimated that three out of five people worldwide, will be impacted by water scarcity by 2050, which will have a negative impact on food and water security as well as affecting socioeconomics (De Bon *et al*., 2010). Globally, the agricultural sector makes use of about 70% of the total usable freshwater, which is the largest user of global freshwater (Becerra-Castro *et al*., 2015). In addition, as the demand for water increases, the demand for food crops also increases, putting more strain on the agricultural sector. De Fraiture et al., 2008 predicted that a 65% increase in global cereal demand will occur around 2050 which is already pressuring the current water resources. The importance of accessible water for agricultural purposes is of great importance as roughly 100 L of water is used to produce 1 kg of tomatoes on farmland (De Bon *et al*., 2010). Given the declining accessibility to valuable water sources, safe irrigation water is a global issue as is not an achievable option without treatment steps (De Bon *et al*., 2010).

Global food security is hugely affected by water scarcity and the occurrence of climate change (Olivier et al., 2015). Apart from the contribution of climate change, is the over-exploitation of water resources by the urban and industrial sectors, exponentially increasing and worsening the issue (Olivier et al., 2015). As the agricultural sector is not the only sector heavily relying on water, various sector will be competing for any available water sources and could result in less water saved for agricultural use (De Fraiture, 2008).

The sudden boom of urbanisation, industrialisation and increasing population in South Africa have cause most of the rural communities to lack in adequate sanitation and safe water sources (Gemmell & Schmidt, 2011). These communities therefore make use of water sources most accessible to them which are most likely contaminated due to poor sanitation conditions, leading to human and environmental health issues in South Africa (Gemmell & Schmidt, 2011). The impact of water scarcity is the hardest hit by particularly arid and semi-arid regions (Nevondo & Cloete, 1999), such as Southern Africa. Most of these arid and semi-arid regions are currently showing more interest in reuse of wastewater as it is becoming an essential component of managing sustainable water resources worldwide (Farhadkhani *et al*., 2018). In addition, the reuse of wastewater not only contributes to supplying irrigation water sources but help conserving valuable resources and relieves the stress on the environment impacted regarding the effluents discharge into water sources (Aiello *et al*., 2007). However, industrial wastewater sources have shown to harbour pathogenic microorganisms, posing a threat to humans and the risk of bacterial infections spreading through crops irrigated with polluted

wastewater (Elgallal *et al*., 2016). The use of microbially unsafe water, potentially resulting in water-borne infections, poses a major risk to not only rural communities, but also to farmer's crop yield and quality. (Keraita *et al.,* 2008). It has been documented that more fresh produce and crops are linked to more foodborne infections as good quality water sources are rapidly declining (Falardeau *et al*., 2017; Olivier *et al*., 2015).

Therefore, correct management of water resources for food security is mandatory as the cost of water usages is also increasing, aside from the growing population (Olivier, 2015). It is estimated that approximately 50% of the globe's river sources are polluted and aquifers are emptied much faster than what they are naturally restored (Hanjra & Qureshi, 2010).

2.2 Microbial state of South African rivers

Microbial situation of South African river water systems has been poorly studied, but the research that has been done shows great concerns of the water quality (Van Rooyen *et al*., 2018). The river quality across most of South Africa has caused a safety concern as industrial, agricultural, and domestic sectors in most cases all make use of the same river, ultimately contributing to severe river contamination (Olivier *et al*., 2015; Paulse *et al*., 2009). The lack of acceptable sanitation, poor management and waste and sewage removal cause the deposition of human and animal waste to flow into river systems (Gemmell & Schmidt, 2013). In South Africa very little is done on improving sanitation, management and upgrading of wastewater treatment plants, and with an exponentially growing population the problem worsens (Gemmell & Schmidt, 2013). Fresh water sources will only keep decreasing with the increase of poor management and limited improving of treatment facilities (Teklehaimanot *et al*., 2014). Rivers and other stormwater runoffs have been identified as key contributors of point and non-point source pollution in urban water (McQuillan & Robidart, 2017). Nutrient input and waste from domestic and agricultural sources fall within non-point pollution sources, compared to point source pollution, which include effluent directly released from industrial and sewage source (Yadav *et al*., 2015). In addition, the practice of discharging polluted or insufficiently treated wastewater into river systems, known as de facto reuse, also occurs in South Africa (Swana *et al*., 2020). Skosana et al. (2015) reported that de facto reuse in South Africa were performed to alleviate water shortages and solve storage space among treated wastewater effluents. Limited research has been performed regarding the extent of de facto reuse in South Africa, however, faecal pollution from these practices poses a considerable challenge regarding the conservation of river water quality. Consequently, the constant detection of high faecal contamination in various rivers of South Africa have become a major public health concern (Paulse *et al*., 2009; Britz *et al*., 2013; Bester, 2015; Olivier, 2015).

In order to tackle this problem, the South African Water Research Commission (WRC) has performed various research studies over the past few years to determine the exact microbiological state of South African rivers. Guidelines from the South African Department of Water Affairs (DWA), as well as World Health Organisation (WHO) standards have been used to evaluate the water contamination levels recorded. *Escherichia coli* (*E. coli*) has been used as the indicator organism, as it is mostly associated with the presence of pathogenic bacteria in water, as well as been strongly associated with faecal contamination from animal production or sewage works (Britz *et al*., 2013).

Muller et al. (2003) reported the presence of *E. coli* 0157:H7 from sewage systems and *E. coli* pathogens in contaminated rivers of South Africa. Momba et al. (2008) isolated pathogens, such as *Salmonella* and *Vibrio cholerae*, from wastewater facilities in the Eastern Cape province. Gemmell & Schmidt (2013) reported that samples from the Msunduzi River in KwaZulu-Natal far exceeded the safety limits (microbiological). Furthermore, Germs et al. (2004) reported that communities and farms surrounding the Chunies River in Limpopo were also at risk at that time as high faecal contamination was detected.

2.3 Western Cape rivers

In light of the microbial state of South African rivers, the Western Cape shows very similar, if not worse outcomes, as various studies have been done over the past few years with concerning results (Paulse *et al*., 2009; Huisamen, 2012; Britz *et al*., 2013; Bester, 2015; Olivier *et al* 2015). Barnes & Taylor, 2004 detected 1.29 x 10^7 *E. coli* per 100 mL from Plankenburg river in 2004. The Plankenburg river proves to have the highest microbial contamination during the summer months as the WRC investigated the river over a five-year period (Van Blommestein, 2012). However, regardless of the month, the Plankenburg river is not suitable for irrigation purposes as various pathogenic microorganism have been detected year-round (Britz *et al*., 2013). Recently studies show no further improvement as Olivier, (2015) reported 6.41 log CFU.100 mL^{-1} of faecal coliforms. Few years later, van Rooyen (2018) reported an average count of faecal coliforms in Plankenburg to be 6.67 log CFU.100 mL $^{-1}$, which still does not comply with safety regulations.

The Berg river, which is also in the same area, reported to have 1.7 x 10^6 *E. coli* per 100 mL (Paulse *et al*., 2007). According to WHO and DWA guidelines, not one of the tested rivers met die safety requirements and these contaminations are most likely due to the effect of informal settlements in the surrounding areas in combination with industrial and sewage waste (Britz *et al*., 2013; Paulse *et al*., 2007). As with the Plankenburg river, various industrial and agricultural runoff contribute to the contamination, as well as the informal settlement, Kayamandi, located on the banks of the river (Britz *et al*., 2013; Paulse *et al*., 2009). Recently, van Rooyen (2018) reported that the

average count for faecal coliforms in Plankenburg was 6.67 log CFU.100 mL^{-1}, which does not comply with safety regulations.

Furthermore, the Mosselbank river in the Kraaifontein area of Cape Town, has also reported concerning microbial levels (Bursey, 2021). It is of great concern as this river acts as the irrigation water source of large-scale commercial farmers further downstream. Previous studies (Bursey, 2021) have reported E. *coli* counts ranging between 4 and 5.5 log CFU.mL^{-1} for this location. These high levels could possibly be attributed to the water site being downstream of a wastewater treatment plant. Another Western Cape River, the Franschhoek river, has previously reported in *E. coli* counts ranging between 2.5 and 4.9 log CFU.mL^{-1} (Bursey, 2021). Even though these counts are lower than the results from Mosselbank river, it is still concerning as the river water serves as irrigation water for vineyards and large-scale commercial tomato farmers in the Franschhoek area.

Furthermore, regarding other Western Cape rivers, limited research and data is available. Overall, Western Cape water sources have also decreased significantly during the extreme drought the province faced between 2016 and 2018 (Godsmark *et al*., 2019). In addition, droughts greatly affect the water availability and quality used for agricultural and domestic use, along with a decrease in crop yield (Godsmark *et al*., 2019)

2.4 Irrigation water guidelines in South Africa and international

As the microbial profile of irrigation water in South Africa has become a concern on behalf of public health (Britz, 2013, Bursey, 2021), water from river water systems is compared to national and international irrigation water guidelines for microbial characteristics. Irrigation water guidelines are used to describe water quality based on microbiological and physico-chemical characteristics.

Steele & Odumeru (2004) stated that water guidelines vary between countries, depending on the history of outbreaks and the current economical state of the country. In South Africa, the DWAF established a set of water guidelines used in the three main water-dependent sectors of industrial, domestic and agriculture. In Table 1 the South African microbiological guidelines for irrigation water is stated, compared to the international irrigation water guidelines of the USA and WHO.

Table 2.1 Microbial limits for irrigation water in South Africa and international (DWAF, 1996a; DWAF 1996c; Uyttendaele *et al*., 2015)

Microorganism	Irrigation water limit (CFU. mL^{-1})		
	South Africa	USA	WHO
Escherichia coli	< 10	< 10	< 10
Listeria monocytogenes	NS	NS	NS
Salmonella species	NS	NS	NS
Protozoan pathogens	< 1 cyst	NS	< 1 cyst

NS – not specified

As seen in Table 2.1, important pathogens such as *Listeria monocytogenes* and *Salmonella* species are not specified in irrigation water guidelines. As Bursey (2020) also stressed, these foodborne pathogens could be underreported as there are no compulsory tests issued by the government regarding the testing of these organisms. A possible reason for microbial limits not included in the irrigation water guidelines could be attributed to knowledge gaps or limited research regarding the importance of these pathogens in an irrigation water setting. However, literature have reported that *L. monocytogenes* and *Salmonella* spps. naturally occur in the environment and are linked to faecal contamination which have polluted river water sources before (Gholipour *et al*., 2020; Eng *et al*., 2015). Sigge et al. (2016) reported that including *L. monocytogenes* and *Salmonella* spps along with *E. coli* in irrigation water guidelines would be of great importance regarding food safety and lessening foodborne outbreaks from contaminated water.

Furthermore, physico-chemical characteristics also contribute to the overall water quality of river water. In addition, guideline limits for physico-chemical characteristics of irrigation water were also developed. As crops and fresh produce are sensitive to certain physico-chemical characteristics, such as soil salinity, guidelines were developed as a control measure regarding plant health. In Table 2.2 the South Africa guideline limits for physico-chemical characteristics of irrigation water (DWAF, 1996a) are stated.

Table 2.2 Guideline limits for physico-chemical characteristics of irrigation water (DWAF, 1996a)

Water quality characteristics	Irrigation water limit
TDS	260 mg.L^{-1}
TSS	50 mg.L^{-1}
pH	6.5 – 8.4
Turbidity [1]	10 NTU
EC	40 mS.m^{-1}
Alkalinity [2]	< 120 mg.L CaCO$_3^{-1}$
UVT%	Not stipulated
COD [3]	< 75 mg O$_2$.L^{-1}.

[1] Not stipulated. No limits indicated for irrigation water, however the Water Quality Guidelines for Domestic Use (DWAF, 1996c) stated that water with turbidity values > 10 NTU, can potentially carry an associated health risk of disease. Therefore, this guideline was used for the irrigation water limit.

[2] Not stipulated. A value of < 120 mg.L CaCO$_3^{-1}$ was selected from the Industrial Water Guidelines (DWAF, 1996b) as crop quality is merely affected by alkalinity range below 120 mg.L CaCO$_3^{-1}$.

[3] Not stipulated. The Industrial Water Guidelines (DWAF, 1996b) stated that an COD level < 75 mg O$_2$.L^{-1} will be an acceptable limit for irrigation water.

As seen above, several physico-chemical characteristics are considered when determining water quality. One of the most important physico-chemical characteristics regarding UV treatment is ultraviolet transmission (UVT). It is important as UVT is a measure of UV light percentage, at 254 nm, that is able to pass through a water sample. As for recommended guideline limits in South Africa, UVT% is not yet stipulated for irrigation water use.

To determine the total dissolved solids (TDS) measurement, the sum of mobile charged ions is measured by the handheld device, expressed in parts per million (ppm) which corresponds to a reading in mg.L^{-1}. The mobile charged ions are also positively related to the sample's electrical conductivity.

Total suspended solids (TSS) refer to all suspended material in the water sample. To determine the TSS measurement, a standard method, which involves a filtering, heating, and weighing step, is used, and expressed in mg.L^{-1}. Furthermore, chemical oxygen demand (COD) refers to the amount of oxygen available in a solution for oxidative reactions to consume. The DWAF (1996a) stated that COD is a key parameter, as high COD levels could cause damage to machinery and affect the efficiency of treatments. With regards to limits in South Africa, there is currently no legislation regarding COD levels

in irrigation water. Therefore, the wastewater guideline limits for industrial use (DWAF, 199b) were consulted.

Turbidity measurements are affected by TSS level, which is expressed in Nephelometric Turbidity Units (NTU). As with COD, turbidity is also an important characteristic, as high levels can damage irrigation water equipment such as sprinkler systems. In addition, no limits are stipulated for irrigation water. However, the Water Quality Guidelines for Domestic Use (DWAF, 1996c) stated that water with turbidity values > 10 NTU, can potentially carry an associated health risk of disease. Therefore, this guideline was used for the irrigation water limit.

Furthermore, alkalinity is also not stipulated for irrigation water use. However, a value of < 120 mg.L $CaCO_3^{-1}$ was selected from the Industrial Water Guidelines (DWAF, 1996b) as crop quality is merely affected by alkalinity range below 120 mg.L $CaCO_3^{-1}$.

These microbial and physico-chemical guidelines, as physical, chemical and biological attributes, were used to provide better interpretation of water quality at river water sites used for the purpose of agricultural irrigation water.

2.5 Food safety concerns of fresh produce and water sources

2.5.1 Fresh produce

The increasing consumption of fresh fruit and vegetables certainly shows some benefits to human health (Dauchet *et al*., 2009). Furthermore, fresh produce normally receives very little microbial intervention other than undergoing a simple washing step (Lim & Harrison, 2016).

However, from a microbiological standpoint, there is major risk and challenge with the increasing consumption of fresh produce as there is a rise in the recent number of produce-related foodborne outbreaks (Uyttendaele *et al*., 2015). Due to the minimal cleaning of fresh produce, alternative strategies should be implemented to decrease possible pathogenic bacteria to the appropriate levels (Lim & Harrison, 2016). Green vegetables have been categorized as the highest priority regarding fresh produce from a global safety viewpoint (WHO, 2008). The safety of fresh produce has become a major topic in the past decade (Uyttendaele *et al*., 2015). Studies concerning the safety of fresh produce and irrigation water has been studied from the early 1900s to this day. The contamination of pathogens can occur at any steps of the farm-to-fork pathway (Uyttendaele *et al*., 2015). The production phase of fresh produce, which include planting and irrigation of the plant, is the first step where contamination can occur as it is exposed to irrigation water and soil (Uyttendaele *et al*., 2015). As soil and water are additional risk areas regarding fresh produce, we investigate each risk area in the following sections.

2.5.2 Soil

Soil is a heterogeneous environment which provides adequate nutrients, water activity, pH and temperature to harbour most pathogenic microorganisms (Smith *et al*., 2018). Soil can therefore be a source of contamination on fresh produce, if the same area was used for animal production and disposal of waste (Suslow *et al*., 2001). Plant products grown to provide food are prone to contamination from pathogenic bacteria as these plants produce root exudates, which improves the nutrients and aeration in the soil, resulting in more soil bacteria (Smith *et al.,* 2018). *Listeria monocytogenes* is a well-known pathogen, acting as a saprophyte in the environment where soil, vegetation and water are present (Locatelli *et al*., 2013). As *L. monocytogenes* naturally occurs in soil, processes where soil contamination is expected to happen, it is likely to be detected and may cause issues related to fresh produce and human health (Smith *et al*., 2018). Listeria *monocytogenes* shares the soil environment with other potential foodborne pathogens such as *E. coli*. Ibekwe et al. (2004) reported that soil texture can significantly affect the growth of the microbial population as different *E. coli* serogroups may be found in different soil environments. Texture may be associated to properties such as cation-exchange capacity and pH which will affect the adhesion ability of microorganisms to soil particles, ultimately indirectly affecting the microbial transport efficiency (Bradford *et al*., 2013). Furthermore, limited information is available about *E. coli* survival rate in the soil environment, it can be narrowed down to be originating from either irrigation water or faeces (Islam *et al*., 2004). In addition, the survival of another pathogen, *Salmonella,* can remain functioning up till a year in the soil environment (Davies & Wray, 1996).

Some research has been done to examine the effect of soil-moisture before rainfall in the context of sediment transport and soil infiltrability during splash and runoff (Ziadat & Taimeh, 2013). A positive linear relationship was noted between antecedent soil moisture and soil impermeability occur, and if both factors increase it will result in the increase in overland flows (Lee *et al*., 2019). Nag et al. (2021) reported that greater antecedent soil moisture is very much associated with increased transport of microbial matter in water through soil, across all directions (vertically and horizontally). Thus, it can be safe to say that soil is a major safety concern as it acts as a harbouring environment for pathogenic microorganisms and the characteristics of soil may inhibit or support the growth of various microorganisms.

2.5.3 Irrigation water

One of the best ways to address future food security issues is by increasing the productivity of the agricultural sector (Wheeler *et al*., 2017). The development of irrigation schemes in African countries have assisted in further development of the agricultural sector and combatting water scarcity (Burney & Naylor, 2012). However, researchers have found that microbially unsafe irrigation water is the main

source of pre-harvest contamination of fresh produce (Warriner *et al*., 2009; Pachepsky *et al*., 2011). Microbiological pathogens harbouring in water are for the most part of faecal origin and sometime even directly from faecal matter (Olivier, 2015), thus an important source of pathogenic contamination, particularly irrigation water (Uyttendaele *et al*., 2015). Major foodborne pathogens such as *L. monocytogenes*, *Salmonella* and *E. coli* may be present in untreated water sources and pose a risk as it can enter the food chain and cause foodborne diseases. The transport of pathogens during irrigation procedures are driven by numerous factors, such as soil type, soil moisture, cell size, pore size, precipitation intensity (Bradford *et al*., 2013) Apart from soil factors and weather patterns, the correct irrigation method should also be of high importance.

The most common irrigation techniques being used include drip and sprinkler irrigation, where some techniques offer more ways of microbial contamination than others. According to several studies, drip or surface irrigation should rather be implemented compared to sprinkler irrigation, as the edible parts will not be in direct contact with contaminated water (Steele & Odumeru, 2004; Adhikari *et al*., 2020). In addition, the FDA stated that spray and flood irrigation techniques cause the highest microbial load transfer onto the edible parts of the plant (FDA, 2011). Furthermore, if contaminated irrigation water is being used, pathogens would be widely spread on the outer surface of these crops, resulting in food safety concerns for consumers (Adhikari *et al*., 2019). To support this statement, various studies have shown that lettuce under sprinkle irrigation has shown to produce higher number of microbial contaminations in comparison with other techniques (Van der Linden *et al*., 2013). In addition, anaerobic conditions may develop during furrow irrigation methods as to the normal sprinkler or drip system, which lower the microbial activity of aerobic organisms in the soil environment (Fonseca *et al*., 2011). Furthermore, Solomon *et al*., (2003) reported that sprinkle irrigation may also damage the surface of the crops, creating favourable conditions for opportunistic microorganisms such as *E. coli*. The bacteria will attach itself to the produce and can remain active for long periods of time, while washing of fresh produce does not significantly remove it, ultimately resulting in foodborne infection risks when it comes to produce consumed raw (Falardeau *et al*., 2017). In contrast, the use of drip irrigation techniques has been showed to be a relatively safe alternative method, particularly in terms of crop cross-contamination (Song *et al*., 2006). However, as bacteria can enter and harbour in various environments, these techniques are never fully successful in preventing microbial contamination. The pathogenic *E. coli* 0157:H7 have been detected on dust particles, indicating that contamination can still occur through drip irrigation as mud and dust are present (Cooley *et al*., 2007). But it is believed that *E. coli* 0157:H7 can possibly internalise the plant in various mechanisms. If *E. coli* 0157:H7 is transferred via water it may potentially internalize the plants (Ge *et al*., 2013). Pathogens, and bacteria in general, have the ability to enter plants through

the stomata, root system and any cracks in the leafy greens (Kroupitski *et al*., 2009). Once a pathogen has gained access to the inside of plants, the use of chemical disinfection is ineffective as they are protected by the plant structure (Ge *et al*., 2013).

Recently, developing countries such as South Africa have stressed the concern with regards to irrigation water quality for agricultural use (Olivier *et al*., 2015). With all these risks in mind, procedures for correct treatments needs to be in place to improve the safety of these irrigation water sources and overall higher quality of the farm-to-fork chain. River water sources will not only improve the safety of the produce, but it can also improve the overall growth and yield of herbaceous species and will be of great importance to farmers as it reduces the need for fertilizers, which bring economic benefits for them (Bedbabis *et al*., 2010).

2.5.4 Bacterial outbreaks associated with fresh produce and irrigation water

Fresh fruit and vegetables are a high-risk food group for microbial contamination due to their natural growth conditions and the processes applied in the farm to fork chain (Santos *et al*., 2012). Consumption of contaminated fruits and vegetables leads to microbial infections which are the biggest obstacle regarding produce-associated diseases (Uyttendaele *et al*., 2015). Several outbreaks have been documented across the globe, from Japan, European Union and United States (Abadias *et al*., 2008). Furthermore, most of the fresh produce outbreaks reported were caused by the presence of *Listeria monocytogenes*, *Salmonella* spp. and *E. coli* 0157:H7 (Mukherjee *et al*., 2006).

Irrigation water quality has also contributed to several fresh produces outbreaks the past few years (Uyttendaele *et al*., 2015). Enterohemorrhagic *E. coli* (EHEC) and *Salmonella enterica* are associated to both environment and food safety, which could elevate food outbreaks in the agricultural sector (Karp *et al*., 2015). In 2005, the United States experienced a widespread *Salmonella* outbreak, where patients suffered from Salmonellosis, from tomatoes that was irrigated with contaminated water (Greene *et al*., 2008). In addition, the United States also experienced a *E. coli* 0157:H7 outbreak related to fresh produce during 2006, hospitalizing 205 people with gastroenteritis (Jay *et al*., 2007). Jay et al. (2007) further reported that three people died, possibly due to haemolytic uremic syndrome. In Sweden, *E. coli* 0157 was detected in contaminated lettuce which was irrigated with polluted river water (Soderstrom *et al*., 2008). Soderstrom et al. (2008) investigated the outbreak and concluded that the *E. coli* found on the lettuce came from an animal production facility upstream and the river passing through, was the route of transmission. Additionally, in 2008 poor agricultural water caused an outbreak of *Salmonella* in peppers (Behravesh *et al*., 2011). Agricultural runoff water also caused an outbreak of (EHEC) on fresh produce in Sweden during 2013 (Edelstein *et al*., 2013). As the years go by, one would expect that outbreaks linked to fresh produce would have been reduced however, this is not the case as so many factors influence the cause of an outbreak. In 2018, two

outbreaks of *E. coli* 0157:H7, once again occurred across various states of North America as contaminated lettuce was consumed, with more than 100 hospitalizations due to mild diarrhoea and gastroenteritis (CDC, 2018). A blueberry grower also caused six infections of *Salmonella* in the state of Minnesota in 2010 (Miller *et al*., 2013). All these outbreaks highlight the risks associated to fresh produce, which is commonly consumed as a raw food product. The use of contaminated irrigation water on fresh produce can therefore be seen as a major risk for foodborne outbreaks. In conclusion, the main pathogens involved in these outbreaks included *E. coli* 0157:H7, *Salmonella* and *Listeria monocytogenes* which can cause haemolytic uremic syndrome, Salmonellosis and Listeriosis, respectively (Chen *et al*., 2020).

Most of the first world countries have updated epidemiological surveillance systems, which will track most outbreaks and provide valuable data on each, compared to underdeveloped countries which lack access to such systems (Uyttendaele *et al*., 2015). Countries without epidemiological surveillance systems lack the reporting of outbreaks, but it does not imply that no outbreaks occur. It has been shown in developing countries such as India, Senegal and South Africa that poor irrigation water leads to microbiological concern of fresh produce (Aborode *et al*., 2021).

2.6 Wastewater treatments on food safety

2.6.1 Untreated wastewater concerns and Consequences of inadequate wastewater treatments in South Africa

The aim of wastewater treatment is to produce effluent that is suitable for the reuse by the agricultural sector and or discharging safe water back into inland and coastal water systems (Chigor *et al.,* 2013). Wastewater mostly contain various microorganisms, which are capable of surviving in the environment and end up being transmitted through fresh produce to humans (Chigor *et al*., 2013, St*e*ele & Odumeru, 2004; Uyttendaele *et al*., 2015). About 1400 species of bacteria, fungi, viruses and parasitic worms have been identified in wastewater, posing a major health risk for humans (CSIR, 2010). Factors that influence the microbial load of wastewater to disinfection include the attachment, biofilms, clustering, and growth condition of these organisms (Okoh *et al*., 2007; LeChevallier & Au, 2004). Ultimately, various health concerning pathogens have been detected in wastewater systems, causing major risk areas for agricultural processes and human health (Edokpayi *et al*., 2018).Reclamation treatment facilities ultimately improves the microbiological quality of water, but the effluents of wastewater treatment plants can transport microbiological and chemical contaminants, affecting the safety of irrigated vegetables (Khalid *et al*., 2018). The consequence of untreated effluents into fresh water sources has many effects on the environment and cause public health concerns (Okoh *et al.,* 2007). Untreated effluent harbour pathogenic bacteria that cause infections and toxic by-products (Paillard *et al*., 2005). Furthermore, the increase of nutrients from

these effluents results in eutrophication processes in water ecosystems, ultimately disrupting aquatic habitats and death of animals (Okoh *et al*., 2007).

In the light of these issues, Iloms et al. (2020) studied the workings of wastewater treatment plants (WWTPs) in South Africa and concluded that most of the facilities discard their effluents into nearby water sources, which is used for many purposes downstream. Edokpayki et al. (2018) investigated WWTPs in the Limpopo Province of South Africa and reported that most of the wastewater is not properly treated and rarely meets the safety requirements. There is limited research regarding wastewater effluents in the rivers resulting in various disease. However, several outbreaks of cholera and diarrhea has been observed in most provinces. In 2003, three of the South African provinces encountered a cholera outbreak where 3901 cases were reported with 45 deaths (Edokpaki *et al*., 2018). And in 2004 another cholera outbreak in Mpumalanga province, claiming 29 lives (Edokpaki *et al.,* 2018). A few years later in 2014 another diarrhea outbreak, linked to water, was observed where 45 people were hospitalized (Edokpaki *et al*., 2018).

Several studies have proven that most of South African WWTPs waste are still not treated properly, and this could be due to poor management, inadequate investment in wastewater treatment (WWT) infrastructure and the possibility of corruption (Edokpayki *et al*., 2018). Enforcing water and environmental laws must be in place and adhered to, to protect the environment and health of our citizens that makes use of these water sources downstream (Edokpayki *et al*., 2018). The Table below (Table 2.3) shows bacterial pathogens commonly found in untreated wastewater along with their corresponding disease (WHO).

Table 2.3 Bacterial pathogens commonly found in untreated wastewater (WHO, 2012)

Bacterial species	Symptoms / Disease
Campylobacter jejuni	Gastroenteritis
Escherichia coli	Gastroenteritis
E. coli 0157:H7	Haemolytic uremic syndrome
Helicobacter pylori	Abdominal pain & gastric cancer
Salmonella spp.	Salmonellosis
Salmonella typhi	Typhoid fever
Listeria monocytogenes	Listeriosis
Shigella spp.	Dysentery
Vibrio cholera	Cholera

2.7 Pathogenic microorganisms related to fresh produce and irrigation water

2.7.1 Pathogen contamination

As already stated, the fact that fresh produce is such a high-risk food group for pathogenic contamination brings a daily challenge to the agricultural and food industries. The points of possible contamination in the food-production chain are endless as contamination can occur anywhere from production, processing, preparation, and distribution (Bartz *et al*., 2017). Each risk area has its own set of factors contributing to these contaminations. Several environmental factors such as weather, season and landscape has shown to correlate with the distribution of foodborne pathogens (Falardeau *et al*., 2017). Having a wide range of environmental variables during outbreaks makes it extremely difficult to determine the exact contaminant or origin. With regards to weather patterns, higher levels of *E. coli* and *Salmonella* has been observed during times of higher precipitation (Gu *et al*., 2013). One possible mechanism may be through transport of pathogens as overland flow occur during rainfall (Lee *et al*., 2019). As with preharvest contamination, pathogens could possibly be detected in soil, faeces, irrigation, human handling and surface water sources. Many pathogens survive through postharvest processing which result in cross-contamination of any of the upcoming processes in the food chain (Jung & Matthews, 2014). *Escherichia coli*, *Salmonella* spp., and *Listeria* spp., have shown the ability to survive in soil, water and edible parts of crops, illustrating an abundance of environments which relate to fresh produce and irrigation water sites (McEgan *et al*., 2013). Contaminated surface or groundwater can therefore transport pathogens to crops via irrigation, and pathogens harbouring in soil contaminate crops also via irrigation water sources or direct contact (Jacobson & Bech, 2012). Foodborne human pathogens do not pose any risks to plants, but they have evolved enabling them to survive in intermediate plant hosts (Schikora *et al*., 2012).

2.8 Major pathogens of concern in fresh produce and irrigation water

2.8.1 *Escherichia coli*

Escherichia coli forms part of the *Enterobacteriaceae* family which includes agents of various gastrointestinal infections (Richter *et al*., 2018). As *E. coli* naturally occurs in the healthy human gastrointestinal tracts, it is therefore also associated with faecal contamination and ever present in contaminated water sources (Richter *et al*., 2018). *E. coli* is beneficial to human health as it contributes to vitamin K production as well as probiotic build-up (Warriner *et al*., 2009). However, certain *E. coli* strains can also cause severe human diseases. As *E. coli* 0157:H7 is the most well-known serotype, other non-O157 serotypes do exist and could also cause illnesses (Lim *et al*., 2010). Data collected from the early 80s to the beginning of the 21st century revealed that more than half of all *E. coli* 0157:H7 outbreaks were associated with food, where 21% of these cases was linked to fruit and vegetables (Rangel *et al*., 2005). Most *E. coli* diseases affecting humans have been linked to the consumption of contaminated water or food including fresh produce and ground beef (Rangel *et al*., 2005). Cattle act as a major source of *E. coli* 0157:H7 for human infections, as the slaughtering and manure of the cattle follow different paths in infecting humans (Mukherjee *et al*., 2007). Cattle manures contaminate water runoff which may transfers to irrigation systems and minimally processed fresh produce, and may cause human infections (Doyle *et al*., 2017). Wright et al. (2013) reported that *E. coli* 0157:H7 can internalize the root systems of lettuce and spinach plants. This is concerning regarding the food safety of fresh produce and crops, which undergo minimal processing further. Furthermore, this poses high risk when fresh produce and crops are washed on the outside, which is most likely the last processing step before consumption. Solomon et al*. (2003) performed a unique study by simulating in-field contamination by purposely spraying *E. coli* 0157:H7 on lettuce and noted that the pathogen was still harbouring the edible parts after 30 days of introducing it.

It is believed that pathogens, and microorganism in general, will thrive in environments with high temperatures, high humidity, and low light intensities. The inactivation of *E. coli* O157:H7 was not significant with regards to humidity and temperature changes (Stine *et al*., 2005). However, the change in light exposure were significant (Stine *et al*., 2005). In addition, Fonseca et al. (2011) reported that parameters such as moisture content is also insignificant when compared to the effect of light exposure.

During the warmer months, it has been demonstrated that *E. coli* has a shorter survival period, but the organism is still frequently detected in a fresh produce setting (Fonseca *et al*., 2011). Additionally, it has been reported that three out of four *E. coli* 0157:H7 outbreaks occur during the summer months (Rangel *et al.,* 2005). Caponigro et al. (2010) suggested that the warmer temperatures and regular occurrence of rain explains the frequent detection of *E. coli*. Seasons will most likely not

be the only factor influencing the occurrence of pathogenic microorganisms as Barker-Reid et al. (2009) noted that windy weather significantly increases the spread of *E. coli* through sprinkle irrigation. *E. coli* will always be an essential obstacle to overcome in these environments, as it is introduced in so many ways to irrigation water sources and has mechanisms to internalize fresh produce and crops.

2.8.1.1 Shiga toxin- producing *Escherichia coli (STEC)*

Shiga toxin-producing *Escherichia coli* (STEC) have been accounted for various gastrointestinal diseases reported in several outbreaks around the world (Parsons e*t al*., 2016). These pathogens are substantially concerning to public health as STEC cause a severe life-threatening condition known as haemolytic uraemic syndrome (HUS) and haemorrhagic colitis (HC) (Byrne *et al*., 2015). Ruminants, specifically cattle and sheep, have been identified as the principal reservoir of STEC (Mughini-Gras *et al*., 2018). The transmission of STEC occurs through the consumption of contaminated food or water along with direct contact with animals and their environments (Vishram *et al*., 2021). Isik et al. (2020) have reported that STEC has been transferred from river water, via irrigation systems, to fresh produce. Vishram et al. (2021) indicated that there are more than 400 serogroups of *E. coli*, whereas 100 have been linked to serious illness and linked to causing outbreaks around the world. The most well-known STEC serogroup is O157, linked to the first reported STEC outbreak in the late 1980s (Byrne *et al*., 2015). However, non-O157 serogroups also poses substantial concern as most infections are under-reported, with similar clinical complications as O157 (Johnson *et al*., 2004). Valilis et al. (2018) also emphasised the importance of non-O157 serogroups as detection and outbreaks of these serogroups are increasingly more common. Apart from the Shiga toxin genes, another virulence factor known as Intimin contributes to the pathogenicity of the organism (Valilis *et al*., 2018). Intimin is seen as the attachment gene that helps STEC to attach and colonise in the gut (Gonzalez-Escalona & Kase, 2019). Farfan et al. (2012) reported that Shiga toxin alone is insufficient in causing severe illness without the adherence of STEC to gut epithelial cells.

2.8.2 *Salmonella* species

In 1855, Theobald Smith was the first to discover *Salmonella* from pig intestines infected with swine fever (Eng *et al*., 2015). The name *Salmonella* comes from an American pathologist, Dr Daniel Elmer Salmon, who helped Smith find it (Eng *et al*., 2015). *Salmonella* belongs to the *Enterobacteriaceae* family, which is a Gram-negative, facultative anaerobe and rod-shaped microorganism (Barlow & Hall, 2002). Around 2600 serotypes have been recognized in the genus, with the majority of them showing abilities to adapt and survive in a wide range of animal hosts, including humans (Allerberger *et al*., 2010). Pathogenic *Salmonella* strains adapt very easily in human cells, they invade and replicate at a significant rate and therefore cause potentially fatal diseases (Eng *et al.,* 2015). Hansen-Wester *et al*.

(2002) documented that *Salmonella* invades non-phagocytic human cells and generates its own phagocytosis in order to control the host cell. The most frequent manifestation of *Salmonella* infection is gastroenteritis (Majowicz *et al*., 2010). Swine, poultry, and cattle has been the main harbouring environments for these organisms, and therefore the main source of most *Salmonella* infections (Eng *et al*., 2015). During the slaughter process at abattoirs, animal gut can be exposed to the edible muscles, which often result in *Salmonella* contamination as the organism is naturally found in the intestines (Gillespie *et al*., 2005). Jung et al. (2014) reported that contaminated irrigation water often harbours *Salmonella* in areas also affected by animal agriculture. It has been established that contaminated fresh fruit and vegetables also contribute to the transmission of *Salmonella* and cause illnesses when consumed (Pui *et al*., 2011). A study identified *Salmonella* spp. and leafy greens as the most common food-pathogen combination which is linked to foodborne outbreaks of nonanimal origin in Europe from 2007 to 2011 (Oblessuc & Melotto, 2020). *Salmonella* causes outbreaks related to food products such as melons, green-based salad, sprouts, and potatoes (DeWaal & Bhiuya, 2007).

Among all *Salmonella* subspecies, *S. enterica* is responsible for 99% of all *Salmonella* infections found in warm-blooded animals and humans (Eng *et al*., 2015). The largest number of bacterial infections, as well as deaths associated with any foodborne disease, in the United States can be traced back to *S. enterica* (Scallan *et al*., 2011). Pregnant women, young children and the immunocompromised are considered to be more susceptible to *Salmonella* infections than healthy individuals (Eng *et al*., 2015).

Ge et al. (2013) studied the effect of UV as a preharvest treatment for leafy greens and noted that *S. typhimurium* from irrigation water did reduce by a 7.3-log reduction, however, the remaining microbial load recovered and showed more resistance to the specific UV dosage. Exposing bacteria, such as *Salmonella*, to UV may result in the development of mutations and therefore increased photoreactivation, making the bacteria more resistant to UV exposure (Ge *et al*., 2013). In depth preparation needs to be done to develop the target UV dosages, in order to decrease any microbial resistance.

2.8.3 Listeria monocytogenes

Listeria species are Gram-positive, facultatively anaerobic, rod-shaped, and non-spore forming microorganisms (Coroneo *et al*., 2016). Twenty-one species of *Listeria* genus has been identified, with a few species being pathogenic (Coroneo *et al.,* 2016). The most well-known species is *L. monocytogenes* as it causes the listeriosis disease in humans since the early nineteenth century, whereas *Listeria ivanovii* is the main animal pathogen of concern (Fallah *et al*., 2012). High-risk groups for listeriosis infection include the elderly, pregnant women, infants, immunocompromised and cancer patients (Abay *et al*., 2017). A major concern about *Listeria monocytogenes* is its ability to grow

at refrigeration temperatures, very low oxygen requirements and its ability to grow in a wide pH range from pH 4 to pH 9.6 (Kurpas *et al.,* 2018). The ability to grow at low temperatures provide a food safety challenge during cold storage, especially when fresh produce undergoes very little processing after been harvested at the farms. *Listeria monocytogenes* can easily contaminate or cross-contaminate food products as it can be found in untreated river water, faeces of animals, soil environment and green foliage crops (Sanlibaba *et al*., 2018). In some cases, manure is used as fertilizer for crops, potentially contaminating the produce with undesirable pathogens (Gholipour *et al*., 2020). It is well established that fresh produce is often treated with manure as fertiliser (Gholipour *et al*., 2020). This is concerning as the manure can harbour *Listeria* species, and when eaten raw it can ultimately result in serious *Listeria* infections (Gholipour *et al*., 2020). In addition, various raw, undercooked foodstuff is a high-risk area, simply because raw products are easily contaminated and undergo minimal treatment (Oyelami *et al*., 2018). In addition, the presence of *L. monocytogenes* in RTE vegetables have been commonly detected before (Olaimat & Holley, 2012).

In arid and semi-arid regions of the world, the reuse of wastewater has been on the increase and the safety of wastewater should therefore be studied (Gholipour *et al*., 2020). Wastewater shows great potential to be associated with the contamination of fresh produce and crops, as irrigation water used for fresh produce and crops are sourced from rivers with wastewater runoff. Gholipour *et al*. (2020) reported that wastewater shows the presence of various pathogens and could therefore act as a potential source for the transmission of bacterial infection such as listeriosis. *Listeria monocytogenes* is seen as an opportunistic human pathogenic bacterium, which can interact with the surface of fresh produce and transport through the root system of the plants (Smith *et al*., 2018). Several treatment methods, such as the use of irradiation, natural antimicrobial compounds, bacteriocins, ultraviolet light disinfection and ultrasound is implemented to target *Listeria* species (Smith et al., 2018).

2.8.4 Other important pathogens

2.8.4.1 Bacillus cereus

As mentioned before, the growing consumption of fresh vegetables raises concern regarding microbial safety of food (Sood *et al.,* 2017). *Bacillus cereus*, a spore-forming organism, belongs to Gram-positive bacteria (Sood *et al*., 2017). *Bacillus cereus* is extensively distributed, from environmental water samples to its sporulation form in various food samples (Fiedler *et al*., 2019). In addition to this statement, Jensen et al., (2003), noted that decaying organic material and fresh water is a common environment to detect *B. cereus*.

Apart from the capacity to cause food spoilage, *B. cereus* contamination can also result in illness and produce toxic chemicals (Begyn *et al*., 2020). In addition, Frentzel et al. (2018) noted that

vegetables such as carrots, lettuce, cucumbers, and salad leaves indicated to be the main carrier of Bacillus *cereus*, indicating that fresh produce is a risk group for contamination.

With regards to bacterial spores, it has shown to be between 20 and 50 times more resistant to UV radiation, compared to vegetative cells (Setlow, 2014). Spores have evolved with various UV resistant characteristics such as dedicated DNA repair mechanisms, and modifications in the DNA's UV photochemistry due to specialised proteins and pigments on the outer layer (Begyn *et al*., 2020; Setlow, 2014).

Furthermore, Begyn et al. (2020) studied the UV-C resistance of *B. cereus* endospores. He reported that if these endospores were repeatedly exposed to UV exposure, the UV-C stress would result in the selection of mutants, yielding more UV-C resistance. In addition, Begyn et al. (2020) noted that the endospores were exposed to UV-C for very short periods of a few minutes, which resulted in increased resistance.

2.9 Antimicrobial resistance and food pathogens

Along with all the possible harbouring environments, is the rising concern of microbial resistance to antibiotics. The effluents from domestic wastewater that enter river water systems have been widely studied for the presence of antibiotic resistant bacteria (ARB) and antibiotic resistance genes (ARGs) (Almakki *et al*., 2019). The disposal of various wastewaters in rivers acts as a contact and exchange location for environmental and human microorganisms, pathogenic and non-pathogenic (Välitalo *et al*., 2017). In addition, the mixing of different chemical compounds and microorganisms imposes selection pressure which favour the emergence of antibiotic resistance among microorganisms (Amos *et al*., 2018).

As microorganisms can show some resistance to antibiotics, *L. monocytogenes* is luckily still sensitive to most antibiotics that impact Gram-positive bacteria (Byrne *et al*., 2016). However, other studies have shown that antimicrobial resistance can drastically increase in *Listeria monocytogenes* strains, as many antimicrobial treatments are used in veterinary medicine and pathogens can be transferred from animal to humans via the consumption of food (Conter *et al*., 2009). *Listeria monocytogenes* is seen as an opportunistic human pathogenic bacterium, which can interact with the surface of fresh produce and transport through the root system of the plants (Smith *et al*., 2018). Several treatment methods, such as the use of irradiation, natural antimicrobial compounds, bacteriocins, UV light disinfection and ultrasound is implemented to target *Listeria* species, in particular *Listeria monocytogenes,* for reduced food safety risks in fresh produce and irrigation water (Bari *et al*., 2005).

Apart from *Listeria monocytogenes*, other important organisms such as Extended spectrum beta-lactamase (ESBL) - producing *Enterobacteriaceae* are also an increasing concern. ESBL –

producing *Enterobacteriaceae* exhibit enzymes, called beta-lactamases, which provide multi-resistance to β-lactam antibiotics, essentially making these organisms harder to treat (Kar *et al*., 2015). The detection of ESBL-producing organisms in river water is of high importance as the increase of antibiotic resistant microorganisms are imminent (Almakki *et al*., 2019). In addition, ESBL-producing organisms are detected at an increasing rate, possibly due to the excessive use of antibiotics in the animal husbandry and agriculture recently (Almakki *et al*., 2019). Furthermore, environmental microorganisms illustrating antibiotic resistance are a major concern as these organisms can potentially enter the food chain through contaminated irrigation water used on fresh produce.

2.10 Treatment methods of contaminated irrigation water

Water treatment refers to the process which water undergoes to achieve certain qualities to meet specified standards (Crittenden *et al*., 2012). When surface water from rivers and reservoir sources are the main water sources, various factors will need to be addressed during treatment. The most important factors will include reducing pathogenic microorganism loads, disinfection by-products (DBPs), natural organic material (NOM) and turbidity (Zhang *et al*., 2020). As with traditional water treatment facilities, the main goal is to make use of mixing, flocculation, sedimentation, and rapid filtering in order to remove suspended and colloidal particles (Zhang *et al*., 2020). These mechanisms remove pathogenic microorganisms to a certain extent, but disinfection is responsible for the inactivation during such a treatment (Zhang *et al*., 2020). The two main aspects which determine the successfulness of a water treatment system, include DBP production and efficiency of inactivation, where both should be considered simultaneously in the system (Zhang *et al*., 2020).

2.10.1 Physical treatment

Besides the use of chemical disinfectants, a range of physical treatment methods can also be used to treat water sources (Olivier *et al*., 2015). Yiasoumi et al. (2005) stated that physical treatment can be placed in two main categories, filtration, and gravity. For irrigation water disinfection, techniques such as adsorption, absorption and straining would be the main focus (Lenntech, 2014). Filtration processes mainly remove suspended solids, chemicals, certain compounds and odours and potential to physically retain microorganism (Momba *et al*., 2008).

Physical treatment may be attributed as a pre-treatment method prior to chemical or UV disinfection, as the main purpose is the reduction of suspended solids, and certain biological and chemical compounds. Establishing a pre-treatment step may improve the efficacy of various chemical and photo-chemical treatments to follow.

2.10.1.1 Slow Sand filtration

Slow sand filtration is a method for the treatment of physical, chemical, and biological contaminants in wastewater (Li *et al*., 2018). In the early 1800s, sand filtration was mainly used to reduce suspended solids and turbidity of water, before being primarily used as a disinfectant (Huisman & Wood, 1974). Sand filtration, as a pre-treatment option, significantly improves the overall water quality by size exclusion and adsorption (Bar-Zeev *et al*., 2012). The first filtration fitted in the United States was specifically requested to lower a microbial pathogen, *Salmonella typhi*, in drinking water (Bitton *et al*., 2005). In later years, various studies were performed to understand the microbial reduction capabilities of slow sand filtration. Additionally, Hijnen et al. (2007) reported that *Giardia* and *Cryptosporidium*, which are common waterborne microorganism, are effectively reduced by slow sand filtration. Overall, it is reported that a mature sand filter can effectively reduce most bacteria, cyst, viruses, and parasitic organisms by a 2 to 4 log reduction (Zheng & Dunets, 2014). Sand filtration is still used for its simple process with straightforward and low-cost operation in tertiary treatment of wastewater plants (Samineni *et al*., 2019). Slow sand filtration can be used as a pre-treatment or stand-alone treatment, and the advantage of simple and low-cost operation is very beneficial to regions and underdeveloped countries without the availability of skilled staff (de Oliveira & Schneider, 2019).

Slow sand filtration not only improves the microbial content of water but also general water qualities. Li et al. (2018) demonstrated that slow sand filter effectively lowers the turbidity to less than 1 NTU. These turbidity values, however, can only be achieved with the most effective sand particle sizes. The most effective sand, for the use of slow sand filtering, range from 0.15 to 0.35 mm in particle size (Huisman & Wood, 1974). Sand particle sizes between 0.15 and 0.35 mm effectively reduces turbidity without the addition of any coagulant chemicals (Huisman & Wood, 1974). Furthermore, Guo *et al*., (2018) reported that sand filtration successfully removed dissolved organic carbons, consequently lowering the formation of DBPs during the process. Pathogens are also captured by these pore sizes, as contaminated water moves through (Olivier *et al*., 2015). As microbial and biological matter accumulate in these filter pores, they soon become inactive and dead cells clog the water channels (de Oliveira & Schneider, 2019). Blocked water channels can only be restored by means of backwashing. Particles being rejected from entering the filter will accumulate on the surface of the filter, resulting in a rich biological active layer called the schmutzdecke (de Oliveira & Schneider, 2019). Mechanisms involved in the removal of microbiological-mediated contaminants include adsorption of bio-oxidation, scavenging and predation (Haig *et al*., 2011).

To protect the schmutzdecke from any physical fluctuations, such as pressure and temperature, the water level should be approximately 0.9 m in depth (Steward-Wade, 2011). The sand

layer should be roughly 80 cm deep with a gravel layer ranging from 5 to 15cm, in order to support the fine sand particles (Zheng & Dunets, 2014). Rocks act as the last layer before leaving the filter, allowing water to move through freely without sand particles seeping out (Olivier *et al*., 2015). In response to the obstacles faced by sand filtration, ultrafiltration (UF) has proven to be an effective alternative for the treatment of drinking water (Moslehyani *et al.,* 2019).

2.10.1.2 Ultrafiltration

According to Crittenden et al. (2012) will membrane filtration be the direction for a new, revolutionized water technology which fulfil all the needed requirements. However, the development and further refinement of membrane filtration will heavily depend on the advances in science and membrane materials, therefore, alternative solutions should be studied (Zhang *et al*., 2020).

Ultrafiltration (UF) is considered state of the art pre-treatment technology as it has a small footprint, easy modular configuration and is highly automotive (Gao *et al*., 2011). As UF has been extensively implemented in old water plant renovations and newer water plant designs, resulting in replacing commonly used sand filtration systems (Wiesner *et al*., 1999). Furthermore, UF which is highly efficient in reduction of microbial matter, has become a favourable alternative to produce microbiologically safe water (Gao *et al*., 2011).

Bourgeois et al. (2011) studied the effects from treatments such as sedimentation with reflocculation, gravity thickening, dissolved air flotation with reflocculation, and the use of UF membranes on residues. Bourgeois et al. (2011) reported that UF performed the best by removing contaminants under normal and challenging conditions. However, the development of UF is very limited by a major problem referred to as membrane fouling, particularly in sludge water where pollutant concentrations are considerably higher (Fan *et al*., 2020).

It is well established that UF is more reliable when working in synergy with other components. The pre-treatment of feedwater needs to alleviate membrane fouling during UF operation (Fan *et al*., 2020). Despite the inefficiency of UF, pre-treatment methods such as adsorption, filtration and oxidation should be considered (Fan *et al*., 2020). The use of coagulation in combination with powdered activated carbon (PAC), acts as a pre-adsorption process for UF (Stoquart *et al*., 2012). In order to increase removal of organic contaminants (specifically humic acid) through UF, the addition of PAC to UF is therefore critical (Xu *et al*., 2019). Thus, the combination of coagulation, sedimentation and adsorption will be of great benefit, significantly improving the effectiveness of UF, providing potable water (Xu *et al*., 2019). Fan et al. (2020) also stated that the use of a single coagulant or oxidation pre-treatment step, will have a limiting effect in controlling membrane fouling. Zhang et al. (2017) suggested combining sedimentation and ozonation to pre-treat activated carbon filter backwash water (ACFBW), to successfully control membrane fouling. However, adding extra

coagulants or oxidation treatments will significantly contribute to increased management and operation costs of the plant (Fan *et al.*, 2020). It is suggested that the operation cost of UF is an important draw-back and would not be a viable option for irrigation water, but rather applied in drinking water treatments.

Treatment prior or in combination with UF operation is potentially the most important aspect of the whole process and would determine the overall effectiveness. If UF membrane integrity is compromised, microbial contamination may potentially be transported into reclaimed water sources (Lee *et al.*, 2019). Many external and internal factors can potentially influence the membrane's efficiency. The degradation of UF fibres can also occur, as chemical reactions, corrosion, poor installation, irregular maintenance, membrane stress, and filter damage by objects not removed in pre-treatment are the most common causes (Antony *et al.*, 2014). Recent studies conducted by Lee et al. (2018) showed that membranes younger than 1 year are much more efficient in removing biological pollutants, such as bacteria and viruses, compared to membranes aged for 2-6 years. Maintenance of the membrane is great importance to keep performance at the highest level for as many years possible.

2.10.1.3. Biochar

One of the products of biomass pyrolysis, biochar, has drawn much attention the past few years as an appropriate medium to filter out pathogenic microorganisms and for the removal of metals, such as copper and lead (Perez-Mercado *et al.*, 2019). Despite the large particle diameter of biochar, the removal capabilities of viruses and bacteria is similar to that of sand filtration (Bradford *et al.*, 2013). The removal capabilities of biochar mostly depend on the filter materials, which obtain different adsorption capacities, as well as different biofilms forming on filter surface and straining in small pore spaces (Cecen & Aktas, 2011; Bradford *et al.*, 2013).

With regards to the different biofilms forming on the filter surface, biochar provides opportunistic pathogens the opportunity to grow, as the environment contain some degree of free nitrogen, making it more suitable for secondary microbial growth (Wu *et al.*, 2016). The formation of different biofilms solely depends on the porosity of the filter media being used (Dalahmeh *et al.*, 2014). In addition, as operation time increase, the process of biofilms formation will increase and take over as filter material decreases, resulting in the mechanism of adsorption to fall away (Cecen & Aktas, 2011).

With regards to biochar applications in the industry, Perez-Mercado (2019) stated parameters such as high hydraulic loading rate (HLR), high electric conductivity (EC) and the use of diluted or partially treated wastewater would influence biochar's efficiency to remove pathogens. The HLR intended to be used for irrigation water should be much higher in on-farm treatments (ranging from

130-6 000 L.m $^{-2}$.d^{-1}), in order to maintain a sufficient flow, compared to conventional wastewater treatments which is much lower (34 L.m $^{-2}$.d $^{-1}$) (Keraita *et al*., 2008). It is expected from low-income countries to dilute raw wastewater effluents intended for irrigation purposes (Keraita *et al*., 2008). These dilutions are meant to reduce organic load rate (OLR) of the water till a certain extent and protecting the workings of biochars (Keraita *et al*., 2008).

When biochars are used for highly concentrated wastewater effluents it may potentially damage the filters and decrease the lifespan. However, lowering the OLR will result in limiting the development of biofilms in the filters, ultimately reducing the ability of removing microorganisms (Bradford *et al*., 2013). Apart from water treatment, biochar is also acknowledged in agriculture to improve water holding capacity, carbon sequestration and soil stability to remove heavy metals and atrazine (Yanala & Pagilla, 2020).

2.10.2 Chemical treatment

Several strategies have been proposed to help reduce the contamination of pathogenic microorganisms in water and river sources, of which chemical treatment is well known and commonly used (Gill *et al*., 2015). Among all chemical disinfectants, chlorine-based compounds are the most commonly used disinfectant for the treatment of irrigation water and disinfection of wastewater, as its dependant on its oxidizing capabilities to destroy microbial cell wall components, ultimately resulting in cell death (Decol *et al*., 2018; Yiasoumi *et al*., 2005). Other than chlorine-based disinfectants, is the use of bromine-based disinfectants, hydrogen peroxide and ozone used for water treatment. The ideal disinfectant should be effective, reliable, and consistent in the specific working environment against targeted microorganisms. In general, disinfectants should be easily transported and stored without concerning safety hazards (Olivier *et al*., 2015).

2.10.2.1 Chlorine

Chlorine, among all other chemical disinfectants, is the most used method for disinfection of wastewater and irrigation water, as it's been used from the early 1800s (Tombini Decol *et al*., 2019). The three most common forms of chlorine used for water disinfection include elemental chlorine, calcium hypochlorite and sodium hypochlorite (Momba *et al*., 2008).

When chlorine is added to water it forms two compounds, hypochlorous acid (HOCl) and hydrochloric acid (HCl) (Olivier *et al*., 2015). LeChevallier & Au, (2004) stated that HOCl then dissociates into a hydrogen (H$^+$) atom and hypochlorite (OCl$^-$). Furthermore, HOCl and OCl$^-$ makes up the free chlorine compounds which is purely responsible for the disinfection capabilities of chlorine (Momba *et al*., 2008). These free chlorine compounds result in disruption and destruction of bacterial cells by three phases (Bitton *et al*., 2005). The destruction of bacterial cells occurs when free chlorine species result in the leakage of DNA, RNA, enzymes, and nucleic acids within the cell and ultimately

cell mortality (Olivier *et al*., 2015). Furthermore, the bacterial cells may undergo insufficient adenylate energy levels due to limited respiration and transportation activities which leads to sulfhydryl oxidation by chlorine (Bitton, 2005). Despite the global use of chlorine, it is unfortunately highly reactive with organic matter which causes organo-halogenated disinfection by-products to accumulate and form (Tombini Decol *et al.,* 2019). However, chlorine dioxide (ClO_2), has proven to be an acceptable alternative to chlorine for agricultural water disinfection (Tombini Decol *et al*., 2019).

Chlorine dioxide is commercially applied in the United States and Spain by leafy greens growers (Lopez-Galvez *et al*., 2017). Farmers apply specific ClO_2^- concentrations in drip irrigated fields to effectively reduce microorganisms, as well as avoiding clogging of irrigation lines (Lopez-Galves *et al*., 2018) Chlorine dioxide has a significant advantage over chlorine as minimal types of organo-halogenated by-products, such as trihalomethanes, are formed (Lopez-Galvez *et al*., 2010). However, chlorine dioxide can still lead to accumulation of some DBPs, such as chlorates (ClO_3^-) and chlorites (ClO_2^-) in treated water (Lopez-Galvez *et al*., 2010). The decomposition of ClO_2^- into chlorate and chloride ions may take very long but it will speed up with an increase in concentrated solutions (Gordon, 1972).

Irrigation water disinfected by chlorine-based agents can possibly be the origin of chlorate residues found in food products (Kaufmann-Horlacher *et al*., 2014). According to European Union law, a maximum residue limit of 0.01 mg per kg of chlorate is accepted in food (EC, 2005). Even though chlorine dioxide still produces some unwanted by-products, it is much more effective in disinfection as it shows better bactericidal capabilities with a higher oxidation capacity over chlorine (Hassenberg *et al*., 2017). Several factors such as water pH, water temperature, organic concentration and type, disinfectant dose and dose exposure time will challenge the bactericidal capabilities of ClO_2^- (Ayyildiz *et al*. 2009). When high ClO_2^- are used for waterborne microorganisms in irrigation water, it may show phytotoxic effects which results in plant damage and a growth delay (Raudales, 2014). In addition, if the physico-chemical quality of irrigation water is above standard, sufficient levels of ClO_2^- may not cause any phytotoxic effects to the plant (Lopez-Galvez *et al*., 2018). Overall, chlorine as a chemical treatment may not be the most suited treatment option, possibly due to by-product formation, which negatively affects the environment.

2.10.2.2 Hydrogen peroxide

Hydrogen peroxide is made up of two hydrogen and two oxygen atoms linked by single bonds to form the molecule H_2O_2 (Newman, 2004). With the high oxidation potential of H_2O_2, a variety of disinfection application can be used in multiple industries such as the control of colours and aromas (Ksibi, 2006). The molecule can be easily broken down, releasing hydroxyl radicals ($\cdot OH$) (Raffelini *et al*., 2011). In

the field of food and water safety, H_2O_2 have been proven to effectively reduce a range of microorganisms that occur in irrigation water sources (Sherchan *et al.*, 2014).

The microorganism is inactivated by means of hydroxyl radicals targeting the lipids and proteins of the organism's cell membrane, which indirectly affect the processes associated with the DNA, ultimately resulting in cell death (Vargas *et al.*, 2013; Linley *et al.,* 2012). Currently, various forms of hydrogen peroxide treatment are used on viruses, bacteria, fungi, and algae (Newman, 2004), however recent studies have reported that viruses cannot be effectively reduced (Sherchan *et al.*, 2014). Considering the versatility of H_2O_2, it can be seen to successfully work on its own or in combination with other disinfectants such as UV or chlorine treatment (Vargas *et al.*, 2013; Olivier *et al.*, 2015).

H_2O_2 has been implemented to treat drinking water and wastewater which showed great success as COD and biochemical oxygen demand (BOD) were reduced, which results in an increased water quality (Ksibi, 2006). As H_2O_2 proved successful as a disinfectant, various researchers have further investigated H_2O_2 to find suitable dosages and the effect it has on microorganisms (Vargas *et al.*, 2013). A concentration of 2.5 mg.L^{-1} sufficiently removed organic matter and when a contact time of 2 hours is used, a three-log reduction of faecal coliforms can be achieved (Ksibi, 2006). Furthermore, Ksibi (2006) reported that a dosage of 2.5 mg.L^{-1} for 2 hours decreased COD from 322 mg.L^{-1} to a preferred level of 44 mg.L^{-1}. When considering the inactivation of environmental and reference *E. coli* strains, it was reported that a positive linear relationship occurs between increased H_2O_2 concentrations and microbial inactivation (Giddey *et al.*, 2015). Giddey et al. (2015) also report that environmental strains of *E. coli* showed significant resistance compared to reference strains. Furthermore, to reduce faecal indicator loads by 99%, a dosage of 125 mg.L^{-1} for 56 minutes of contact time is recommended (Ronen *et al.*, 2010). Although most research have been done on *E. coli*, which is a Gram-negative microorganism, it's been reported that the H_2O_2 treatment successfully applies for most other Gram-positive microorganisms (Koivunen & Heinonen-Tanski, 2005).

The disinfection potential of H_2O_2 has proven to be a stand-alone disinfectant, but many internal and external factors will determine the lethality of the treatment applied, which is a disadvantage of the treatment. Factors such as temperature, contact times, pH and dosage concentration can lower the overall efficacy of H_2O_2 (Raffelini *et al.*, 2011)

2.10.2.3 Ozone

The discovery of unwanted DBPs from chlorine products is harmful to human health, which has forced researchers to find new alternative disinfectants for wastewater treatment, including ozone treatment (Martinez *et al.*, 2011). Ozone produces far fewer organic by-products derived from

chlorine than any chlorine-based disinfectants and is also rapidly effective compared to chlorine, as it shows no residual effect due to zero stability in water.

It is believed that the oxidizing effect of ozone is 1.52 times more effective than chlorine (Khadre *et al.,* 2001). During the early 1990, ozone was used to purify drinking water, and to this day it is still applied in certain fields (Martinez *et al*., 2011). The application of ozone treatment can be used in aquaculture, sea water treatment, cyanide removal, colour pollution removal and the destruction of phenols and hydrocarbons from oil refineries (Rice, 1997).

Ozone is seen as a universal disinfectant as it has high oxidation potential along with properties to effectively reduce and deactivate microorganisms (Langlais *et al*., 1991). Ozone in aqueous solutions produce free radicals and secondary oxidants, ultimately improving the oxidizing effect further (Martinez *et al*., 2011). Free radicals and secondary oxidants release in the presence of oxygen, which results in no formation of by-products in food products (Khadre *et al*., 2001). Compared to the uses of UV and chlorine, ozone is significantly more efficient in elimination of viruses as less contact time is needed (Tyrrell *et al*., 1995).

However, despite various advantages, ozone is much more expensive compared to UV and chlorine treatment, as well as being more hazardous as it is unstable. It is, thus, recommended to produce ozone in a stable environment with the idea to use as soon as possible as possible (Martinez *et al*., 2011). Khadre et al. (2001) along with Graham (1997) indicated that ozone treatment produces no harmful by-products. Nawrocki et al. (2003), on the other hand, said that the generation of some secondary compounds, such as nitrogen oxides and nitric acids, take place along with the formation of aldehydes. Some secondary compounds such as oxides, acids and aldehydes may be seen as harmful compounds, however, further research needs to be performed to determine their toxicity. Nevertheless, according to the FDA, ozone has been established as a safe disinfection option for the food industry, together with treating drinking water (Graham, 1997). Furthermore, various other studies have revealed that ozone treatment also reduces COD, turbidity, colour, pH and TOC (Total organic carbon) levels in the water (Gardiner & Montgomery 1968). These observations prove that ozone treatment not only reduces microbial load in water but also improves the water quality, which is a big advantage compared to chlorine-based treatment.

Ozone acts on various cellular substances including peptidoglycans and lipids from bacteria, enzymes, and viruses (Martinez *et al*., 2011). With these characteristics, ozone can be seen as a very powerful tool in the disinfection of viruses, fungi and bacteria, and can therefore be applied to the best standards (Tyrrell *et al*., 1995). Furthermore, it has been documented those organisms such as *E. coli*, faecal streptococci, faecal and total coliforms are effectively reduced by ozone (Voidarou *et al*., 2007). As with waterborne microorganisms, it has been reported that *Giardia lamblia* is moderately

affected by ozone, whereas *Cryptosporidium parvum* shows no effect to the treatment at all (Liberti *et al*., 2000).

As with any treatment process, various factors influence the effectiveness on microbial inactivation, and with ozone is it no different. The solubility, reactivity and stability of ozone is significantly affected by temperature changes (Martinez *et al*., 2011). Furthermore, the changes in pH, relative humidity, turbidity, presence of organic material and specific additives, dosage, contact time and the type of microorganism all influence the activity and efficiency of ozone treatment (Voidarou *et al*., 2007). It is therefore necessary to calculate the correct contact time and taking into consideration all the other factors to achieve the optimum dosage. The optimum dosage to effectively reduce pathogens is mostly related to its content in the input effluent and the specific disinfection goal (Martinez *et al*., 2011). In the industry, it has been established that the optimal disinfection dosage for ozone treatment ranges from 10 to 15 mg.O_3.L^{-1} (Martinez *et al*., 2011). To determine the effectiveness of the treatment, the residual ozone should be isolated and measured (Martinez *et al*., 2011). The residual ozone simply refers to the ozone left behind in the water, after ozonation has taken place to ensure the adequate dose (Martinez *et al*., 2011). Residual ozone content of 0.4 mg.O_3.L^{-1} is sufficient for water disinfection, while 0.2 mg.O_3.L^{-1} is the minimum required to transfer the ozone (Langlais, 1988).

Overall, ozone provides various advantages, however, the treatment cost is a significant disadvantage compared to UV and chlorine treatment, along with being more hazardous as it is unstable.

2.10.2.4 Peracetic acid

Peracetic acid (PAA) has been around from the mid nineteenth century, showing the potential to be an upcoming disinfectant (Kitis, 2004). PAA comprises of acetic acid, peracetic acid and hydrogen peroxide, which makes up a clear liquid solution (Van Rooyen *et al*., 2018) that is non-toxic (Wagner *et al*., 2002). In recent years PAA has become a very popular disinfectant in the food and beverage industry as it shows great antimicrobial characteristics towards a range of microorganisms, such as bacteria and fungi (Kitis, 2004). With regards to the disinfection of water, PAA has shown great potential as it reduces indicator microorganisms from various water sources to the required levels according to safety guidelines (Gehr *et al*., 2003). When compared to the disinfectant abilities of chlorine, PAA shows comparable microbial reduction capabilities (Crebelli *et al*., 2005), which means PAA may potentially be used as a stand-alone disinfectant. However, studies have proven that viruses, bacterial spores, and protozoan cysts show high resistance to PAA, whereas some bacterial species are far less resistant (Park *et al*., 2014). The fact that some microorganisms show resistance towards PAA should not be a deciding factor as any many have some degree of resistance to most treatment

techniques currently used. One exceptional characteristic of PAA is that disinfection performance will not be compromised, when using low concentrations and reduced contact times (Crebelli *et al.*, 2005). PAA targets the functioning of lipoproteins in the cytoplasmic membrane of Gram-positive microorganisms and the outer membrane lipoproteins of Gram-negative microorganism (Leaper, 1984). As with most other chemical treatments, genetic material of microorganisms is also affected, leading to cell death (Kitis, 2004).

When PAA exceeds a concentration of 15% (m.v^{-1}) it may be hazardous as it becomes unstable and dangerous when handled (Luukkonen *et al.*, 2014). From a practical point of way, studies have shown that a low PAA concentration of 4 mg.L^{-1} with contact time under an hour sufficiently reduces wastewater microorganisms, initially started at 4-5 log cfu.100 mL^{-1}, to match recommended water guidelines of 1 000 cfu.100 mL^{-1} (Gehr *et al.*, 2003; DWAF, 1996). Caretti & Lubello (2003) completed a study where three different PAA dosages and contact times were tested on total coliforms (TC), faecal coliforms (FC) and *E. coli.* The results obtained indicated that, the microbial count was reduced by increase in PAA dosage and extended contact times, and that combination of 30-minute contact time and 8 mg.L^{-1} PAA concentration was the most effective (Caretti & Lubello, 2003).

Although a short contact time of PAA treatment may be effective against microorganisms, a disadvantage of the treatment is its sensitivity to the various physico-chemical characteristics of water, ultimately influencing the overall efficacy.

2.10.2.5 Ferrate (VI)

Ferrate (VI) (i.e., FeO_4^{-2}) shows the potential to act as a new water treatment agent to revolutionize existing water treatment systems (Zhang *et al.*, 2020). The oxyanions in ferrate (VI) contain iron, in its +6-oxidation state, representing a very high reduction potential of 2.2 V (Sharma, 2002). Apart from being a strong oxidant, Ferrate (VI) initiate the process of coagulation, targeting and collecting colloidal particles in water along with the destruction reactions of harmful inorganic pollutants (Dar *et al.*, 2021). In addition, the end products of ferrate (VI) consist of non-toxic iron (hydr)oxides, holding certain absorption capabilities for water pollutants such as cationic heavy metals (Cui *et al.*, 2018).

The characteristics of Ferrate (VI) can be seen as revolutionary, as it consists of various treatment mechanisms within one treatment agent (Zhang *et al.*, 2020). Additionally, these mechanisms include coagulation, disinfection, precipitation, and adsorption to effectively reduce water pollutants (Zhang *et al.*, 2020). Furthermore, the first known research done on ferrate (VI) was by implementation as an oxidizing agent of organic pollution in water (Zhang *et al.*, 2020). As with coagulation, it can successfully remove algal cells and particulate matter within water (Deng et al., 2017). The success of removal can only be achieved as long as the optimum pH ranges persist (Zhang *et al.*, 2020).

When it comes to the disinfection of microorganisms, ferrate (VI) is high demand, as it removes various indicator organisms and bacteriophages (Cui *et al*., 2018). Jiang et al. (2006) documented that ferrate (VI) can achieve a >6 log reduction of *E. coli* with 6 mg.L^{-1} ferrate (VI) across vast pH ranges in water treatments, which is more than adequate in reducing the microorganism to required safety levels. However, Zhang et al. (2020) reported that ferrate (VI) will be less reactive at a higher pH value, but the stability of the compound would be improved resulting in a longer lifetime, ultimately resulting in a greater exposure to kill of microorganisms. Ferrate (VI) produces low levels of regulated DBPs and can thus be considered a safe option for alternative disinfecting agents (Sharma *et al*., 2005).

Ferrate (VI) can achieve the basic required functions of the conventional treatment of water by reducing of pathogens and turbidity (Zhang *et al*., 2020). The degradation of endocrine disrupting compounds and pharmaceuticals and personal care products (PPCPs) can take place, which is normally poorly treated in traditional WTPs (Zhang *et al*., 2020). Overall, Ferrate (VI) is a good chemical treatment option, however, the fact that it is sensitive to physico-chemical characteristics, such as pH, it may not be the best option for treating water of varying pH levels. In addition, the formation of DBPs, even if low, may be disadvantages for the environment.

2.10.3 Other water treatment options

2.10.3.1 Ultrasound

Ultrasound recently showed the potential to be used in various fields to clean, de-gas, homogenise, and disintegrate a range of materials (Vazquez-Lopez *et al*., 2018). In the last decade ultrasound proved to be a promising and emerging technology for the disinfection of water (Mason & Peters, 2002). The application of ultrasound inactivates microorganisms such as bacteria through the process of cavitation, which can be divided into mechanical, physical and chemical action (Vazquez-Lopez *et al*., 2018). As for mechanical, the ultrasonic waves cause a disturbance in the medium, forming positive and negative pressure zones (Vazquez-Lopez *et al*., 2018). The low-pressure zones form bubbles which become unstable and result in the release of high forces shattering microbial cell walls (Cheeke, 2012). Dolas et al. (2019) described the mechanical effect as formed by strong shear forces, due to acoustic shocks induced by stable and transient cavitation. Physical action refers to the continuous implosion of bubbles and cavitation, ultimately releasing high shearing forces causing a temperature increase in the medium (Vazquez-Lopez *et al*., 2018). Furthermore, sonochemical reactions are generated which originate from various pathways, such as the oxidation by hydroxyl radicals produces by water undergoing thermal dissociation and pyrolytic decomposition inside hotspots (Hoffmann *et al*., 1996). An increase in negative pressure zones favour cavitation, while high temperatures are believed to modify certain properties such as vapour pressure of the medium being treated. Another important

factor affecting the effectiveness of bacterial inactivation is the frequency emitted, as it is inversely proportional to efficiency of conversion of kinetic energy to heat (Hua & Thompson, 2000). Therefore, it is believed that as temperatures increases in a shorter space of time, the bactericidal effect would be more significant.

Cavitation is not exclusively used to influence microorganisms; however, it will also affect some water parameters (Vazquez-Lopez *et al*., 2018). Cavitation can oxidize molecules to H_2O, CO_2, H_2O_2 and nutrients to their PO_4^{3-} and NO_3^- forms when ammoniacal nitrogen, proteins and phospholipids are present (Borea *et al*., 2017). Vazquez-Lopez et al. (2018) reports that ultrasound can be used as an alternative disinfection methods as microbial levels were achieved according to guidelines from many countries. In addition, the water quality can also be improved with ultrasound, however, a disadvantage is that very limited research has been performed on irrigation water scenarios.

2.10.4 UV disinfection as treatment option for study

2.10.4.1 Introduction to Ultraviolet light (UV)

Ultraviolet (UV) light represents wavelengths between 100 – 400 nm, as it forms part of the electromagnetic spectrum (Dai *et al*., 2012). UV light emits wavelengths shorter than visible light (>400nm) and longer than X-rays (<100nm) (Koutchma, 2009). The UV spectrum consist out of four spectral groups, vacuum UV (100-200 nm), UV-C (200-280 nm), UV-B (280-315 nm) and UV-A (315-400 nm) (Olivier *et al*., 2015), as seen in Fig 2.4 below. The spectral group of UV-C shows the greatest potential in regard to microbial reduction, also taking in account the effects of UV-B and UV-A.
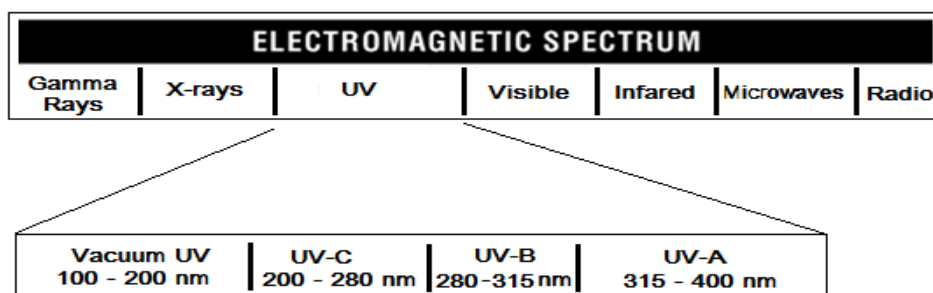
Figure 2.4 Ultraviolet light is made up of four sub-categories, which also forms part of the broader electromagnetic spectrum (USEPA, 2006).

Since the discovery of UV light in the late 1800's (Bolton & Cotton, 2008), in-depth research followed in order to understand the workings of UV light as a possible disinfection alternative. As UV light proved to have potential germicidal properties, it was further developed. The first application of UV light to disinfect drinking water, was in France during the early 1900's (Olivier *et al*., 2015). UV

disinfection has been implemented across various fields, such as the water industry, as a multipurpose, well-accepted disinfection treatment option (Poepping *et al*., 2014). Several studies reported that a wide range of pathogenic and spoilage microorganisms can be effectively reduced by UV light disinfection (Gayan *et al.,* 2014). Another promising finding is that well-known chlorine resistant waterborne pathogens, *Giardia* and *Cryptosporidium,* can be effectively reduced, where they show resistance to most previous disinfection methods (Hijnen *et al*., 2006). UV does not only reduce pathogenic microorganisms, but also effectively reduce grey mould, black mould, and *Rhizopus* soft rot (Lim & Harrison, 2016). However, several external and internal factors could influence the germicidal effect of UV. Application potential of UV should therefore be thoroughly investigated to optimise disinfection efficacy.

2.10.4.2 UV disinfection mechanism

Fundamentally, UV irradiation achieves microbial disinfection based on various photochemical processes as microbial cellular structures can absorb the incident UV light (Koutchma, 2009). The advantages of UV are rooted in the wavelength range of 100-400 nm, where UV photons have significantly high energy owing to wave-particle duality and strong photochemical reactions and reactivity (Yang *et al*., 2020). After the exposure to UV light, biochemical mechanisms will occur, ultimately resulting in the damage and destruction of multiple cellular components with emphasis on the alteration and damage of genetic material, resulting in cell mortality (Premi *et al*., 2015). Furthermore, as the damage and destruction of cellular compounds occur, no detrimental by-products are generated during the process (Gayan *et al*., 2014). Understanding the basic principle of photochemistry where light energy, in the form of photons, are transferred to microbial cells, thus changing the receiving molecules from ground to excited state, is of great importance (Gayan *et al*., 2014).

As UV is divided into four different sub-categories, each group contains different characteristics and therefore different chemical reactions will take place. UV-C or short-wave UV radiation (200-280nm) is considered to be the most effective UV region to effectively act as a germicide (Gayan *et al*., 2014). The optimal photo absorption range of nucleotide bases, which make up the microbial DNA, is around 254-260 nm (Van Rooyen *et al*., 2018) (Fig. 2). Therefore, the range of UV-C is ideal for photons to be absorbed by the nitrogenous bases, generating the formation of cross-linking photoproducts, ultimately preventing transcription and replication of the organism and eventually die off (Lopez-Malo & Palou, 2005).

However, water shows the ability to absorb UV light at lower UV wavelengths (< 230 nm) and therefore resulting in a poorer disinfection (Van Rooyen *et al*., 2018). Well-known pathogens such as *E. coli* 0157:H7 and *Salmonella* have been effectively reduced in the 200-280 nm wavelength range of

UV light (Ge *et al.,* 2013). Irrespective of the fact that UV-C light is the best suited for microbial disinfection, the efficacy of the light will greatly depend on the dosage, contact time, type of microorganism and the quality of the water medium. Figure 2.5 illustrated the wavelengths at which proteins and nucleotides absorb UV light.
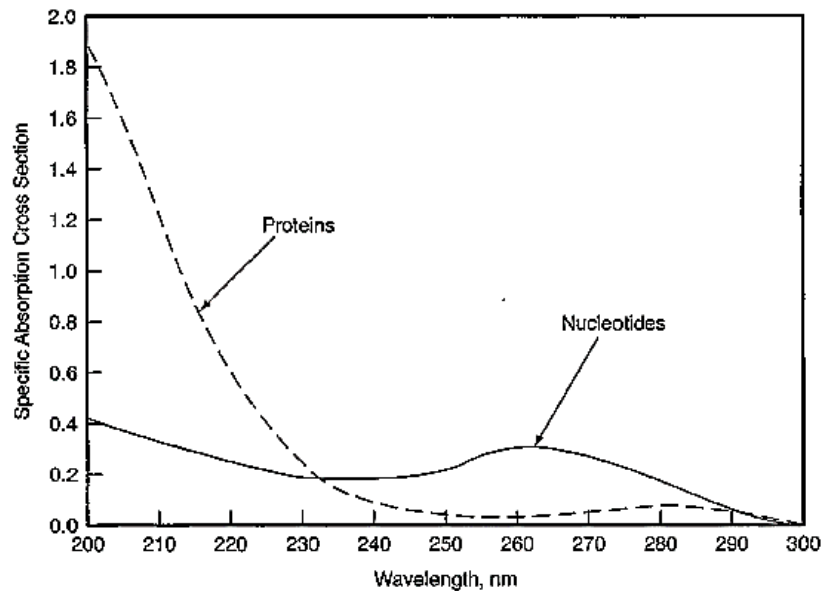
Figure 2.5 Proteins and nucleotides absorbing UV light at different wavelengths (Bolton & Cotton, 2008)

2.10.4.3 General difference between medium-pressure UV and low-pressure UV systems

UV disinfection systems used in water treatment installations usually come in the form of a closed-pipe or open-channel system where UV irradiation is facilitated by vapour lamps (Howe *et al*., 2012). Systems placed directly into water pipelines for disinfection can be referred to as closed-pipe systems, which can be further grouped as single-lamped and multi-lamps parallel to water (Bolton & Cotton, 2008). Single-lamped systems usually make use of low-pressure UV light for treatment of water at a home environment, whereas multi-lamps systems can be used with either low- or high-pressure lamps for small communities or bigger treatment plants (Bolton & Cotton, 2008). These systems consist of UV sensors, UV lamps, and cleaning apparatus intended for the quartz sleeves (Olivier *et al*., 2015). As for open-channel systems, lamps are placed in perpendicular order to the water flow. Furthermore, open-channel systems are largely implemented at wastewater disinfection sites (Bolton & Cotton, 2008).

Mercury vapour lamps can be categorized as low intensity (low-pressure) and high intensity (medium-pressure) light sources (Zimmer-Thomas *et al*., 2007). Low-pressure (LP) UV systems emit a monochromatic wavelength, emitting 85% of wavelengths at 253.7 nm, which is commonly used for the disinfection of drinking water and water at wastewater treatments (Zimmer-Thomas *et al*., 2007).

The medium-pressure (MP) UV system has been more recently developed, making use of a polychromatic wavelengths, with a broader range, ranging from 185 to 1367 nm (Mofidi *et al.,* 2002), covering both UV-B and UV-C (Guo *et al*., 2009). The MP UV covers a wider range of wavelengths which may be advantageous, as the output energy and power density is significantly higher, enabling the construction of a compact system, ultimately resulting in a smaller footprint (IJpelaar *et al*., 2010). In addition, MP UV systems are more likely to be implemented where large scale systems are required. As MP lamps uses polychromatic wavelengths, it may result in targeting more structures such as micro pollutants and cause more irreversible physiological changes involved in the repairing process (Zimmer & Slawson, 2002). However, Guo et al. (2009) reported that a UV dosage of 40 mJ.cm^{-2} in both LP- and MP-UV lamps achieved the required coliform levels in water. In addition, Oguma et al (2005) studied the effect of LP and MP UV systems on non-pathogenic *E. coli*, reporting that both systems produced similar inactivation results but for MP the DNA repair, through photoreactivation, reduced significantly. The characteristic of MP UV systems to reduce the process of photoreactivation is a major advantage over LP UV systems to the food safety environment.

Despite the high energy output of MP systems, only about 12% is converted to the required UV-C light output (IJpelaar *et al*., 2010). The lifetime of MP lamps is usually around 5 000 hours, which is half the lifetime of LP lamps (Kostyuchenko & Giller, 2005). However, the installation, maintenance and footprint of LP UV systems will potentially be higher than MP, as it makes use of a much lower power output (< 1 000 W), ultimately resulting in more lamps to be installed in order to achieve the power output of MP systems (IJpelaar *et al*., 2010).

2.10.4.4 UV disinfection effect on microorganism

Specific mechanisms and pathways are contributing to the inactivation of microorganisms and their components. Photolysis, consisting of several photochemical mechanisms, target the DNA and RNA of microbial cells, including proteins and other cellular components (Gayan *et al*., 2014; Shafaei *et al*., 2017). The exposure of microbial DNA to UV light generates the formation of harmful crosslinks between adjacent pyrimidine nucleobases, namely pyrimidine (6-4) pyrimidone photoproducts and cyclobutene pyrimidine dimers (Yamamoto *et al*., 2017; Shafaei *et al*., 2017). These photoproducts also include pyrimidines nucleic acids, which comprises of cytosine, thymine and alternatively purine nucleic acids such as guanine and adenine (Rodriguez *et al*., 2014). The nucleic acids from the pyrimidine derived photoproducts, absorb incident UV light significantly stronger in comparison to the purines (Gayan *et al*., 2014). The genetic material of microorganisms absorbs photos of high energy via the main absorption band, focussed at the 265 nm mark of the UV-C range (Yamamoto *et al*., 2017). Furthermore, these nucleobases become photoexcited, which undergo several chemical reactions,

ultimately resulting in alteration and modification of the genetic information or simply the inhibition of DNA processes such as transcription and replication in microbial cells (Shafaei *et al*., 2017).

Other compounds such as sugars and phosphate molecules are not targeted, as the UV wavelength range is too high (Koutchman, 2009). Bolton & Cotton (2008) reported that impaired microbial replication can be linked to alteration of genetic material and the presence of photoproducts, ultimately leading to pathogens with no capability to cause illnesses and eventually undergo cell death. As pyrimidine (6-4) pyrimidone and cyclobutene pyrimidine dimers (CPDs) are the two dominant photoproducts, making up 75% of microbial DNA, damage through UV light is by means of CPDs reactions (Premi *et al*., 2015).

2.10.4.5 DNA repair mechanism post UV disinfection

As mentioned before, UV light exposure disrupts the DNA of microorganisms by forming lesions which hinder DNA replication and transcriptions, ultimately ending in cell death. However, its been documented that most microorganisms have the potential to repair their UV-induced damaged cells through a few repair systems (Friedberg *et al*., 2003). For microorganisms to maintain their genetic integrity, various DNA repair systems have been developed (Yamamoto *et al*., 2017). Among all repair systems, nucleotide excision repair (NER) is the most common (from bacteria to humans), as well as a versatile machinery for UV lesion repairing and other bulky DNA lesions (Friedberg *et al*., 2003). During the process of NER, various proteins link to excise a small part of the DNA strand, containing the UV lesion (Sancar, 2003). Furthermore, gap-filling DNA synthesis by means of DNA polymerase will occur, followed by ligation to restore the intact DNA (Sancar, 2003).

In 1949, Kelner (1949) discovered a phenomenon named photoreactivation. Bacteria inactivated by UV light showed abilities to be reactivated by the exposure of visible light (Kelner, 1949). Rupert (1962) demonstrated that a specific enzyme, called DNA photolyse, is the cause for this phenomenon. After the discovery, it was found that DNA photolyse was discovered in organisms from all kingdoms of life, except for placental mammals (Van der Spek *et al*., 1996). Furthermore, photoreactivation, which is a light dependent mechanism, occurs when CPDs are disintegrated with the DNA photolyase, resulting in the formation of dimers under visible- (320-480 nm) and near UV-light (UVA) (Salcedo *et al*., 2007). It was also discovered that a similar process to photoreactivation exist, called dark repair. In contrast, dark repair makes use of a DNA repair mechanism which is light independent (Guintini *et al.,* 2017). The fact that microorganism can repair UV-induced damages could pose a biological safety concern as the UV disinfection efficiency will be significantly poorer.

In the past decade, the phenomenon of photoreactivation and dark repair has been widely studied in the aspects of where bacteria is present in wastewater and drinking water (Shafaei *et al*., 2017). Photoreactivation influences the UV system by reducing disinfection efficiency, often resulting

in higher UV doses to be supplied, resulting in a higher overall energy output of the entire system (Hijnen *et a*l., 2006).

Microorganisms such as *E. coli*, *Salmonella typhimurium*, *Shigella dysenteriae* and *Listeria monocytogenes* showed the ability to undergo photoreactivation after UV treatment (Hu *et al*., 2012). However, apart from the specific microorganisms, the correct UV dosage is widely considered as one of most significant factors in the research of photoreactivation (Wen *et al*., 2019). Guo et al. (2009) reported that a UV dosage of 15 mJ.cm^{-2} results in no photoreactivation mechanism in *E. coli*. It has also been proven that higher UV-C dosages results in an overall decreased photoreactivation of microorganisms (Nebot Sanz *et al*., 2007). In addition, Safaei *et al*., (2017) reported that the application of sufficiently high inactivation fluence will best control bacterial photoreactivation. As UV doses greater than 15 mJ.cm^{-2} has shown limited photoreactivation of *E. coli*, total coliforms and faecal coliforms, compared to a lower inactivation fluence, if kept constant (Safaei *et al*., 2017). Li et al. (2017) reported that at 280 nm UV light, photoreactivation and dark repair mechanisms are noticeably reduced for *E. coli*. Furthermore, the application of MP UV dosages over LP UV have been a big advantage, as photoreactivation is reduced remarkably (Oguma *et al*., 2002; Zimmer & Slawson, 2002).

In addition, most waterborne bacteria make use the 310-480 nm UV range and the enzyme photolyase to repair damaged DNA (Shafaei *et al*., 2017). Microorganisms' potential for photoreactivation is a major concern in larger scale UV disinfection systems, as in most cases water is exposed to sunlight, excelling photoreactivation and ultimately leading to treated water with increased microbial loads (Shafaei *et al*., 2017). Furthermore, a previous study conducted by Berger et al. (1996) documented a decrease in microbial load, after being exposed to solar UV-B radiation, with the wavelength range of 280-315 nm. The UV-B portion of sunlight provide more than double the inactivation of *E. coli* compared to UV-A (315-400 nm) (Sinton *et al*., 1994). Previous studies have also shown that increased temperatures, in the range of 25-35°C, will also increase bacterial photoreactivation (Safaei *et al*., 2017). Authors encourage that nutrients in receiving water sources be investigated during bacterial photoreactivation (Safaei *et al*., 2017). Considering the time required for microorganism reactivation and regrowth to take place, Gilboa & Friedler (2008) suggested that in the first three hours post UV disinfection most reactivation will occur, followed by a poorer regrowth rate.

2.10.4.6 UV inactivation efficacy

The efficacy of UV treatment has been established in various scientific research and years of safe public-health practices (Zimmer & Slawson, 2002). Many of these studies have made use of culture-based methods, and therefore based on the fact that culturable parameters can effectively indicate the various risk levels of bacterial pathogens (Yang *et al*., 2020). However, microorganisms have been

recorded to maintain their viability to a certain extent as UV treatment does not have direct effect on various viability parameters as compared to culturability parameters (Yang *et al*., 2020). In support of this statement many parameters did not have any direct effect such as the respiration rate (Blatchley *et al*., 2001), mRNA (Yang *et al*., 2020), adenosine triphosphate (ATP) levels (Xu *et al*., 2018) and permeability of the bacterial membrane (Nie *et al*., 2016). It is therefore of great importance to select the most suitable analytical methods and correct interpretation of outcomes when efficiency and performance of UV disinfection is investigated (Yang *et al*., 2020). It is difficult to compare past research of microbial inactivation by UV radiation, as the geometry and conformation of UV equipment, optical properties of the liquid, and flow patterns play various important roles in the efficacy and lethality of the UV radiation (Muller *et al*., 2011).

2.10.4.7 Importance of physiochemical properties of UV treated water

It is of great importance to include a pre-treatment step prior to UV disinfection, ensuring the most effective UV dose is applied (Farrell *et al*., 2018). The ability of the UV treatment to inactivate microorganisms will mainly depend on the water quality achieved before the disinfection process (Farrell *et al*., 2018). The parameters associated with water quality and UV transmission include, UVT%, TSS, TDS, COD, turbidity, and particle size (Farrell *et al*., 2018). UV transmittance (UVT%) simply refers to the amount of UV light absorbed by different constituents of water. A high UVT% value indicates low absorption and therefore resulting in a higher UV dosage of the water sample (Olivier *et al*., 2015).

Turbidity is also one of the most recognized parameters for the measurement of water quality prior to disinfection (Farrell *et al.*, 2018). The World Health Organisation (WHO) encourages a turbidity level of 0.2 NTU, whereas the United States and European countries are satisfied with a level of <1 NTU (WHO, 2011). UV disinfection recently attracted a lot of attention as it reduces formations in current regulated DBPs and a high success rate of UV disinfection on typical chlorine resistant pathogens (Hijnen *et al*., 2006). However, turbidity causing materials (TCMs) greatly impact the effectiveness of the UV disinfection (Farrell *et al*., 2017; Jones *et al*., 2014). These materials mostly consist of organic and inorganic matter including biological matter such as microorganisms and algae (Farrell *et al.,* 2017). As these materials can differ from water sources and possibly provide the same turbidity levels, the effectiveness of the UV treatment may differ for each (Passantino *et al*., 2004). It is well established that TCMs can affect the working of the UV system. The efficiency of the UV system can be lowered as TCMs cause scattering and absorption of the incoming UV light, along with microorganisms shielding against the UV light, resulting in insufficient UV dosages to eliminate pathogens (Carre *et al.,* 2018). Howe et al. (2012) referred to these scattering and shielding as encasement and shading mechanisms of interference. As inorganic matter from TCMs, together with

organic compounds, absorb incident UV light, it is believed that chemical oxygen demand (COD) and total dissolved solids (TDS) will increase with an increase in UVT% (Olivier *et al*., 2015). Furthermore, Adhikari et al*.* (2019) reported the presence of suspended particles in a contaminated water sample, where the suspended particles are considerably bigger compared to the *E. coli* cells, showed no changes in efficacy while increasing the UV-C dosage. Suspended particles may provide shielding to microbial cells from incident UV-C light (Cantwell & Hofmann, 2011).

Another parameter closely associated with TCMs is the microbial attachment (Liu *et al*., 2013). The attachment of microorganisms to these materials can occur, as microorganisms can aggregate or bind to organic materials, ultimately lowering the inactivation efficiency of the UV light (Liu *et al*., 2005). Microbial attachment is driven by van der Waals forces and electrostatic interactions (Hassard *et al*., 2016). Factors that will influence microbial attachment include hydrophobicity, surface roughness and tension (Hassard *et al*., 2016). In addition, microorganisms attaching to material particles pose a great risk to UV inactivation as it is more mobile in water compared to static biofilms produced by bacteria (Liu *et al*., 2005). TDS is therefore a concern as it potentially acts as a microbial transmission vehicle during the UV disinfection step and result in microbial infections (Farrell *et al*., 2018).

As the size of UV treatment plants differ, the size of pumps and flow rate capacities can also differ, potentially resulting in change of the disinfection effectiveness. UV dosages can be increased by reducing the water flow rate, therefore, increasing the contact time for possible pathogens in the water (AbdelRahim *et al*., 2013). Laminar flow will follow if the water flow rate is decreased, resulting in notable reduction of mixing (AbdelRahim *et al.,* 2013). However, reduced mixing causes a reduced UV disinfection as the pathogens are not properly exposed to the UV to inactivate them (Crittenden *et al*., 2003). The correct ratio of mixing and flow rate should be investigated to achieve the maximum UV exposure with the lowest possible flow rate.

In order to ensure proper efficiency of any UV system implemented for water disinfection, the inclusion of a pre-treatment or prefiltration step, with the most suitable water quality parameters, is mandatory for effective results (Adhikari *et al*., 2019).

2.10.4.8 Cost analysis and feasibility for farm scale UV treatment

All the issues regarding DBP formation from chlorination have given rise to investigate alternative disinfection methods to chlorination (Tak & Kumar, 2017). As UV systems are considered as an alternative method for chlorination, one of the most important factors that should be considered on full scale application, is the cost difference (Tak & Kumar, 2017). Three parameters form part of the cost analysis of UV disinfection, i.e., power- and capital cost as well as operation and maintenance (O&M) cost (Tak & Kumar, 2017). These O&M costs refers to the manpower required, quartz sleeve

cleaning and maintenance of the UV lamp (Tak & Kumar, 2017). For smaller scale plants there is a significant difference in initial capital cost compared to the total annual O&M cost, but when used in the long run the lower O&M cost of a UV system will make up for the high initial capital cost (Tak & Kumar, 2017). Tak & Kumar's (2017) study concluded that UV saves up to 63% of the total annual O&M cost in comparison to chlorination. A study by Gomez- Lopez et al (2009) also concluded that UV disinfection is a more cost-effective approach in the long run and suggested that UV is the better option in terms of performance and cost.

2.11 Concluding remarks

Access to clean, safe water is essential for human health, social development and the water dependant sectors of the world (Ao *et al*., 2019). However, with the increase in global population and rapid urbanisation, pollution and water shortages have also increased to concerning levels (Ghassemi & White, 2007; Gunda *et al*., 2019; Jiang, 2015). Furthermore, as water sources change, the variation in water quality is also significant, resulting in water related problems for the agricultural sector (Ao *et al*., 2019). Jongman et al. (2017) indicated that various water sources, such as surface water, ground water and municipal water, is regularly used as irrigation water on farms for fresh produce and crops.

This is a major public health concern, as the consumption of fresh fruits and vegetables have significantly increased over the past decades (Alegbeleye *et al.,* 2018). In addition, Pachepsky et al. (2011) noted that irrigation water with poor microbial profiles has often been implicated as the primary source of pathogenic contamination of fresh produce. As fresh produce has marginal processing and is normally consumed raw (FDA, 2011), the risk for microbial transport from polluted water to fresh produce is significant. In support of this statement, Painter et al. (2013) reported that during 2013, 51% of foodborne infection outbreaks in the United States occurred among plants, along with fresh fruit and vegetables causing 45.9% of the outbreaks in the plant category. Various sources may be the origin of these outbreaks; however, the Food Safety Modernization Act (FSMA) has identified agricultural irrigation water as the primary source of bacterial contamination.

With regards to South Africa, river water is the favoured water supply for irrigation water use on farms. Previous research has shown that river water profiles, specifically the Western Cape rivers, varies significantly, due to the various microbial and physico-chemical characteristics (Bursey, 2020; Paulse *et al*., 2009; Huisamen, 2012; Britz *et al*., 2013; Bester, 2015; Olivier *et al*. 2015). However, limited research is available regarding the difference in physico-chemical and microbial characteristics within one river system, which is also important when establishing the overall state of the river. Furthermore, continued research should be performed regarding the river profiles to determine the condition of rivers over longer periods. In addition, this research would provide more data concerning the optimisation of water disinfection treatments of irrigation water, ultimately improving food safety.

Universal food washing processes have shown to reduce microbial contamination on fresh produce, however, pathogenic organisms are not fully eliminated (FDA, 1998). In order to prevent microbial contamination, various treatment method have been developed for disinfecting irrigation water. Several methods such as filtration techniques (Koivunen *et al*., 2003), ozone treatment (Kim *et al*., 1999), peracetic acid disinfection (Kitis, 2004), and a variety of chlorine compounds have been used. However, these methods have illustrated some limitations, ranging from effectiveness, complexity, negative effects on crops, formation of DBPs in the environment, high maintenance cost and microorganisms showing increased resistance to treatments (Adhikari *et al*., 2019; Hua & Reckhow, 2007; LeChavallier & Au, 2004).

One method that shows potential to overcome these limitations, is the use of UV-C light (Hijnen *et al*., 2006). The mode of action for UV disinfection is based on the formation of pyrimidine dimers compounds in the microorganism's DNA, where these compounds hinder the process of replication, transcription, and translation, ultimately resulting in cell death (Koutchma *et al*., 2009). The short-contact time, operation simplicity and minimal microbial resistance to UV disinfection makes it popular in irrigation water scenarios. In addition, when compared to chlorine treatments, UV disinfection have gained popularity in recent years due to treatment being environmentally friendly with no by-product production (Hijnen *et al*., 2006). However, the phenomenon of photoreactivation has been documented. Bacteria inactivated by UV light have shown the ability of reactivation by the exposure of visible light (Koutchma *et al*., 2009). Considering that photoreactivation is a limitation of UV disinfection, has medium-pressure UV lamps and sufficient UV doses illustrated to be effective in reducing these organisms.

Research regarding UV radiation on microbial strains have been limited, as most research investigated the application of UV radiation at laboratory-scale along with testing reference microbial strains, mostly *E. coli.* When considering the complexity of upscaling UV radiation to treatment plants at farm level, various factors should be considered. Investigation would be necessary regarding the working of a pilot-scale UV system, with the use of large volumes of river water where environmental microbial strains could be put to the test. In addition, as seen in literature, the physico-chemical characteristics could influence the working of such systems, which could influence the efficacy of the UV disinfection. Establishing a better understanding of the complexities of upscaling UV disinfection plants would be imperative for the success of irrigation water treatment.

Furthermore, as seen with the irrigation water guidelines of South Africa, important pathogens such as *Listeria monocytogenes* and *Salmonella* spps. is not included in testing, due to no legislative pressure. These organisms should be investigated to establish the risk associated to irrigation water safety. Therefore, knowledge gaps also occur concerning the effectiveness of UV

irradiation on these pathogens. The inclusion of different river profiles, with varying physico-chemical and microbial characteristics, in UV disinfection studies would be vital for further research. As this would contribute to the overall optimisation of UV radiation of river water along with determining a UV dose response for various microorganism of concern.

The main purpose of this current study was to address certain knowledge gaps in literature and constructing a stronger foundation regarding previous research in this field, which involved the investigation of different water treatment techniques for different microbial organisms. Lastly, providing recommendations for the use of a river source as irrigation water, with the goal to ultimately improve food safety in South Africa and worldwide.

2.12 References

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CHAPTER 3

CHARACTERISATION OF THE PHYSICO-CHEMICAL AND MICROBIAL PROFILE OF THE PLANKENBURG RIVER SYSTEM

3.1 Abstract

The variation in microbial and physico-chemical characteristics of the Plankenburg river system over a longer distance was investigated during this study. Five different water sampling sites along the Plankenburg river were chosen, where various microbial and physico-chemical tests were conducted. The microbial tests included *Escherichia coli* (*E. coli*), coliform, Shiga toxin-producing *E. coli* (STEC) and Extended spectrum beta-lactamase (ESBL) – producing *Enterobacteriaceae.* Characterising the Plankenburg river system in terms of physico-chemical characteristics included tests such as ultraviolet transmission (UVT), total dissolved solids (TDS), total soluble solids (TSS), chemical oxygen demand (COD), pH and electrical conductivity (EC).

Physico-chemical results and guideline limits along with the microbial results, show that water from various sites in the Plankenburg river system exceed guideline limits for safe irrigation water. It was established that several point and non-point pollution sources could have resulted in poorer river profiles at specific points. In addition, STEC was also detected by genotypical confirmation using the Pall GeneDisc Top 7 test, however, selective isolation of STEC strains was challenging during this study. Furthermore, ESBL – producing organisms was also successfully isolated and confirmed from a few water sites, through selective agar, ESBL testing procedures and species identification by MALDI-TOF analysis. The findings of this study were concerning, suggesting that water treatment, such as UV disinfection, is required before river water is used for irrigation of fresh produce, ultimately to improve food and crop safety through treated irrigation water. The physico-chemical and microbial results also supported in determining correlations of water quality and UV treatment efficacies.

When considering the main aim of this study, physico-chemical and microbial results suggest that a site-specific approach should be followed when implementing on-farm UV irrigation plants. This could be implemented on a river system with water quality variations at different water sites. Custom system design could be beneficial from an economic feasibility view, as UV disinfection and pilot plant optimisation could be cost saving and most efficient.

3.2 Introduction

The quality of river water has recently become one of the biggest environmental concerns worldwide (Taoufik *et al*., 2017). This is a major issue as many river systems represent as an important source of water use for human consumption, industrial purposes and for the agricultural sector as irrigation water (Barakat *et al*., 2016). Due to the high demand and over exploitation of water sources various sectors influence the water quality. Various anthropogenic activities such as agricultural runoff, chemical spills, discharge of domestic and industrial wastewater have negative impacts on the water quality of rivers (Regier *et al*., 2020; Wang *et al*., 2013; Barakat *et al*., 2016; Mustapha & Nabegu, 2011). Anthropogenic activities refer to elevated concentration of pollutions in the atmosphere and environment (Dai *et al.,* 2018). In addition, the occurrence of natural processes such as erosion will also affect the water quality poorly (Wang *et al*., 2013). Each of these anthropogenic activities will impact the water quality differently as the physical, chemical, and biological characteristics vary between pollution sources (Regier *et al*., 2020). Overall, river water is highly susceptible to pollution as it is easily accessible for the discharge and disposal of various pollution sources.

Due to a semi-arid climate, South Africa experiences an average rainfall of 450 mm per year, which is well below the global average of 860 mm per year (Otieno & Ochieng, 2004). As the country is currently water stressed, it is important to preserve the current water sources. Analysis of river water would be essential to detect key contributors affecting water quality.

River water quality is the composite of physical, chemical, and biological characteristics, which are subjected to local and temporal variation and the water flow volume (Mandal *et al*., 2010). In addition, Barakat et al. (2016) stated that water sources can be efficiently managed if river water quality and variability is known. Regular monitoring will also provide valuable data on temporal conditions as well as the effect of seasonal and geographical evolution of the river system (Simenov *et al*., 2003). As rivers and other stormwater runoffs have been identified as key contributors of nonpoint source pollution in urban water, investigating the sources of faecal microorganisms in runoff water will provide valuable information on the water quality of a river (McQuillan & Robidart, 2017). It is well established that coliforms are key indicators of microbial water quality, and therefore it is important to assess variation of coliform concentrations (Jeon *et al*., 2019).

Various pathogens such as STEC can be transferred from contaminated river water via irrigation systems to fresh produce, ultimately increasing the health risk for consumers (Isik *et al*., 2020). Farm animals such as sheep and cattle have been identified as the most common source for STEC to be detected, indicating that these pathogens are generally from faecal contamination (Mughini-Gras *et al*., 2018). STEC has also been identified from a range of wild animals, such as bird,

other mammals and insects, indicating a wide environmental distribution of the pathogen (Pires *et al*., 2019).

Effluents from domestic and agricultural wastewater in river water systems have also been widely studied for the presence of antibiotic resistant bacteria (ARB) and antibiotic resistance genes (ARGs) (Almakki *et al*., 2019). The disposal of various wastewaters in rivers acts as a meeting and exchange location for environmental and human microorganisms, both pathogenic and non-pathogenic (Välitalo *et al*., 2017). The mixing of different chemical compounds and microorganisms imposes selection pressure which favour the emergence of antibiotic resistance (Amos *et al*., 2018). Furthermore, human activities generate a range of pollutants that can include disinfectants and heavy metals, which further apply selective pressure on bacteria, contributing to the selection of ARB in an environment (Baker-Austin *et al*., 2006). One of the principal techniques by which bacteria resist antibiotics is through beta-lactamase production (Vital *et al.,* 2018). Beta-lactamases are enzymes which hydrolyse beta-lactam antibiotics, such as cephalosporins and penicillins (Vital *et al.,* 2018). It has been reported that some *E. coli* strains can produce ESBLs which is particularly significant as a wider range of antibiotics can be targeted (Vital *et al*., 2018; Tissera *et al*., 2013). Apart from *E. coli*, other *Enterobacteriaceae* such as *Klebsiella pneumoniae* can also produce ESBLs and is seen as an important human ARB (Pitout, 2012).

Although the presence of various potential pathogens, along with microbial indicator loads and specific physico-chemical parameters at certain sites in the Plankenburg river have been reported before (Britz *et al*., 2013; Lamprecht *et al*., 2014), the monitoring of the river system over a longer distance is still limited. The first objective of this study was to characterise and compare the physico-chemical characteristics of the Plankenburg river system by including five sampling sites inside and outside of Stellenbosch from November-December 2020 on three separate sampling occasions. The second objective was to also characterise and compare general indicator microorganism counts (*E. coli* and coliforms) between the different sampling sites in the river system. Lastly, the third objective was to screen for the presence of other important organisms such as STEC and ESBL-producing *Enterobacteriaceae* that could potentially be at risk to consumers.

As the Plankenburg river flows through domestic, industrial, and agricultural settings, various potential pollution sources exist that could result in variations in river water quality. Determining the water quality variation within a river system will provide valuable data on the possible effect different potential pollution sources have on a river. Furthermore, the data can be used for UV treatment optimisation of water with different physico-chemical and microbial profiles.

3.3 Materials and Methods

3.3.1 Research Design

The aim of this study was to characterise the Plankenburg river system by investigating the water quality at 5 different sites of the river. The experimental design of this study was structured around three main objectives. Firstly, characterising the Plankenburg river in terms of physico-chemical characteristics by measuring ultraviolet transmission (UVT), total dissolved solids (TDS), total soluble solids (TSS), chemical oxygen demand (COD), pH and electrical conductivity (EC). Secondly, characterising the Plankenburg River in terms of *E. coli* and coliform loads. The last objective was to investigate the occurrence of (STEC) and (ESBL) - producing *Enterobacteriaceae*. All three objectives were evaluated on three separate sampling occasions between November 2020 – December 2020. The Irrigation Water Guidelines (1996a) served as reference for results interpretation, in order to determine if water was safe and acceptable for agricultural irrigation usage. Figure 3.1 indicates a schematic diagram of the post-sampling process per river to achieve the three objectives mentioned.
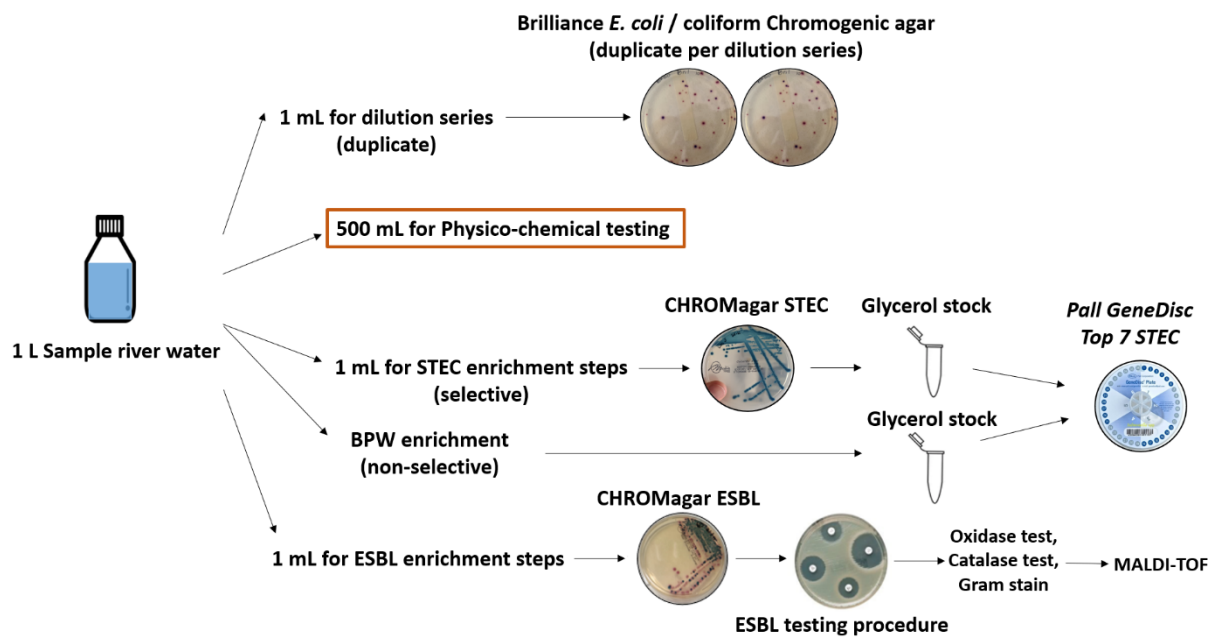
Figure 3.1 Schematic diagram of the post-sampling process per river sample location per round

3.4 General materials and methods

3.4.1 Site selection

The Plankenburg river was chosen as the starting point for this study as multiple previous studies have shown very concerning levels of microbial loads at one of the sites included in this study. To better understand the influence of the microbial status of the Plankenburg river, especially after it merges with the Jonkershoek river, five sites were selected. These five selected sites represent environmental changes that occur as the river flows through the Stellenbosch district. Table 3.1 provides a short description and reference term for each of the five sampling locations.

Table 3.1 Five river sampling location names and descriptions of this study

River sample location	Location description	Reference term	Coordinates
Location 1	First sampling point on Plankenburg river	Plank 1	33°53′29.22″ S 18°49′41.46″ E
Location 2	Merging of Plankenburg river with Kromrivier	Plank 2	33°55′57.65″ S 18°51′06.58″ E
Location 3	Jonkershoek river before merging with Plankenburg river	Jonkers	33°56′37.20″ S 18°50′48.54″ E
Location 4	Merging of Plankenburg river with Jonkershoek river to form Eerste river	Plank 4	33°56′36.52″ S 18°50′37.96″ E
Location 5	Last sampling point on Eerste river	Eerste	33°58′00.06″ S 18°48′13.02′′ E

The Plankenburg river originates in the north-west (NW) of Stellenbosch central. Since the exact origin point of the river is unknown, the first river sampling location (Plank 1) was chosen roughly ± 5km in the NW direction upstream of Stellenbosch central (33°53′29.22″ S 18°49′41.46″ E). River sampling location two (Plank 2) was at the point just after the Kromriver joined the Plankenburg river, which had flowed through the Kayamandi informal settlement and Plankenburg Industrial area (33°55′57.65″ S 18°51′06.58″ E). River sampling location two has been frequently tested for physico-chemical and microbiological characteristics as part of previous research (Bursey, 2020; Olivier, 2015; Sivhute, 2019; van Rooyen, 2015), and now acts as a "control" and comparison to this study. The

Plankenburg river then merges with the Jonkershoek river to form the Eerste river, moving in a southwestern (SW) direction away from Stellenbosch central. The Jonkershoek river, before merging with the Plankenburg river, acts as river sampling location three (Jonkers) (33°56'37.20" S 18°50'48.54" E). As the Jonkershoek river doesn't flow past any informal settlements and industrial areas, this location can be compared to Plank 2 to investigate the possible effects thereof. Furthermore, the Eerste river confluence point serves as river sampling location four (33°56'36.52" S 18°50'37.96" E) named Plank 4. Further downstream the Eerste river is joined by a smaller river, named the Veldwagters river.The Veldwagters river flows past a wastewater treatment plant before joining the Eerste river (Meek *et al.,* 2010). The last sampling point of the study, river sampling location five (Eerste), is situated downstream of both the confluence point and an agricultural area that is used for   livestock and game farming (33°58'00.06" S 18°48'13.02'' E). In conclusion, each of the five water sampling locations were carefully selected to test for differences in river environments and the effects of agricultural, domestic, and industrial effluents into the river. Below Figure 3.2 and 3.3 depict the fiver river sampling sites in and outside Stellenbosch, Western Cape.

3.4.2 Sample collection method

Each of the five river locations were sampled on three separate occasions, with the use of a sampling rod containing a sterile 1 L Schott bottle before the samples were transported to the Department of Food Science laboratory for analysis. The South African National Standards (SANS) method 5667-6 for water sampling (SANS, 2006) was followed for each sampling occasion. Microbiological tests were performed on the same day of sampling, within six hours of sampling. Physico-chemicals tests were performed the next day, within 24 hours of the sampling. The water samples were stored in a fridge at 5°C for 24 hours.
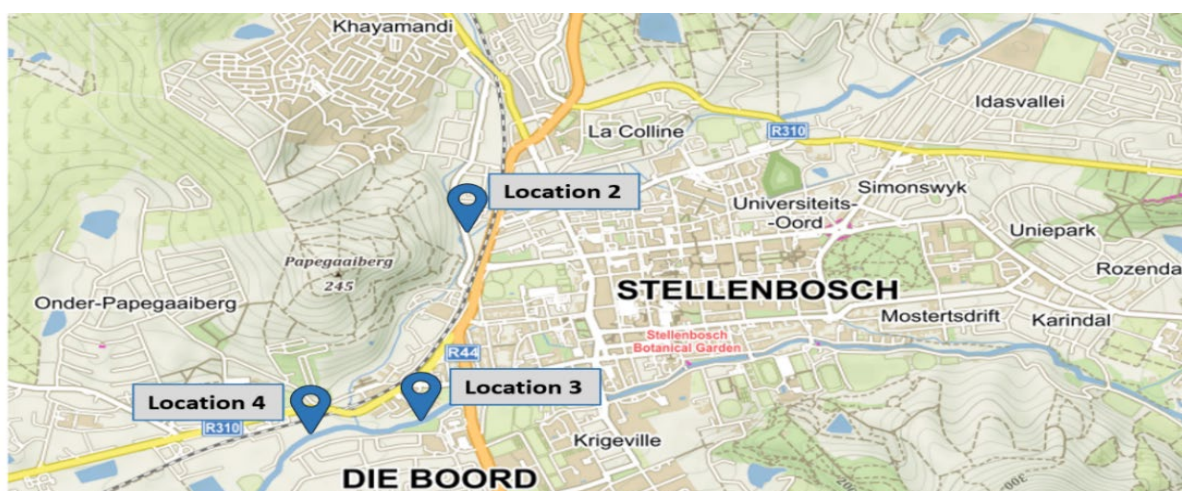
Figure 3.2 Close-up the river water sampling locations for Location 2 (Plank 2), 3 (Jonkers) & 4 (Plank 4) within the town of Stellenbosch, Western Cape

Figure 3.3 The river water sampling locations for Location 1 (Plank 1) and 5 (Eerste) outside the town of Stellenbosch, Western Cape

3.5 Physico-chemical analysis of river water samples

3.5.1 Ultraviolet Transmission percentage (UVT%)

To determine the UVT% of the water sample a Sense T254 UV Transmission (%) Photometer was used according to the manufacturer's instructions (Berson, Netherlands). Distilled water was used for the calibration of the photometer as it represented a UVT% of 100%. The analysis was performed in duplicate for each sample, after which the average values was determined.

3.5.2 Total Dissolved Solids (TDS)

A (TDS)-3 meter (HM Digital) was used to the determine the total dissolved solids (TDS) of the water sample. To determine the TDS measurement, the sum of mobile charged ions is measured by the handheld device, expressed in parts per million (ppm) which corresponds to a reading in $mg.L^{-1}$. The mobile charged ions are also directly proportional to the sample's electrical conductivity. The analysis was performed in duplicate for each sample, after which the average values was determined.

3.5.3 Total Suspended Solids (TSS)

The total suspended solids content of each water sample was determined by following the instructions set out by Standards Methods (APHA, 2005). The procedure was performed by filtering the river water sample through a glass microfiber filter (Munktell, Sweden). After filtration, the filter was weighed and heated in a crucible for 2 hours at 105°C. After heating, the crucible was cooled in a desiccator and weighed. The weight before and after heating was used to determine the TSS measurement, expressed in $mg.L^{-1}$. The analysis was performed in duplicate for each sample, after which the average values was determined.

3.5.4 Chemical Oxygen Demand (COD)

A Spectroquant Nova 60 COD cell test (Merck Millipore, South Africa) was used to photometrically measure the COD of each river water sample. Chemical oxygen demand (COD) refers to the amount of oxygen available in a solution for oxidative reactions to consume. As per the standard testing procedure, three millilitres of each water sample were transferred to a COD cell test (Merck, South Africa). The test cell was thoroughly vortexed and placed in a thermal reactor (Hach, USA) to digest for 2 hours at 148°C. After digestion, samples were cooled to room temperature, followed by measuring the COD values with the Spectroquant NOVA 60 Spectrophotometer (Merck Millipore, South Africa). COD values were expressed in units of $mg.O_2.L^{-1}$. The analysis was performed in duplicate for each sample, after which the average values was determined.

3.5.5 Turbidity

An Orion AQ3010 Turbidity Meter (Thermo Scientific, USA) was utilised to measure the turbidity of the water samples. The turbidity meter was calibrated with control samples with known turbidity values. Turbidity is expressed in Nephelometric Turbidity Units (NTU). The analysis was performed in duplicate for each sample, after which the average values was determined.

3.5.6 pH

A portable pH meter (WTW, Germany) was used to measure the pH of water samples, according to the manufacturer's instructions. Prior to testing, the pH meter (WTW, Germany) was calibrated by using standard pH solutions of pH 7, pH 4 and pH 10. The analysis was performed in duplicate for each sample.

3.5.7 Alkalinity

The alkalinity of the water samples was measured according to the instructions set out by Standard Methods (APHA, 2005). A solution of 0.1 N H_2SO_4 was prepared and transferred to a glass burette. The solution was titrated into a glass beaker containing 50 mL of a water sample and a pH probe. The titration was performed until a pH of 4.3 was reached. The volume of H_2SO_4 needed to record a pH 4.3 were used to determine the alkalinity with the use of a standard calculation. Alkalinity is expressed in units of $mg.L\ CaCO_3^{-1}$. The analysis was performed in duplicate for each sample, after which the average values was determined.

$$\text{Calculation:} \quad \frac{50\ 000\ x\ 0.1}{20\ x\ amount\ used\ in\ titration} = x\ mg.L\ CaCO_3$$

3.5.8 Electrical Conductivity (EC)

A portable HI 8733 Conductivity Meter (Hanna Instruments, USA) was used to determine the electrical conductivity (EC) of the water samples. The instrument measured the quantity of dissolved salts in the samples to obtain a value in mS. m^{-1}. The analysis was performed in duplicate for each sample, after which the average values was determined.

Table 3.2 Suggested limits for physico-chemical characteristics of irrigation water (DWAF, 1996a)

Water quality characteristics	Irrigation water limit
TDS	260 mg.L^{-1}
TSS	50 mg.L^{-1}
pH	6.5 – 8.4
Turbidity [1]	10 NTU
EC	40 mS. m^{-1}
Alkalinity [2]	< 120 mg.L CaCO$_3^{-1}$
UVT%	Not stipulated
COD [3]	< 75 mg O$_2$.L^{-1}.

[1] Not stipulated. No limits indicated for irrigation water, however the Water Quality Guidelines for Domestic Use (DWAF, 1996c) stated that water with turbidity values > 10 NTU, can potentially carry an associated health risk of disease. Therefore, this guideline was used for the irrigation water limit

[2] Not stipulated. A value of < 120 mg L CaCO$_3^{-1}$ was selected from the Industrial Water Guidelines (DWAF, 1996b) as crop quality is merely affected by alkalinity range below 120 mg L CaCO$_3^{-1}$.

[3] Not stipulated. The Industrial Water Guidelines (DWAF, 1996b) stated that an COD level < 75 mg O$_2$.L^{-1} will be an acceptable limit for irrigation water.

3.6 Microbiological analyses of river water

3.6.1 *Escherichia coli* and coliform enumeration and identification

The membrane filtration (MF) method was used as part of the enumeration step of *E. coli* and coliforms of the river water samples. A serial dilution ($10^0 – 10^{-6}$) was prepared in duplicate according to the South African National Standards (SANS) method 6887-1 (SANS, 2011), in 90 mL Ringer's solution volumes. One 100 mL volume of each dilution were filtered through a sterile cellulose nitrate membrane filter with a pore size of 0.45 μm and diameter of 47 mm (Millipore, South Africa) as specified by the U.S Environmental Protection Agency (USEPA) method 1604 (USEPA, 2003). The membrane filter was then placed onto Brilliance *E. coli* / coliform selective agar (Oxoid, South Africa) and incubated at 37°C for 24 hours. After incubation, *E. coli* colonies were counted with a colony

colour of purple. A total coliform colony count was also determined by counting both purple and pink colonies.

3.6.2 Detection and isolation of presumptive positive STEC colonies

A 100 mL river water sample was filtered through sterile cellulose nitrate membrane filter with a pore size of 0.45 µm and diameter of 47 mm (Millipore, South Africa). The membrane filter was then transferred with sterile forceps to 20 mL of sterile buffered peptone water (BPW) and incubated at 37°C for 2 hours. After incubation, 1 mL of the BPW was transferred to 9 mL of EC broth (Oxoid, South Africa), and further incubated for 24 hours at 37°C. After incubation, a loopful suspension of the EC broth was streaked onto selective CHROMagar STEC plates (MediaMage, South Africa) and incubated at 37°C for 24 hours. Presumptive positive STEC colonies (mauve colour) were picked and streaked onto other CHROMagar STEC plates (MediaMage, South Africa) and incubated to ensure purity of colonies. As a further-control measure, one mauve coloured colony was also picked and streaked onto Levine's Eosin Methylene Blue (LEMB) Agar (Oxoid, South Africa) and incubated at 37°C for 24 hours. Metallic green colonies were considered as indicative of *E. coli* and were then picked and streaked onto non-selective Nutrient Agar and incubated at 37°C for 24 hours. After incubation, straw-coloured colonies from Nutrient Agar plates were picked and transferred to 5 mL of sterile Tryptic Soy Broth, mixed, and incubated at 37°C for 24 hours. After incubation, 800 µL of the TSB suspension was transferred to 800 µL of sterile 50% (v.v⁻¹) glycerol solution in a sterile Cryo.s™ Freezing tube (Greiner Bio-one, Austria), followed by the tube being thoroughly mixed. The Cryo.s™ Freezing tube contained a final glycerol stock solution concentration of 25% (v.v⁻¹), which was stored at -80°C until further analysis.

3.6.3 Detection and isolation of presumptive positive ESBL colonies

A 100 mL river water sample was filtered through sterile cellulose nitrate membrane filter with a pore size of 0.45 µm and diameter of 47 mm (Milipore, South Africa). The membrane was then transferred with sterile forceps to 20 mL of sterile BPW and incubated at 37°C for 2 hours. After incubation, 1 mL of the BPW were transferred into 9 mL of EE broth (Oxoid, South Africa), and further incubated for 24 hours at 37°C. After incubation, a loopful suspension of the EE broth was streaked onto selective CHROMagar ESBL plates (MediaMage, South Africa) and incubated at 37°C for 24 hours. Presumptive positive ESBL colonies (pink colour) were picked and streaked onto another CHROMagar ESBL plates (MediaMage, South Africa) to ensure purity of colonies, which were further incubated at 37°C for 24 hours. As a further-control measure, pink coloured colonies were picked and streaked onto VRBGA agar (Oxoid, South Africa) and incubated at 37°C for 24 hours. Purple/pink colonies, indicating presumptive positive *Enterobacteriaceae*, were picked and streaked on non-selective Nutrient Agar, followed by incubation at 37°C for 24 hours. After incubation, straw colour colonies from Nutrient

Agar plates were picked and transferred into 5 mL of sterile Tryptic Soy Broth, thoroughly mixed with a vortex, and incubated at 37°C for 24 hours. After incubation, 800 μL of the TSB suspension was transferred to 800 μL of sterile 50% (v. v⁻¹) glycerol solution in a sterile Cryo.s^{TM} Freezing tube (Greiner Bio-one, Austria), followed by the tube being thoroughly mixed. The Cryo.s^{TM} Freezing tube contained a final glycerol stock solution concentration of 25% (v. v⁻¹), which was stored at -80°C until further analysis.

3.6.4 Preparation of presumptive ESBL strains for ESBL confirmation testing

The bacterial stock isolate was removed from the -80°C and defrosted. After reaching room temperature, 20 μL inoculum was suspended in five mL of sterile TSB and incubated at 37°C for 24 hours. After incubation, the TSB-bacterial suspension was streaked onto CHROMagar ESBL plates (MediaMage, South Africa) for phenotypic confirmation. Following confirmation on the CHROMagar ESBL plates, presumptive positive strains were streaked on non-selective Nutrient Agar (Oxoid, South Africa) and incubated for 24 hours at 37°C, inversely. A single colony from each Nutrient Agar plate was transferred to a MacCartney bottle with 25 mL sterile distilled H_2O ($_dH_2O$), to reach an approximate cell density similar to the 0.5 McFarland Standard (BioMèrieux, South Africa). A Spectroquant Prove 600 Spectrophotometer (Merck, South Africa) was used as a confirmation to test for bacterial cell optical density (OD) of the suspension. The spectrophotometer was zeroed with sterile distilled water before measuring the absorbance of each suspension at 600 nm, with adjustments to obtain a final absorbance reading of 0.2 (approximately 8×10^8 cells per ml) for all suspensions prior to testing.

3.6.5 ESBL confirmation testing procedure

To test whether *Enterobacteriaceae* strains were ESBL producers, the EUCAST (2020) disc diffusion testing procedure was followed. Mueller-Hinton agar (Oxoid, South Africa) was prepared and inoculated with each of the isolated *Enterobacteriaceae* strains, in duplicate. This was done using a sterile cotton swab, which had been soaked in the respective suspensions, prior to inoculation. Excess fluid was removed from the swab by pressing it against the sides of the swab tube provided. According to the inoculation procedure set out by EUCAST (2020), the swab was moved in horizontal, vertical, and diagonal directions to ensure that the surface of the agar plate was completely covered with the inoculum. Following the inoculation procedure, a disc dispenser (Thermo Scientific, South Africa) was used to dispense discs onto each plate, in duplicate. The discs of ceftazidime [30 μg], cefotaxime [30 μg] and cefepime [30 μg], each individually and in combination with clavulanic acid [10 μg] were used for each strain (Davies Diagnostics, South Africa). Each plate was then inversely incubated at 37°C for 24 hours. After incubation, the zone diameters were measured. EUCAST (2020) indicated that if the

inhibition zone diameter of discs containing clavulanic acid are ≥ 5 mm larger than discs with without the clavulanic acid, a strain can be considered an ESBL producer.

Figure 3.4 indicates the laboratory procedure to detect and isolate presumptive positive colonies of STEC and ESBL-producing Enterobacteriaceae from a river water sample
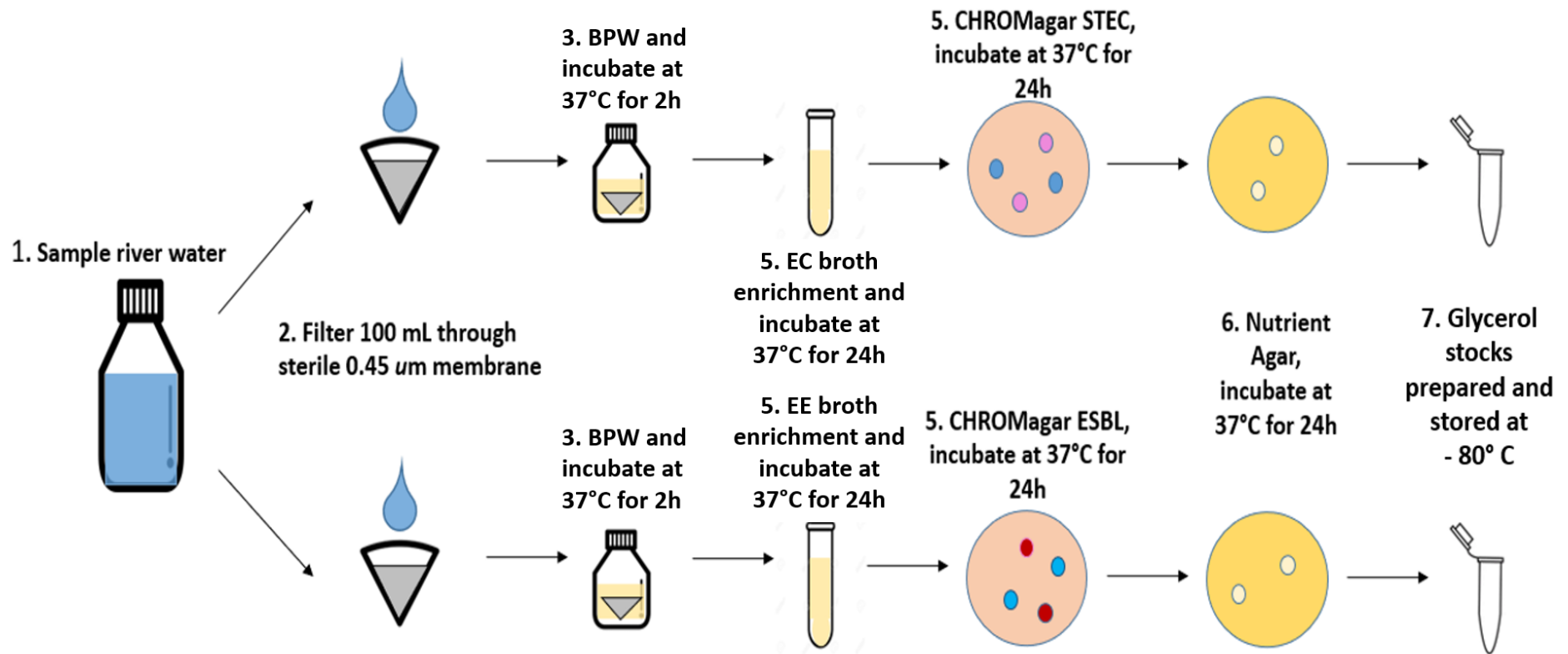
Figure 3.4 Laboratory procedure to detect and isolate presumptive positive colonies of STEC and ESBL-producing *Enterobacteriaceae* from a river water sample

3.6.7 Characterisation of bacterial strains using the Oxidase- and Catalase test

The oxidase reaction test was used for the characterisation of bacterial isolates. Although there are many method variations to the oxidase test, the Filter Paper Spot Method was used for this study. The oxidase reagents used was 1% tetra-methyl-*p*-phenylenediamine dihydrochloride (g.v^{-1}), in water. A disposable loop was used to pick a well-isolated, fresh colony (18-to-24-hour culture) from Nutrient Agar plates (Oxoid, South Africa). The picked colony was rubbed onto filter paper, followed by one to two drops of 1% tetra-methyl-*p*-phenylenediamine dihydrochloride on the bacterial smear. Oxidase positive microorganisms changed to a dark purple colour in about 10 seconds, compared to oxidase negative microorganisms which showed no colour changed.

The catalase test detects the presence of the catalase enzyme, differentiating catalase-positive from catalase-negative microorganisms. The catalase test reagent used was hydrogen peroxide (H_2O_2), as H_2O_2 is broken down by the catalase enzyme, resulting in the formation of bubbles (Ripolles-Avila *et al*., 2018). The Slide (drop) Method was used for this study. A well-isolated, fresh colony (18-to-24-hour culture) from Nutrient Agar plates (Oxoid, South Africa) was picked and transferred to a microscope slide with a disposable loop. Using a sterile dropper, one drop of the 3% H_2O_2 was placed on the bacterial smear. The petri dish was covered with the petri dish lid and kept still. For catalase-positive microorganisms immediate bubble formation was observed, compared to catalase-negative microorganism that showed no bubble formation.

3.6.8 Characterisation of bacterial strains using Gram staining

Gram staining is fundamental to the phenotypic characterisation of bacteria. The staining procedure differentiates bacteria according to their cell wall structure. Gram-positive bacteria have a thick peptidoglycan layer which stains dark purple. Moreover, Gram-negative bacteria stain pink as they have a thin peptidoglycan layer. The Gram stain procedure was followed according to the protocol of Smith & Hussey (2005). A heat fixed smear was prepared by transferring a small amount of a bacteria colony to a drop of water on a microscope slide, followed by heating the microscope slide over a Bunsen burner for two seconds. The slide was flooded with crystal violet staining reagent for one minute, followed by rinsing with tap water for five seconds. The slide was then flooded with Gram's Iodine for another one minute, followed by rinsing for five seconds. The slide was then flooded with a decolorising agent for 15 seconds, followed by rinsing for five seconds. Lastly, the slide was flooded with a counterstain, safranin, for one minute, followed by rinsing. The slide was blot dried with absorbent paper and observed with a microscope under oil immersion with a 100x magnification. As mentioned, Gram-positive bacteria stained dark purple and Gram-negative bacteria-stained pink.

3.6.9 Identification of bacterial strains using MALDI-TOF

Following the isolation and characterisation, environmental strains were prepared for MALDI-TOF analysis. Microbial cell extracts were prepared with sterile distilled water and HPLC-grade ethanol according to the standard procedures (Zvezdanova *et al*., 2020). After preparation, samples were identified with the MicroFlex LT Matrix-Assisted Laser Desorption/Ionisation Time of Flight (MALDI-TOF) mass spectrometer (Bruker Daltonics, Germany; Zulu, Z. 2021, Researcher, Faculty of Natural and Agricultural Sciences, University of Pretoria, Pretoria). MALDI-TOF is a technique used to detect and characterise organic molecules based on their mass. In addition, the technique uses a laser to disperse and ionise the sample into different molecules, which results in the molecules moving through an electric field vacuum before being detected by a membrane (Feucherolles *et al*., 2019). Feucherolles et al. (2019) further reported that the time of flight of molecules depends on their electrical charge and mass, which results in specific spectra for each organism. The spectra obtained from the samples were compared to spectra of reference strains in the database of the MALDI Biotyper 3.0 software, to determine each isolate's identity. For each isolate, a logarithmic score was obtained that indicated similarity between a reference strain and tested isolate (Zulu, Z. 2021, Researcher, Faculty of Natural and Agricultural Sciences, University of Pretoria, Pretoria). According to the manufacturer's guidelines, these logarithmic scores are classified into four value ranges that serve as indications of the reliability of the MALDI-TOF identification (Table 3.3) (Zvezdanova *et al*., 2020). A logarithmic score lower than 1.700 would for instance indicate a mixed culture or the absence of reference spectra on the database for the tested isolate, score above 2.3 will be reliable up to species level.

Table 3.3 Logarithmic score interpretations of MALDI-TOF results (Zvezdanova et al., 2020)

| Log value range	Logarithmic score interpretation
<1.700	No identification possible
1.700 – 1.999	Only genus identification
>2.000	Probable specie identification
>2.300	Species identification with high accuracy

3.6.10 Screening for Shiga toxins with Pall GeneDisc STEC Top 7 test

The Pall GeneDisc STEC Top 7 discs enables the detection of DNA from Shiga-toxin *E. coli* (STEC) belonging to O-serogroups O26, O103, O111, O145, O45, O121 and O157 using real time polymerase chain reaction analysis. The wells of the disc are preloaded with specific reagents for PCR analysis including internal inhibition controls and specific primers and probes for the detection of virulence genes and *E. coli* Top 7 serogroups (O26, O103, O111, O145, O45, O121 and O157*).

As mentioned before, presumptive positive STEC colonies and non-selective BPW suspensions were stored in glycerol solution (25% v. v⁻¹) at -80°C, for Strategy 1 and Strategy 2, respectively. These isolates and suspensions were resuscitated by defrosting to room temperature, followed by transferring 100 µL of each suspension to 5 mL of sterile BPW (Oxoid, South Africa) tubes. The BPW tubes were then incubated at 37°C for 24 hours.

After incubation, 50 µL of BPW enrichment (containing isolate suspension) was streaked onto a CHROMagar STEC (MediaMage, South Africa) plate and further incubated at 37° for 24 hours, according to Strategy 1 (Figure 5). After incubation, presumptive STEC colonies were picked and transferred to a lysis tube (Pall Corporation, France). Furthermore, for strategy 2 (Figure 5), 50 µL of BPW enrichment (containing non-selective suspension) were directly transferred to a lysis tube (Pall Corporation, France).

The lysis tubes for both strategy 1 and 2 were then incubated in a heating block at 102°C ± 2°C for 10 minutes, according to the manufacturer's instructions. DNA lysed samples were placed in a fridge at 5°C ± 3°C, until PCR analysis (one colony per lysis tube). For storage longer than 6 hours, samples were frozen at -20°C ± 2°C. The figure (Figure 3.5) below shows the post-isolation procedure for both Strategy 1 and 2 of screening for Shiga toxins with the Pall GeneDisc STEC Top 7 test
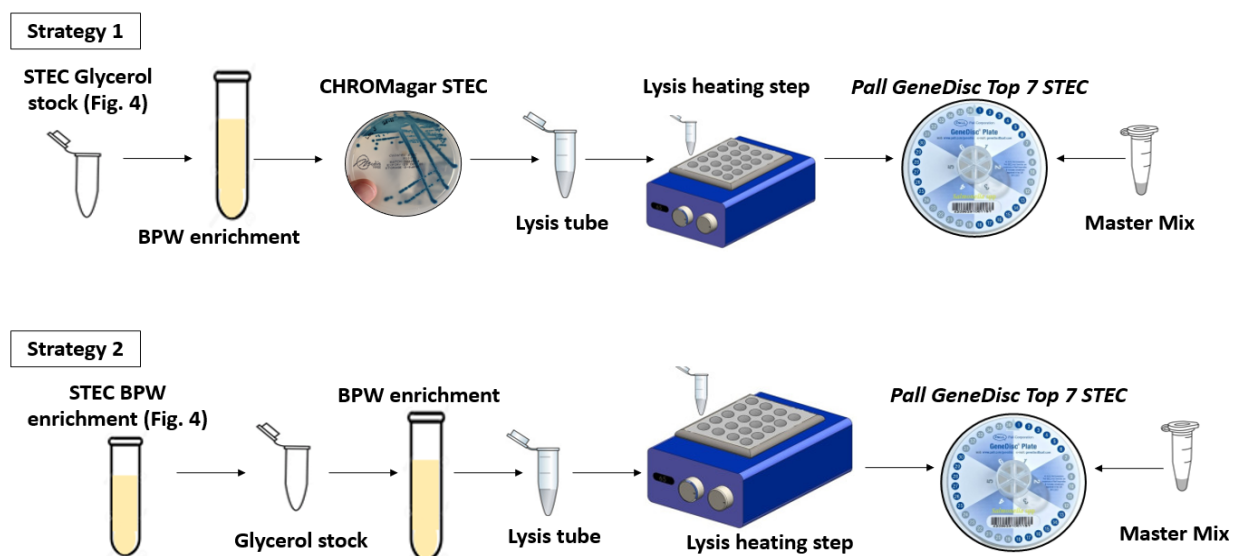
Figure 3.5 Schematic diagram of STEC screening procedure

3.6.11 Statistical analysis

Statistical analysis was performed by using Statistica software (13.5.0). Significant differences (p<0.05) were determined by utilising a 95% confidence interval. Furthermore, Statistica was used for the calculation of means, standard deviations and Pearson correlation coefficients. Microsoft Excel 2013 were used for tables and graph construction in figures.

3.7 Results and Discussion

3.7.1 Objective 1: Characterise the physico-chemical variation of the Plankenburg river water at different sites

In this section of the study, the variation in the physico-chemical characteristics of the Plankenburg river system were studied at five water sampling locations. Each of the five locations were sampled on three separate occasions and the characteristics of the river water samples were determined within 24 hours of sampling. The results of the physico-chemical analyses are presented in Table 3.4.

Determining the physico-chemical variation of a river system could potentially indicate the effects of different pollutions sources of a river.

The results presented in Table 3.3 show how the physico-chemical characteristics vary between different water sites of the Plankenburg river system. The Irrigation Water Guidelines (DWAF, 1996a) indicate that an acceptable level for TDS is 260 mg.L^{-1}. The TDS of water is considered as one of the most important parameters for the measuring of water quality as it is directly correlated and influenced by elevated levels of turbidity and conductivity (Kothari *et al*., 2021). Irrigation water with high TDS levels can cause the root systems of fresh produce to collapse due to plant cells not being able to maintain osmotic balances (Saiyood *et al*., 2012), resulting in plant death. Location One (Plank 1) and Location Two (Plank 2), both exceeded the guideline limit with average values of 560.33 mg.L^{-1} and 261.33 mg.L^{-1}, respectively. At a 95.0% confidence interval, there were significant differences ($p<0.05$) between Plank 1 and Plank 2, as Plank 1 was significantly higher. Plank 1 was situated downstream from a brickworks site, which could have potentially contributed to the high TDS reported. This conclusion was based on previous work by Oraeki et al. (2018) that reported a TDS range of 541- 709.5 mg.L^{-1} from wastewater samples collected directly in an effluent outlet of a clay brick production plant. The TDS value of Plank 1 in this study falls within the range indicated by Oraeki et al. (2018).

Table 3.4 Physico-chemical results obtained from each of the three sampling occasions during the months of November 2020 – December 2020

Characteristics	Plank 1 1	2	3	Avg. / SD	Plank 2 1	2	3	Avg. / SD	Jonkers 1	2	3	Avg. / SD	Plank 4 1	2	3	Avg. / SD	Eerste 1	2	3	Avg. / SD
UVT%	28.00	21.90	29.30	28.00 / 3.95	67	49	78	64.70 / 11.95	97	90.70	98.70	90.40 / 3.44	88	72.40	96.40	85.60 / 9.94	81.60	82.70	87.20	83.83 / 2.42
TDS (mg. L^{-1})	564	533	584	560.33 / 20.98	289	255	240	261.30 / 20.49	91	74	71	78.60 / 8.80	110	98	137	115 / 16.31	255	249	258	254 / 3.74
TSS (mg. L^{-1})	39.20	46.30	34.30	39.90 / 4.92	52.73	61.00	21.00	44.91 / 17.24	11.86	14.76	7.30	11.3 / 3.07	19	27	25	23.7 / 3.39	23	29	31	27.70 / 3.39
COD (mg O$_2$. L^{-1})	55	35	28	39.30 / 11.44	31	27	15	24.30 / 6.79	10	10	10	10 / 0	15	10	10	11.7 / 2.35	24	18	10	17.30 / 5.73
pH	7.32	7.43	7.42		7.50	7.56	7.37		6.89	7.00	7.09		7.01	7.12	7.19		7.41	7.40	7.53	
Turbidity (NTU)	25	31	31	29.00 / 2.82	8	12	17	12.30 / 3.68	0.50	1.80	1.10	1.1 / 0.53	1.50	2.00	3.00	2.2 / 0.6	1.80	2.50	30	2.43 / 0.49
EC (mS.m^{-1})	0.26	0.27	0.28	0.27 / 0.01	0.29	0.28	0.31	0.29 / 0.01	0.32	0.38	0.35	0.35 / 0.02	0.32	0.32	0.32	0.32 / 0	0.33	0.37	0.41	0.37 / 0.03
Alkalinity (mg.L CaCO$_3$$^{-1}$)	75	76	79	76.70 / 1.69	91	92	91	91.30 / 0.47	97	96	99	97 / 1.24	90	86	88	88 / 1.63	100	108	105	104.3 / 3.29

*UVT – ultraviolet Transmittance   *TDS – Total Dissolved Solids   *TSS – Total Suspended Solids   *COD – Chemical Oxygen Demand   *EC – Electrical Conductivity   *SD – Standard deviation

Of all the sites tested Location 3 (Jonkers) had the lowest overall TDS value of 78.67 mg.L^{-1}, which was significantly lower than Plank 1 and Plank 2 (Table 4). As Jonkers flowed into the main river system at the confluence point of Location 4 (Plank 4), an average TDS value of 115 mg.L^{-1} was observed, indicating a significant difference (p≤0.05) between the two points. A possible dilution effect may also have occurred at the confluence point as the TDS value at Jonkers was lower than Plank 2.

Furthermore, it should be noted that Location 5 (Eerste) is situated downstream from the Stellenbosch Wastewater Treatment plant (WWTP) (Loff *et al*., 2014). The Veldwagters river also flows past the WWTP and into the Eerste river (Meek *et al*., 2010). The WWTP and Veldwagter river water could potentially have contributed to the higher levels of TDS between Plank 4 and Eerste with values of 115 mg.L^{-1} and 254 mg.L^{-1}, respectively. At a 95.0% confidence interval, there were significant difference (p≤0.05) between Plank 4 and Eerste. The increase in TDS values might be linked to the biodegradation of the organic load by microorganisms in the wastewater (Salaudeen *et al*., 2018). Additionally, wastewater treatment plants also often discharge high levels of heavy metals and different inorganic salts, which may also elevate the TDS levels in the effluent (Sun *et al*., 2017). In addition, there was no significant differences (p>0.05) between Plank 2 and Eerste. This could possibly be attributed to both locations being exposed to industrial effluents, even though these locations are on different locations of the river.

The total suspended solids (TSS) content was calculated by filtering a specific amount of river water through a 0.6 μm glass fibre filter paper. The procedure follows the American Public Health Association (APHA, 2005) standard method for TSS content. The final TSS value represents both inorganic and organic material present in the water sample.

The Irrigation Water Guidelines (DWAF, 1996a) indicate that an acceptable level for TSS is 50 mg.L^{-1}. Each of the five sampling locations fall within the limits and are therefore acceptable for irrigation water use in terms of the TSS content in the water. Plank 2 showed the highest TSS content with a value of 44.91 mg.L^{-1}, however, there were no significant differences (p>0.05) between Plank 2 and that of Plank 1, with value of 39.93 mg.L^{-1}. The environmental pollution around the Plank 2 site was very noticeable upon all sampling rounds which could possibly have contributed to the highest value. Furthermore, Jonkers had indicated the lowest TSS content with a value of 11.31 mg. L^{-1}. At a 95.0% confidence interval, there were significant differences (p<0.05) between Plank 2 and Jonkers, which was expected. This observation could possibly be supported by their geographical differences, as the Jonkershoek river flows from the Jonkershoek mountain range with little exposure to domestic and industrial effects.

On the other hand, Plank 2 was potentially exposed to effluents from the informal settlement (Kayamandi & Enkanini) and Plankenburg industrial area which are in the vicinity of the water sampling

location. As for Plank 4 and Eerste, there were no significant differences ($p > 0.05$) obtained, with values of 23.67 $mg.L^{-1}$ and 27.67 $mg.L^{-1}$, respectively. However, there were significant differences ($p < 0.05$) observed between Jonkers and the confluence point of Plank 4. It was observed that water flow at the confluence point was visually stronger than that of Jonkers, which could possibly indicate the significantly higher TSS values, due to stirring of river sediment. In support of this statement, Leonard & Reed (2002) indicated that TSS concentrations generally increase with velocity peaks of open water flow due to the agitation and stirring of sediment.

Table 3.4 indicated that the TSS content for all five sampling locations ranged from 7.30 to 61 $mg.L^{-1}$, indicating that TSS content varies remarkably throughout the Plankenburg River. The geography of each location varies and therefore a variation in TSS values could potentially occur. The location of Plank 2 showed the highest TSS fluctuation between the three sampling rounds, as it reported the highest overall standard deviation of 17.24 (Table 3.4).

The COD test is based on the principle of a strong oxidising agent under acidic conditions, that fully oxidises all organic compounds in one litre of water to carbon dioxide (Wu *et al*., 2011). The COD value ultimately provides an indication of the available oxygen present in the water sample. The guideline limits for industrial use stated that the COD limit for irrigation water should be < 75 mg O_2. L^{-1} (DWAF, 1996b), to be acceptable for use.

The COD values of the river water samples showed that it ranged from <10 mg $O_2.L^{-1}$ to 39.33 mg $O_2.L^{-1}$, with an overall average of 20.53 mg $O_2.L^{-1}$ for the five locations. Plank 1 and Plank 2 showed the highest COD levels with values of 39.33 mg $O_2.L^{-1}$ and 24.33 mg $O_2.L^{-1}$, respectively. However, there was a significant difference ($p < 0.05$) between the two locations, as Plank 2 was significantly lower. Increased COD levels indicate increased levels of organic pollution in the river (Mamun *et al*., 2020). Furthermore, Park et al. (2013) indicated that sites with increased COD concentrations often receives inputs from domestic and industrial wastewaters. This statement supports the higher COD levels at Plank 2, which was potentially at the receiving end of effluents from either an informal settlement or industrial area. The Jonkers site had COD levels of <10 mg $O_2.L^{-1}$, which is lower than the lowest detectable limit for the Spectroquant NOVA 60 Spectrophotometer (Merck Millipore, South Africa). Furthermore, at a 95.0% confidence interval, there was a significant difference ($p < 0.05$) between Plank 2 and Jonkers, correlating to the TSS content between the same two locations.

An interlinking relationship between COD levels and TSS content of the water sampling locations was observed, as a moderately positive correlation of 0.65 was obtained with the use of the Pearson correlation coefficient. In support of this observation, Calderon et al. (2019) indicated a direct relationship between COD and TSS values measured at different points of a river. Calderon et al. (2019)

further indicated that the formation of biofilms induces an increase in the amount of TSS as the overall organic matter concentration increases.

Turbidity causing materials (TCMs) comprises of natural organic matter, inorganic particles, and biological particles (Farrell *et al.*, 2018). The guideline limit for domestic use (DWAF 1996c) indicates a turbidity value of <10 NTU will be acceptable for use; it was used for irrigation water scenarios in this study. The results obtained revealed that both Plank 1 and Plank 2 exceeded the recommended guideline limit with values of 29 NTU and 12.3 NTU, respectively. Plank 1 showed the highest overall turbidity of 29 NTU, which is one of the main characteristics resulting in the poorest UVT% observed. Jonkers on the other hand, showed the lowest turbidity of 1.10 NTU, indicating a high negative correlation to the highest UVT% measured from the five sampling locations. These values can be supported by the significantly high negative correlation observed between turbidity and UVT%, with a Pearson correlation coefficient of -0.92, indicating an inverse relationship. Furthermore, at a 95.0% confidence interval, there were no significant differences ($p > 0.05$) in turbidity between the three locations of Jonkers, Plank 4 and Eerste.

Ultraviolet transmittance (UVT) is one of the key parameters of water quality. UVT is the measurement of light in percentage passing through a water sample at 254 nm. The UVT varied remarkably between the five sampling locations, indicating different levels of water quality in one river system. Jonkers showed the highest overall UVT% of 95.47 %, which is significantly higher than any of the other four river sampling locations. At a 95.0% confidence interval, a significant difference ($p < 0.05$) in UVT% was seen between the three locations of Plank 1, Plank 2 and Jonkers. However, there were no significant difference ($p > 0.05$) between Plank 4 and Eerste.

Furthermore, the presence of suspended particles and soluble organic molecules in water absorb and disperse UV light, resulting in the reduction of UVT. Xie & Hung (2020) indicated that an increase in TSS and turbidity will decrease the UVT%. This correlation can be observed in Table 3 as UVT% indicated a high negative correlation with TSS and turbidity as Pearson correlation coefficient values of -0.71 and -0.92 were measured, respectively. In addition, organic matter, which is indicated by COD levels, can absorb UV radiation and reduce the initial UV dose applied (Xie & Hung, 2020). A high negative correlation of -0.84 was observed between COD and UVT%, supporting the statement made by Xie & Hung (2020). Furthermore, TDS, TSS and other volatile suspended solids all correlate positively with one another, however, they negatively correlate with UVT% (Shahawy *et al.*, 2018). This statement can be supported by the findings of this study as TDS and TSS negatively correlated with UVT%, with Pearson correlation coefficients of -0.91 and -0.71, respectively. A moderately positive correlation of 0.60 was seen between TDS and TSS, as stated by Shahawy et al. (2018). Plank 1 and Plank 2 indicated similar TSS values with significant difference ($p < 0.05$) in the UVT%. Therefore,

the high TDS of Plank 1 can potentially be the nominator for the significantly lower UVT%, as a high negatively correlations was measured between the two characteristics.

The Irrigation Water Guideline limit (DWAF, 1996a) for pH ranges between 6.5-8.4, indicating that all five water sampling locations are deemed acceptable for use, as their pH values fall within the range. However, there were significant difference ($p < 0.05$) between the locations of Plank 1, Plank 2, Jonkers and Plank 4. This supports the findings from the previous characteristics, indicating that the water profiles vary significantly between different sites. Furthermore, no significant differences ($p > 0.05$) were observed between the Eerste and Plank 1.

As for electrical conductivity (EC), the Irrigation Water limit (DWAF, 1996a) is 40 mS.m^{-1} which indicated that all water samples were deemed acceptable. At a 95.0% confidence interval, there were significant differences ($p < 0.05$) between Plank 1, Plank 2, Jonkers, Plank 4 and Eerste. However, no significant difference ($p < 0.05$) between Jonkers and Eerste.

According to the Irrigation Water Guidelines (DWAF, 1996a), the alkalinity content should be < 120 mg.L CaCO$_3$[1] to be deemed acceptable for the use in agricultural irrigation. The alkalinity values measured from the five sampling locations ranged from 77 mg.L CaCO$_3^{-1}$ to 104 mg.L CaCO$_3^{-1}$, indicating that all locations are deemed safe for use. At a 95.0% confidence interval, there were significant differences ($p < 0.05$) between all five locations of the river. In addition, alkalinity showed a high positive correlation with EC and UVT% with a Pearson correlation coefficient of 0.83 and 0.77, respectively. Furthermore, a high negative correlation was observed between alkalinity and turbidity, with a value of -0.72.

In conclusion, each of the five sampling locations indicated water with different physico-chemical profiles. The varying physico-chemical profiles could perhaps be attributed to the effect from different pollution sources on the river. TDS and turbidity values at both Plank 1 and Plank 2 exceeded the recommended guideline limits.

3.7.2 Objective 2: Microbial characterisation of different sites in the Plankenburg river system

For the last few decades, coliforms have been used as indicator organisms to serve as a measure of faecal contamination in food and water (Dogan-Halkman *et al*., 2003). Coliforms can be further categorised into two groups, faecal and non-faecal. *Escherichia coli* is considered the most common faecal coliform, while organisms such as *Citrobacter freundii*, *Enterobacter aerogenes* and *Klebsiella pneumoniae* are considered common non-faecal coliforms (Dogan-Halkman *et al*., 2003). In this study, *E. coli* and coliforms levels were determined during three separate sampling occasions of the Plankenburg river during the summer months of November and December 2020 and are represented in Figures 6 and 7, respectively. As mentioned before, for each water sample, a serial dilution (10^{-1}-10^{-6}) was performed for the *E. coli*/coliform test, with each dilution plated out in duplicate. In addition, plates with colonies ranging between 10 and 300 were counted, whereas plates with more than 300 and less than 10 colonies were excluded. The same water samples used for microbial analysis were also used to determine the physico-chemical profile of the water at each site (Table 4)

The *E. coli* level in Figure 3.6 ranged from 2.322 to 3.913 log CFU.mL^{-1} and the coliform counts (Fig 3.7) ranged from 3.414 to 5.591 log CFU.mL^{-1}, showing high variability between the five river water locations. The coliform counts in Fig. 7 were much higher than the *E. coli* counts. The reason for this observation can be supported by Bird et al. (2020), which stated that *E. coli* is a subgroup of coliform bacteria.

Overall, it was expected that the Plank 1 site (Fig 3.3) would have the lowest microbial counts and overall best physico-chemical characteristics, as it the nearest point to the origin of the river and upstream of Stellenbosch town centre. However, in terms of physico-chemical characteristics this was not the case, as it showed some of the poorest profiles of the five sampling locations (Table 3.4)

From a microbial point, Plank 1 showed the lowest average *E. coli* counts of 2.453 log CFU.mL^{-1} during the sampling period. As indicated by the Irrigation Water Guidelines (DWAF, 1996a), *E. coli* should be at a limit of 1 log CFU.mL^{-1} (<1 000 CFU.100.mL^{-1}) to be deemed safe for use. Plank 1, with the lowest average *E. coli* counts, was not deemed acceptable for use as irrigation water as it exceeded the guideline limit by 1.453 log CFU.mL^{-1}, translating to 28 colonies over the limit per millilitre water (Fig 3.6).
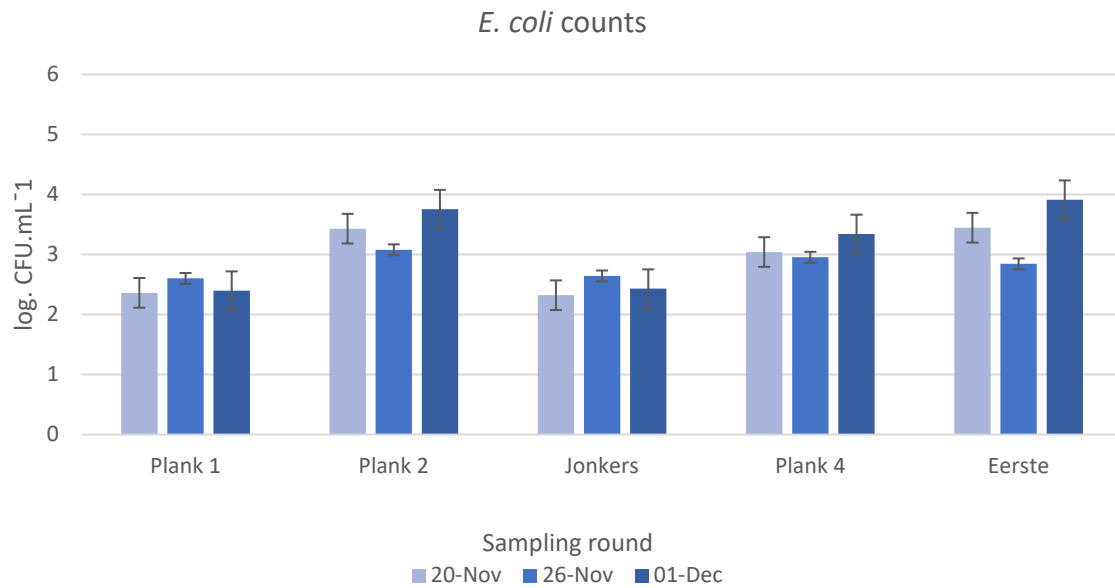
Figure 3.6 Average *E. coli* colony counts from each sample location, after three repeats performed in duplicate, expressed in log CFU.mL⁻¹, with standard deviation error bars for indication of deviation across three sampling rounds
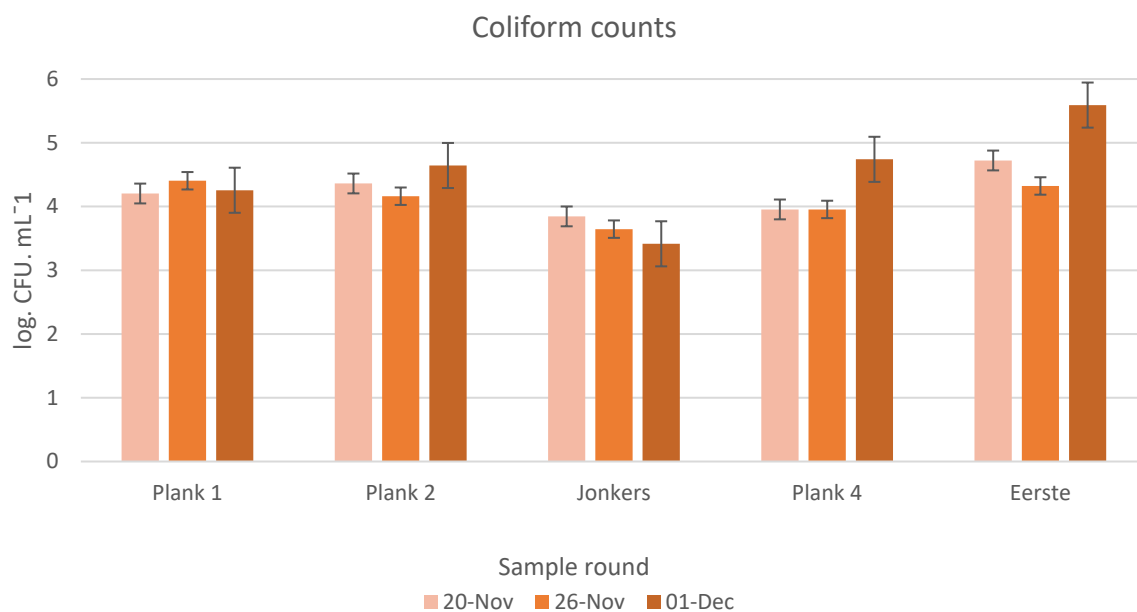
Figure 3.7 Average Coliform colony counts from each sample location, after three repeats performed in duplicate, expressed in log CFU.mL⁻¹, with standard deviation error bars for indication of deviation across three sampling rounds

As for coliforms, an average of 4.287 log CFU.mL^{-1} was obtained at this site, indicating no significant difference (p>0.05) with the counts from Plank 2, which is located further downstream after an informal settlement and industrial site (Fig 3.7). Suzuki et al. (2018) indicated that total coliforms do not represent an accurate index of faecal contamination as many coliforms are derived from the environment, such as soil and natural water bodies. Furthermore, the data shows that Plank 2 showed the highest overall *E. coli* count with an average of 3.422 log CFU.mL^{-1} (Fig. 3.6), exceeding the limit by 2.422 log CFU.mL^{-1}. Since there was a significant difference (p<0.05) between the *E. coli* counts of Plank 1 and Plank 2, it could be suggested that the source for the significant increase can be assigned to possible faecal contamination from the industrial area and informal settlement close to Plank 2 (Fig 3.1 and 3.2). These findings could support the statement from Suzuki et al. (2018), which reported that total coliforms are not a correct indication of the *E. coli* colony count, as seen between the two water sites of Plank 1 and Plank 2.

The microbiological profile of Plank 2 has been studied previously (Britz *et al*., 2013; Bursey, 2020; Paulse *et al*., 2009; Sivhute, 2019). The *E. coli* and coliforms counts were compared to previous microbial data and indicated that the results fall within the same ranges, in log CFU.mL^{-1}. Sivhute (2019) reported an *E. coli* count of 3.16 log CFU.mL^{-1} in her study during the summer irrigation period, which corresponds to the average count of 3.42 log CFU.mL^{-1} measured during the three sampling rounds of this study (Fig 3.6). Plank 2 has therefore been used as a control and comparison for the findings of the current research. Furthermore, it can be suggested that the microbial quality of Plank 2 have not improved over the last 10 years. In addition, Paulse et al. (2009) and Britz et al. (2013) also indicated that Plank 2 is situated downstream of an informal settlement and an industrial area, which can possibly be the main sources of the pollution, as mentioned before.

The third water sampling location, Jonkers (Fig 3.2), was not situated downstream of any industrial area or informal settlement and had of the lowest overall *E. coli* and coliform counts with averages of 2.465 and 3.634 log CFU.mL^{-1}, respectively (Fig 3.6 & 3.7). These results support the observation that an informal settlement and industrial area before Plank 2 could have been key contributors to the river pollution, which resulted in such elevated counts. At a 95.0% confidence interval, there was a significant difference (p<0.05) in *E. coli* and coliform counts between Plank 2 and Jonkers. Seo et al. (2019) also investigated the microbial difference upstream and downstream of industrial and domestic sites. Seo et al. (2019) noted that total and faecal coliform counts were significantly lower at upstream water sites and gradually increased to the downstream sites.

The results presented in Figures 3.6 and 3.7 further indicated a significant increase in *E. coli* and coliform counts between Jonkers and the confluence point at the Plank 4 site (Fig 3.2). This supports the previous observation that contamination potentially occurred before the Plank 2 site in

the river system. As observed at the first water sampling point (Plank 1), there were no significant difference (p>0.05) in *E. coli* counts when compared to Jonkers. The Jonkershoek river therefore has minimal effect on the microbiological state of the Plankenburg river system.

The Plank 4 site (Fig 3.2) showed an average *E. coli* count of 3.112 log CFU.mL⁻¹ and an average coliform count of 4.216 log CFU.mL⁻¹. One would expect that the merging of Plank 2 and Jonkers would dilute the microbial loads measured to a certain degree. Britz et al. (2013) stated that the merging of rivers would interfere with the initial microbial counts as bacteria can die or congregate as changes in the geography of the river occur, which in turn causes fluctuations in microbial counts. As for the results obtained at Plank 4, there was a significant difference (p<0.05) between the *E. coli* counts of Plank 2 and Plank 4 (Fig 3.6). The significant decrease in counts could therefore possibly be attributed to bacterial decay and settling due to geographical changes in the river, as mentioned by Britz et al. (2013). The change in river size and water velocity could also cause a dilution of the microorganisms present in the water.

The last river sampling location was the point of Eerste, which was downstream of all the locations. In addition, the Eerste sampling site is also situated downstream of the confluence points with the Veldwagters river (that flows past a WWTP) and agricultural land used for livestock and game farming. It was expected that the merging of the Veldwagters river and the possible exposure to run-off from animal farming could possibly influence the microbial profile further downstream. Water from the Eerste site proved to have the second highest overall *E. coli* counts, with an average of 3.401 log CFU.mL⁻¹ (Fig 3.6). When considering the high *E. coli* counts of Plank 2, there was no significant difference (p>0.05) between the two locations. In addition, the Eerste site also had the highest overall coliform counts (Fig 3.7), which was significantly higher than any of the four locations upstream.

On the third sampling occasion, a coliform count of 5.591 log CFU.mL⁻¹ was measured (Fig 3.7). As a significant increase in *E. coli* and coliform counts was observed between Plank 4 and Eerste, it could be suggested that the microbial contributions from the Veldwagters and the possible exposure to run-off from animal farming in the area resulted in the elevated counts. Blaak et al. (2014) reported that *E. coli* is a common inhabitant of aquatic sources, however, the detection of these organisms is remarkably higher in wastewater effluents. This statement could both support the elevated counts observed both at Plank 2 and the Eerste sites.

A study performed by Osuolale & Okoh (2017) investigated the microbiological profiles of various WWTPs in South Africa. Osuolale & Okoh (2017) indicated that faecal coliforms, which included *E. coli,* were detected downstream from all five WWTP studied. The findings by Osuolale & Okoh (2017) can be supported by the findings of this study at the Eerste site. The contribution of faecal

contamination through WWTPs is a form of point source faecal pollution, influencing the water quality of the river system (Park *et al.*, 2021).

Kay et al. (2008) has showed that faecal indicator loads remain high in river water, specifically when derived from livestock-farming activities. Kay et al. (2018) further noted that unfenced, streamside pastures grazed by cattle, significantly contribute to faecal contamination of rivers. With regards to livestock-farming activities, surface runoff and soil leaching into the river is a form of non-point source faecal pollution (Ouattara *et al.*, 2011). The origin of the faecal pollution can be from grazing livestock, wild animals and animal manure spread throughout farmed land (Ouattara *et al.*, 2011). These reports could further support results obtained for water from the Eerste site, as the river moves through animal pastures where potential farm run-off could increase faecal contamination in the river water prior to reaching the last sampling location.

Previous research has shown that anthropogenic activities alter the quality of river water through different pollution sources (Regier *et al.*, 2020; Wang *et al.*, 2013; Barakat *et al.*, 2016; Mustapha & Nabegu, 2011). The findings of this study are in line with previous research. Anthropogenic activities could potentially have altered the quality of the Plankenburg river through point and non-point source faecal pollution, ultimately resulting in physico-chemical and microbial variation at different water sites. Due to informal settlements, WWTPS and industrial areas surrounding the Plankenburg river system, high levels of *E. coli* and coliform were reported, most exceeding the irrigation water guideline limits. This conclusion is also further supported by the significantly lower microbial counts observed at the sampling site with minimal exposure to informal settlements and industrial areas (Jonkers site).

The findings of this study would suggest that pre-treatment of river water at certain sites is necessary to reduce risk to the consumer if it is used untreated for irrigation. Furthermore, due to the variability observed between the different river sites, it should be noted that UV treatment application, specifically site-specific optimisation, might need to be explored further. Microbial count data is very valuable as the concentration of indicator organisms is highly variable, which in turn can cause variability of the process efficiency with respect to the desired outcome (Foschi *et al.*, 2021). In addition to the statement by Foschi et al. (2021), the microbiological characterisation of the Plankenburg river will provide valuable data for the overall objectives of the study, which include UV optimisation for water with varying microbiological profiles.

3.7.3 Objective 3: *Isolation and confirmation of STEC and ESBL – producing Enterobacteriaceae*

The third objective was to screen water from the five river sampling locations for the presence of STEC and ESBL-producing *Enterobacteriaceae*, providing more in-depth microbial information for the Plankenburg river system. STEC and ESBL-producing *Enterobacteriaceae* are considered important pathogens, which can cause severe illness (Isik *et al*., 2020; Pitout, 2012). For this objective, microbial presence was determined on a presence/absence basis, compared to Objective 1 and 2, where colonies were counted. Two different selective chromogenic agars (CHROMagar STEC and CHROMagar ESBL) (MediaMage, South Africa) were used for the detection of STEC and ESBL-producing *Enterobacteriaceae,* respectively.

3.7.3.1 Objective 3.1 Isolation and confirmation of STEC

Determining the presence of STEC is vital, as it has been associated to food outbreaks and severe hospitalisation, which is of major public health importance over the past few years (Paletta *et al*., 2019). Moreover, farm animals such as cattle, goats and pigs are the main carriers of these organisms, where their faeces are transferred through agricultural soils and water runoff, ultimately ending in nearby river systems (Iwu *et al*., 2021). While most major foodborne outbreaks are associated to the O157 serogroup, other important non-O157 strains have also demonstrated the ability to cause serious diseases, such as thrombotic thrombocytopenic purpura, haemolytic-uremic syndrome and haemorrhagic colitis (Kintz *et al*., 2020). Strachan et al. (2001) states that a small number of STEC bacteria, less than 100 organisms, can cause illness.

As for the results obtained during this study, Table 3.5 below shows the presence/absence of presumptive STEC and other *Enterobacteriaceae* isolates from the five river water sites in the Plankenburg river system that was obtained as part of strategy 1 detection method (Figure 3.5)

Table 3.5 Growth observed on CHROMagar STEC (MediaMage, South Africa) for presumptive positive STEC and other *Enterobacteriaceae* on three separate sampling occasions, for each of the five locations. Growth is indicated by '+' and no growth by '-'

Organism	Plank 1			Plank 2			Jonkers			Plank 4			Eerste		
	20-Nov	26-Nov	01-Dec	20-Nov	26-Nov	01-Dec	20-Nov	26-Nov	01-Dec	20-Nov	26-Nov	01-Dec	20-Nov	26-Nov	01-Dec
STEC	+	-	+	+	-	+	+	-	+	-	-	-	+	-	+
Other *Enterobacteriaceae*	+	-	+	+	+	+	+	+	+	+	+	+	+	-	+

Although these results (Table 3.5) indicated the presence of presumptive STEC at all sites except Plank 4, none of the isolates could be confirmed as STEC using the Pall GeneDisc STEC Top 7 test. The figure below (Fig 3.8) is an example of one of the typical negative confirmation results obtained during Strategy 1. All five water sampling sites showed the same results after the Pall GeneDisc STEC Top 7 test.
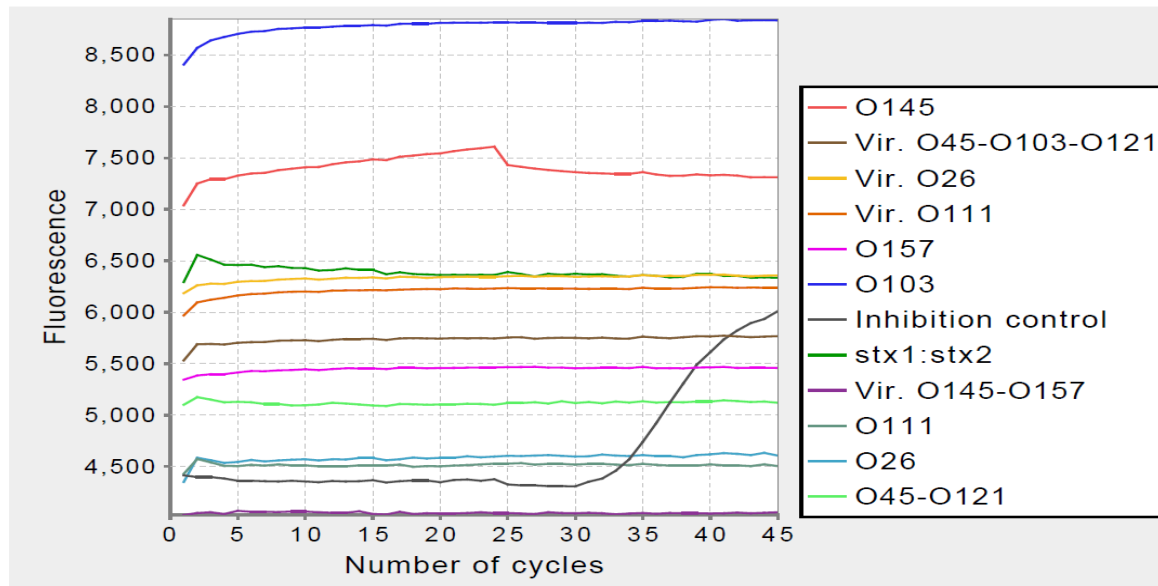
Figure 3.8 No fluorescence signal observed, apart from the inhibition control, illustrating a negative test where no pathogenicity-related genes could be detected in a presumptive STEC isolate

In contrast with Strategy 1 (Fig 3.5), non-selective BPW-enrichments were also tested directly with the Pall GeneDisc STEC Top 7 test as part of Strategy 2 (Fig 3.5). The non-selective BPW-enrichments from each site were pooled together before the Pall GeneDisc STEC Top 7 tests were conducted. The pooled BPW-suspension that showed the presence of STEC strains were re-tested as separate, individual BPW-suspensions. These individual BPW-suspensions were then confirmed with the Pall GeneDisc STEC Top 7 test, meaning the positive results were further validated by a second genotypic screening. Table 3.10 shows the summary of all results obtained during Strategy 2 from the Pall GeneDisc STEC Top 7 test. This data revealed that a different picture emerged using the Strategy 2 for STEC detection, as three of the fiver river water sites (Plank 2, Plank 4 and Eerste) tested positive for the presence of STEC gene sequences with the Pall GeneDisc STEC Top 7 test (Table 3.10).

Figure 3.9 is an example of one of the typical positive results obtained during Strategy 2.

Table 3.10 Summary of all the o-serogroup, *stx* genes and virulence genes detected from each of the five sites during the genotypic confirmation of pooled BPW by the Pall GeneDisc Top 7 test *(*strategy 2) with individual figures on Appendix A

Target	Plank 1	Plank 2	Jonkers	Plank 4	Eerste
	Figure A1	Figure A2	Figure A3	Figure A4	Figure A5
O103	Not detected	**Presence**	**Presence**	**Presence**	**Presence**
O111	Not detected	**Presence**	Not detected	**Presence**	**Presence**
O145	Not detected	**Presence**	Not detected	**Presence**	**Presence**
O157	Not detected	**Presence**	Not detected	**Presence**	**Presence**
O26	Not detected	**Presence**	Not detected	**Presence**	**Presence**
O45-O121	**Presence**	**Presence**	Not detected	**Presence**	**Presence**
stx1: stx2	Not detected	**Presence**	Not detected	**Presence**	**Presence**
Vir. O111	Not detected	**Presence**	**Presence**	**Presence**	**Presence**
Vir. O145-O157	Not detected	Not detected	Not detected	**Presence**	**Presence**
Vir. O26	Not detected	**Presence**	Not detected	**Presence**	**Presence**
Vir. O45-O103-O121	Not detected	**Presence**	Not detected	**Presence**	**Presence**
STEC (Yes/No)	No	**Yes**	No	**Yes**	**Yes**
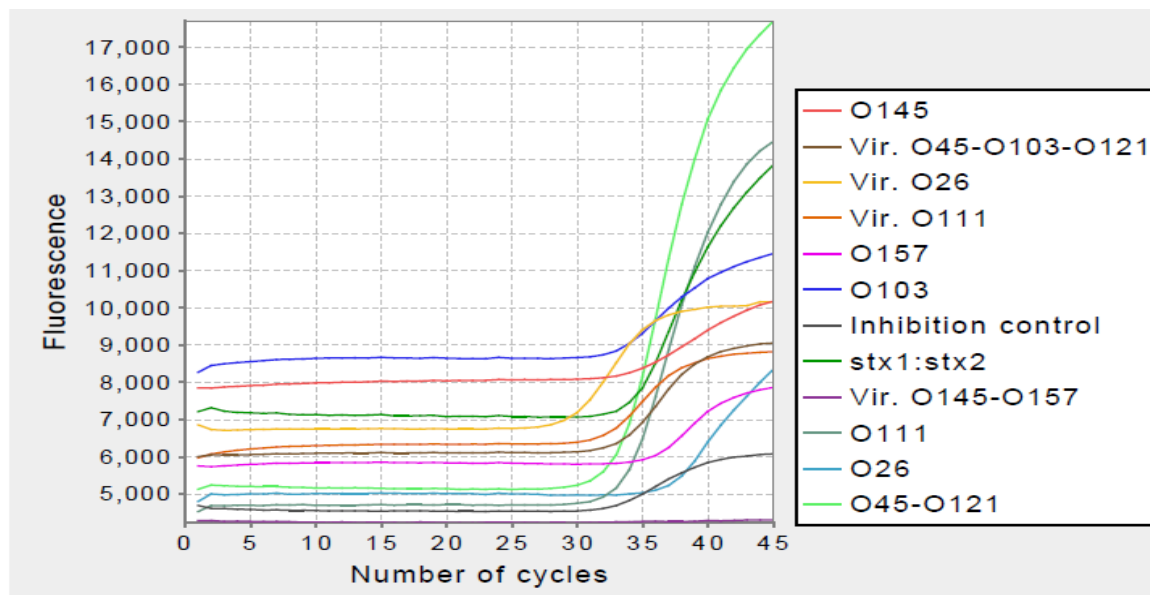
Figure 3.9 Fluorescence signals observed, with the results indicating a positive test where pathogenicity-related genes could be detected in river water samples of Plank 2

The presence of STEC and other non-Shiga toxigenic *Enterobacteriaceae* that was observed in this study confirm previous observations by Gu et al. (2013), Johnson et al. (2003) and Straw et al. (2013), that reported the frequent detection of STEC and other *Enterobacteriaceae* in river water.

The isolation of STEC from food and water samples continues to be challenging, as STEC and other non-pathogenic *E. coli* or *Enterobacteriaceae* are often present in the same environment (Gill *et al.,* 2014). Gill et al. (2014) reported that no selective conditions have been found which are exclusive to the STEC pathotype of *E. coli*, other than the verotoxin expression. Isolation for certain STEC subgroups have been used, which are based on the principle of selective characteristics common in the subgroup (Gill *et al.*, 2014). These selective characteristics include hemolysin activity and antimicrobial resistance (Tzschoppe *et al.*, 2012). This approach can also be limited to detecting all STEC strains, as the technique will only isolate strains with known features (Gill *et al.*, 2014).

Gill et al. (2014) indicated that the high sensitivity of selective media can easily provide false positives (similar to what was observed during strategy 1), as the colony morphology of STEC strains are so diverse and not always distinct in the presence of other *E. coli.* Furthermore, Wylie et al. (2013) stated that chromogenic STEC agar shows a low positive predictive value of 20-60%, ultimately meaning that there is a 20-60% probability that colonies with a positive STEC colour, truly are not STEC strains. This statement could possibly support the negative confirmatory results obtained with the Pall GeneDisc Top 7 test during Strategy 1. In addition, the general CHROMagar STEC media contains tellurite, which has been previously shown to inhibit a significant proportion of STEC strains (Tzschoppe *et al.*, 2012). Tzschoppe et al. (2012) further reported that CHROMagar STEC media

inhibited the growth of 25% of STEC strains tested during his study. In addition, these strains included *E. coli* O103, O121, O145, O157 and other diverse strains (Tzschoppe *et al*., 2012), indicating that the most general STEC serogroups can be inhibited. Furthermore, Gill et al. (2014) also established that chromogenic STEC plates in general are not suitable for the application of the isolation of STEC strains, due to the high proportion of strains being inhibited and should therefore not be the primary screen for these organisms.

Apart from the chromogenic agar limitation, the selective EC broth could also potentially have limited the growth of STEC strains. Baylis et al. (2000) reported that when comparing EC broth and BPW with each other, it was noted that BPW has a stronger buffer capacity, which promote the recovery and growth of bacteria significantly more. In addition, the non-selective BPW is the only validated method for the Pall GeneDisc procedure (Pall Corporation, France), which may possibly suggest that using EC broth may interfere with the system. The water samples used for strategy 2 did not include a selective enrichment (EC) broth, or the use of chromogenic selective STEC agar before and after the storage of strains in glycerol stocks at -80°C, resulting in a non-selective process.

As indicated by the results for strategy 2 (Table 3.10), the BPW-suspensions of Plank 2, Plank 4 and Eerste showed the presence of STEC, with Plank 1 and Jonkers showing no STEC being present (Table 10). The individual spectra figures (Appendix A1-A5) can be viewed for each of the five river sites at the end of this chapter.

With regards to Plank 2 (Table 10 & Fig. A2), it was signalled that *stx* genes were detected at this water site. The o-serogroups of the top seven were all present with the corresponding virulence genes, except for *E. coli* O145 and O157*.

Previous epidemiological studies reported that, independent of the o-serogroup, the genes encoding for the adherence factor intimin (*eae*), the virulence genes, and *stx*2 are mostly associated with severe diseases (Tzschoppe *et al*., 2012). It was also reported by Werber et al. (2002) that some strains can lack genes encoding for virulence factors that are required for the development of severe disease. Therefore, the detection of any virulence and *stx* genes at these water sites can be considered as important.

As mentioned before, Jonkers is the sampling point on Jonkershoek River before it joins the Plankenburg river system. As the Jonkershoek river's source is in Jonkershoek Mountains it possibly has minimal exposure to domestic and industrial effluents, and water with a good microbial profile was expected. As STEC is commonly detected from farm animals and wastewater (Pires *et al*., 2019), it was also expected that Jonkers would have a lower potential for STEC, specifically when compared to Plank 2. This assumption was based on the fact that the location of Jonkers (Fig 3.2) is situated upstream of animal pastures, informal settlements and the industrial area with potential wastewater

effluents. The GeneDisc analyses confirmed this as no *stx*-genes were detected at the river sampling site (Table 3.10 & Fig A3). The virulence gene commonly associated with O111 were detected in the absence of *E. coli* O111 (Table 10). Limited information with regards to this finding is available, however, Hua et al. (2020) reported that the virulence gene of O111, also known as *eae* subtype Θ (theta), can be found in certain other non-O111 o-serogroups such as O121, O103 and O157. The results of this study could support the finding of Hua et al. (2020) as O103 was present along with the virulence gene of O111 (Table 3.10). In addition, certain eae subtypes, such as subtype ε (epsilon), β (Beta), γ (gamma) and Θ (theta) can be carried by more than one STEC o-serotype (Samuels, E. 2021, GeneDisc Specialist, Pall Corporation). Furthermore, Velez et al. (2021) reported that *E. coli* O111 can carry the virulence gene with or without Shiga-toxin, as he further indicated that the pure strains of *E. coli* O111-hSTEC 08 and *E. coli* O111-F-A 790.1 carried these characteristics.

According to the results of isolation Strategy 1 (Table 3.5), no presumptive positive STEC were detected at the Plank 4 sampling site. After performing the second isolation strategy, strategy 2 (Fig 3.5), it was reported that pathogenic STEC gene sequences were indeed present at this water site (Table 3.10 & Fig A4). Furthermore, results from the Plank 4 sampling site indicated the presence of all top seven o-serotypes along with all virulence factors and the *stx* genes detected (Fig A4). The results from the water sampling site of Eerste (Table 3.10 & Fig A5) also indicated the presence of all top seven o-serogroups along with all virulence factors and the *stx* genes detected.

In conclusion, positive STEC colonies could not be isolated during this study on the Plankenburg river system, although the presence of STEC pathogenic genes were successfully confirmed with the Pall GeneDisc STEC Top 7 test.

Pathogenic STEC gene sequences were detected at three of the five river sites. Results of each of the three sites (Plank 2, Plank 4 and Eerste) indicating the presence of STEC, are situated downstream of animal farm activity and wastewater effluents. Mughini-Gras et al. (2018) and Pires et al. (2019) can support these findings, as both reported that STEC were frequently detected downstream from animal farm and wastewater effluents. As mentioned before, Table 3.1 described the geographical location of these three sites, supporting that these locations are influenced by agricultural and industrial pollution sources.

With regards to future studies, Pall Corporation (France) suggested that a technique, named immunocapture, should be used as a confirmation step after the Pall GeneDisc STEC Top 7 test in an attempt to obtain STEC strains. Immunocapture is a highly advanced method in terms of isolation sensitivity (Triplett *et al.,* 2019), which uses magnetised antibodies to isolate specific microbial cells, in this case o-serogroup specific STEC, from a complex and mixed sample. Triplett et al. (2019) further reported that the detection of distinctive antigens or genomic structures through immunocapture,

provide significant advantage for rapid detection of presumptive STEC strains. Future work should investigate immunocapture as a confirmation step for STEC strains in water samples after positively screened with the Pall GeneDisc system.

3.7.3.2 Objective 3.2 Isolation and confirmation of Extended spectrum beta-lactamase (ESBL) – producing *Enterobacteriaceae*

The excessive use of antibiotics in animal husbandry and agriculture in the past decades, caused a global spread of antibiotic resistance (Almakki *et al.*, 2019). The detection of ESBL-producing organisms in river water is of high importance since the increase of antibiotic resistant microorganisms has become a serious threat to public health because the ability to treat infectious diseases is compromised (Almakki *et al.*, 2019). Microorganisms with antibiotic resistance can therefore be a major concern as these organisms can possibly enter the food chain through contaminated irrigation water used on fresh produce, which is then consumed by consumers. In terms of the distribution of ESBL-producing organism, Li et al. (2015) investigated the spread of ESBL-producing microorganisms from animal farms to receiving water, in which he concluded that ESBL-producing microorganisms detected downstream of these activities were significantly higher than upstream. Moreover, animal farms may be a possible contributor of ESBL-producing microorganisms in river systems through the farm sewage system (Li *et al.*, 2015; Kar *et al.*, 2015). During this study, the use of CHROMagar ESBL plates were used as a phenotypic confirmation method for presumptive ESBL-producing organisms. Table 11 below shows the growth observed on CHROMagar ESBL (MediaMage, South Africa) on a presence/absence basis for each of the five river sampling locations.

Table 3.11 Growth observed on CHROMagar ESBL (MediaMage, South Africa) for presumptive positive ESBL *E. coli* and other ESBL *Enterobacteriaceae* on three separate sampling occasions (Nov-Dec 2020), for each of the five locations. Growth is indicated by '+' and no growth by '-'

Organism	Plank 1			Plank 2			Jonkers			Plank 4			Eerste		
	20-Nov	26-Nov	01-Dec	20-Nov	26-Nov	01-Dec	20-Nov	26-Nov	01-Dec	20-Nov	26-Nov	01-Dec	20-Nov	26-Nov	01-Dec
ESBL *E. coli*	+	-	+	-	+	+	-	-	+	+	+	+	+	+	+
ESBL *Enterobacteriaceae*	-	-	+	-	+	+	-	+	-	+	+	+	+	+	-

As seen above, Table 3.11 illustrated that there was a consistent detection of presumptive positive ESBL *E. coli* colonies using CHROMagar ESBL plates (MediaMage, South Africa). Other ESBL *Enterobacteriaceae* were also regularly detected at the five sampling locations.

The results from Plank 4 and Eerste showed the presence of ESBL *E. coli* at all three sampling occasions (Table 3.11). Moreover, Plank 2 results also indicated the detection of presumptive positive strains of both ESBL *E. coli* and ESBL *Enterobacteriaceae* during two of the three sampling rounds*.* Furthermore, Jonkers had the lowest occurrence of ESBL *E. coli* during this study (Table 3.11).

The presumptive positive *ESBL E. coli* and ESBL *Enterobacteriaceae* strains isolated from the five river sampling sites were prepared for MALDI-TOF analyses which is aimed at species identification. Table 3.12 indicates the species identification results after the MALDI-TOF analyses, with individual spectra in Appendix A6-A13. For the scope of this study, only presumptive ESBL *E. coli* was sent for MALDI-TOF species identification.

Table 3.12 Presumptive positive ESBL-producing *Enterobacteriaceae* isolated from the CHROMagar ESBL plates were identified using MALDI-TOF analysis

Water sample location	Date isolated	MALDI-TOF score[a]	MALDI-TOF species identification	Appendix A spectra
Plank 1	20/11/2020	2.404	*Escherichia coli*	A6
Plank 1	01/12/2020	2.530	*Serratia fonticola*	A7
Plank 2	01/12/2020	2.271	*Escherichia coli*	A8
Jonkers	01/12/2020	2.384	*Escherichia coli*	A9
Jonkers	26/11/2020	1.711	*Kluyvera* genus	A10
Plank 4	20/11/2020	2.279	*Escherichia coli*	A11
Plank 4	01/12/2020	2.397	*Escherichia coli*	A12
Eerste	20/11/2020	2.209	*Escherichia coli*	A13

[a]MALDI-TOF Score. A logarithmic score lower than 1.70 would indicate a mixed culture or the absence of reference spectra on the database for the tested isolate, score between 1.70 – 1.99 indicate low-confidence identification and score between 2.00 – 3.00 indicate high confidence identification.

The MALDI-TOF analyses identified all isolates as part of the *Enterobacteriaceae* family, as *E. coli, Serratia fonticola* (*S. fonticola*) and the *Kluyvera* genus were detected. The isolate containing the *Kluyvera* genus organism resulted in a non-reliable identification, as indicated by the low score (<1.999) seen above. The low MALDI-TOF score can possibly be an indication of a mixed culture (Zvezdanova *et al.*, 2020).

After all isolated strains were identified as *Enterobacteriaceae,* each of the strains were prepared for the phenotypic ESBL testing procedure to confirm ESBL production (EUCAST, 2020), as described earlier. According to the method, strains are considered ESBL producers if the inhibition zone diameter is ≥ 5 mm larger around antimicrobial discs containing additional clavulanic acid when compared to antimicrobial discs without clavulanic acid. In addition, as part of the ESBL testing procedure, a positive control, *Klebsiella pneumoniae ATCC 700603* and a negative control, *E. coli ATCC 25922*, were included for each testing round to ensure accuracy of the test. As seen below, the ESBL testing results from eight *Enterobacteriaceae* isolates from the Plankenburg river system are presented in Table 3.13.

Table 3.13 ESBL testing results of all *Enterobacteriaceae* isolates

Location	Organism	Zone diameter (mm)												ESBL producer
		CPM		CPM/CV		CTX		CTX/ CV		CAZ		CAZ/ CV		
		1	2	1	2	1	2	1	2	1	2	1	2	(Yes/No)
Plank 1	*Escherichia coli*	17	17	21	22	13	15	21	20	24	23	28	28	Yes
Plank 1	*Serratia fonticola*	15	14	20	20	15	15	21	20	19	19	21	21	Yes
Plank 2	*Escherichia coli*	14	15	21	20	12	14	24	23	23	20	28	25	Yes
Jonkers	*Escherichia coli*	22	23	27	28	14	14	25	23	22	21	27	27	Yes
Jonkers	*Kluyvera* genus	29	28	32	30	26	26	30	29	33	30	33	34	No
Plank 4	*Escherichia coli*	20	21	25	27	13	15	29	25	25	25	27	29	Yes
Plank 4	*Escherichia coli*	19	20	26	26	14	17	28	25	19	19	26	24	Yes
Eerste	*Escherichia coli*	19	21	30	28	13	15	29	26	23	24	28	30	Yes
	Klebsiella pneumoniae ATCC 700603	22	21	27	26	18	20	26	26	17	17	22	22	Yes
	Escherichia coli ATCC 25922	32	32	32	33	33	32	32	32	31	31	30	31	No

*CPM – Cefepime, CTX – Cefotaxime, CAZ – Ceftazidime, CV – Clavulanic Acid

The results (Table 3.13) showed that seven of the eight isolates were ESBL producers. Each of the five river sites on the Plankenburg river showed the presence of ESBL producers during this study.

The seven ESBL producers only consisted of two different microorganisms, *E. coli* and *S. fonticola* (Table 3.13). However, both *E. coli* and *S. fonticola* are well known ESBL-producing *Enterobacteriaceae* and are commonly classified as multidrug-resistant (Schill *et al*., 2017). Schill et al. (2017) indicated that all *S. fonticola* isolated during his study obtained a chromosomal FONA-6-like gene, which is a minor ESBL. Furthermore, Erb et al. (2018) noted that urban informal settlements have very inadequate sanitary conditions in which ESBL-producing organisms were detected on a constant basis. The statement by Erb et al. (2018) could support the regular detection of ESBL-producing microorganisms at Plank 2 (Table 3.13), which is downstream of an informal settlement (Britz *et al*., 2013) and industrial area (Bursey, 2021). The location of Jonkers had the lowest occurrence of ESBL *E. coli* (Table 3.11)*,* which can be supported by the findings of Li et al. (2015) and Erb et al. (2018), which stated that the presence of ESBL- producing organisms is much higher downstream of animal farm activities and informal settlements.

In conclusion, ESBL – producing organisms were successfully isolated and confirmed from river sites of the Plankenburg river. The outcomes of this study are in line with by the findings of Li et al. (2015) and Erb et al. (2018), suggesting that ESBL-producing organisms can be transferred from animal farm effluents and informal settlement wastewater.

3.8 Conclusion

In this study, the variation in microbial and physico-chemical characteristics of the Plankenburg river system over a longer distance, has been successfully studied. Previous research has shown that anthropogenic activities alter the quality of river water through different pollution sources (Regier *et al*., 2020; Wang *et al*., 2013; Barakat *et al*., 2016; Mustapha & Nabegu, 2011). The findings of this study correlate to previous findings done on other rivers. Anthropogenic activities could potentially have altered the quality of the Plankenburg river system through point and non-point source faecal pollution, ultimately resulting in the physico-chemical and microbial variations observed at different water sites.

Informal settlements, WWTP and the industrial area near the Plankenburg river system could have contributed to the high levels of *E. coli* and coliforms observed, most exceeding the irrigation water guideline limits. As for physico-chemical characteristics, TDS and NTU also exceeded the recommended limits at two of the five river sampling sites (Plank 1 & Plank 2). Furthermore, there were significantly lower microbial counts observed at the sampling site of Jonkers, which was upstream from informal settlements and the industrial area, supporting the statement that anthropogenic activities alter the river water quality.

Seo et al. (2019) conducted a study of the correlation between coliform bacteria and various physico-chemical characteristics, where he indicated a significant positive correlation between coliforms and COD. These findings can be supported by the results indicated in Table 3.4 and Figure 3.7, as a high positive correlation with p-value (Pearson correlation coefficient) of 0.75 was observed between coliforms counts and COD levels of the five water sampling locations. These results could suggest that river water with poor physico-chemical characteristics could probably also have a poor microbial profile.

Overall, the physico-chemical results (Table 3.4) and guideline limits (Table 3.2), along with the microbial results (Fig 3.6 and Fig 3.7), show that water from most sites tested (Plank 1, Plank 2, Plank 4 and Eerste) in the Plankenburg river system exceed guideline limits for safe irrigation water. Minimising the potential transfer risk of pathogens from contaminated river water to fresh produce, would ultimately improve food safety for consumers. Based on the findings of this study, it is suggested that water treatment, such as UV disinfection, is necessary before river water is used for irrigation of fresh produce.

Considering the application of UV irradiation of irrigation water, Carré et al. (2018) indicated that the physico-chemical characteristics of river water are highly important as particles, suspended- and dissolved solids, can diffuse UV light and ultimately result in a reduced UV transmittance of water. In addition, turbidity is one of the most used parameters for measuring water quality prior to UV

disinfection (Farrell *et al.*, 2018). As stated before, TCMs can impact UV inactivation through various mechanisms, such as absorption and scattering of incident UV light and through the shielding of microorganisms (Huber *et al.,* 2011). Reducing turbidity to acceptable limits would increase UV treatment efficacy, as Liu et al. (2005) indicated that a 33% reduction in the initial UV dose will take place when turbidity is increased from 1-10 NTU. In addition, the scattered light is directly proportional to the turbidity measured (Farrell *et al.*, 2018).

As there is a significant difference in UVT% percentage between three of the five locations, UV dose time will also vary significantly, suggesting the importance of UV optimisation. As for Plank 1, a UV dose of 20 mJ. cm^{-2} on the collimated beam will take significantly longer to complete, compared to that of Jonkers which will be shorter. The UV dose time could potentially be lower on the larger scale pilot plant, where the flow rate can also be adjusted to achieve a specific UV dose.

With regards to STEC, STEC colonies (correlating with a positive genotype) could not be cultured during this study. Gill et al. (2014) stressed that the isolation of STEC from food and water samples continues to be challenging, as was demonstrated during this study. However, the second isolation strategy (Strategy 2) did result in the detection of STEC through genotypical confirmation using the Pall GeneDisc Top 7 test. In addition to this finding, Holvoet et al. (2014) also reported during his study that no STEC strains could be isolated, however, it was considered to take all positive GeneDisc results as pathogenic positive samples. With regards to the decision-making of Holvoet et al. (2014), the same conclusion was considered for this study.

Pathogenic STEC was detected at three of the five river sites. Each of the three sites (Plank 2, Plank 4 and Eerste) at which STEC was detected, were situated downstream from either animal farm activity or wastewater effluents, which have been shown by previous studies to be sources of STEC (Pires *et al.*, 2019; Mughini-Gras *et al.*, 2018). It was suggested that future work should investigate the highly advanced method of immunocapture as an isolation step for environmental STEC strains after their presence is detected in water samples using the Pall GeneDisc system.

In addition, ESBL – producing organisms were also successfully isolated and confirmed from river sites of the Plankenburg river. ESBL – producing organisms were phenotypically confirmed by chromogenic ESBL agars, after which all strains were also characterised to genus and species level by MALDI-TOF analysis. Most of the ESBL producing strains sent for MALDI-TOF analysis were identified as *E. coli*. The outcomes of this study can be supported by the findings of Li et al. (2015) and Erb et al. (2018), which suggested that the presence of ESBL- producing organisms is much higher downstream of animal farm activities and informal settlements.

When considering the overarching aim of this study, the results could suggest that a site-specific approach should be followed when implementing on-farm UV irrigation plants. This could be

implemented on a river system with a variation in microbial and physico-chemical characteristics at different water sites. In addition, a site-specific approach and custom system design could be beneficial from an economic feasibility view, as correctly sizing the system in terms of plant flow rate, lamp output energy and water quality will be cost saving and most efficient.

3.9 References

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3.10 Appendix A
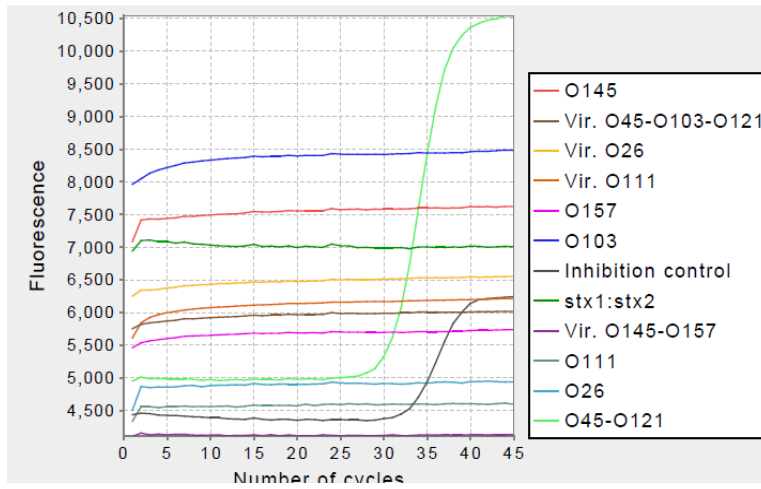
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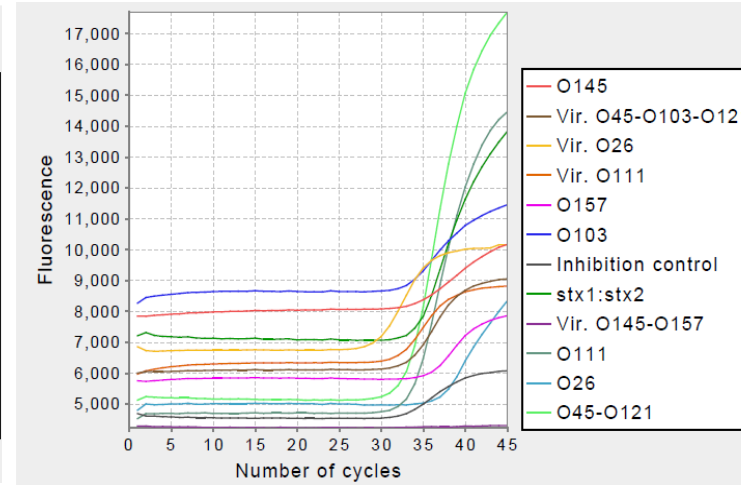
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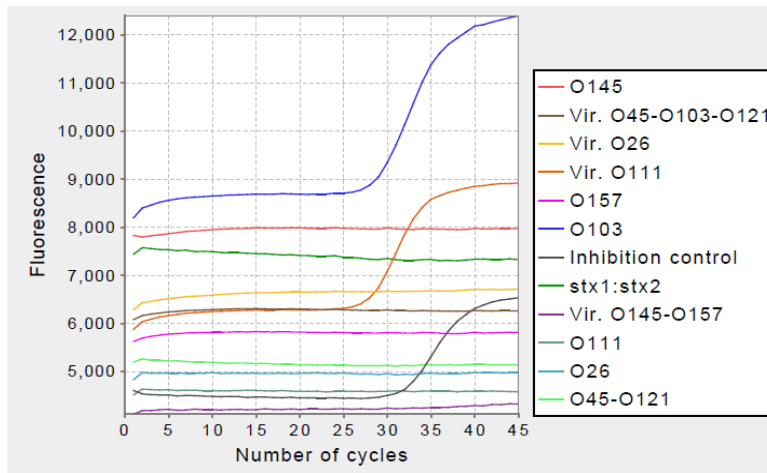
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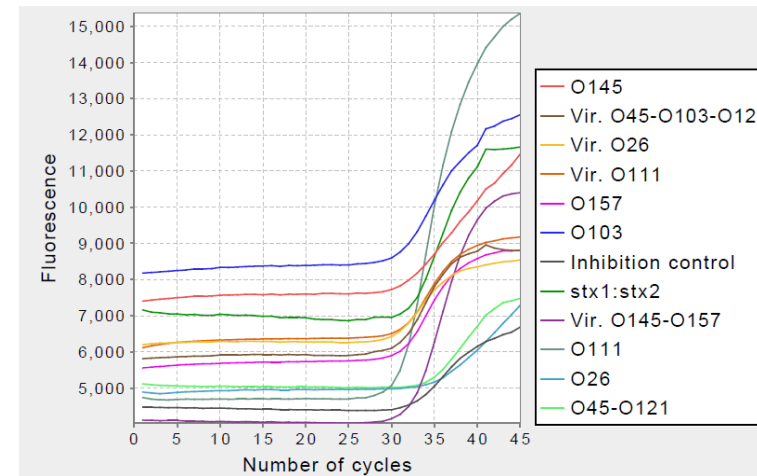
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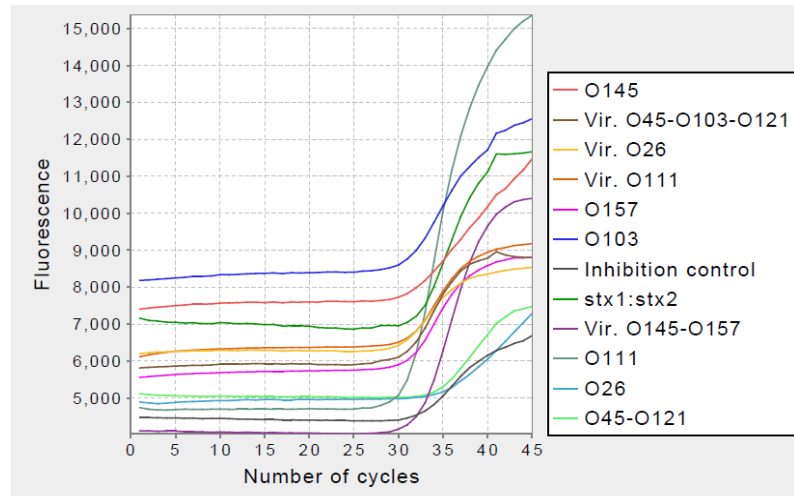
Fig. A5

Figures A1-A5 Representation of the spectra obtained from the Pall GeneDisc STEC Top 7 test results (objective 3.2.1), where A1 = Plank 1, A2 = Plank 2, A3 = Jonkers, A4 = Plank 4 and A5 = Eerste

123
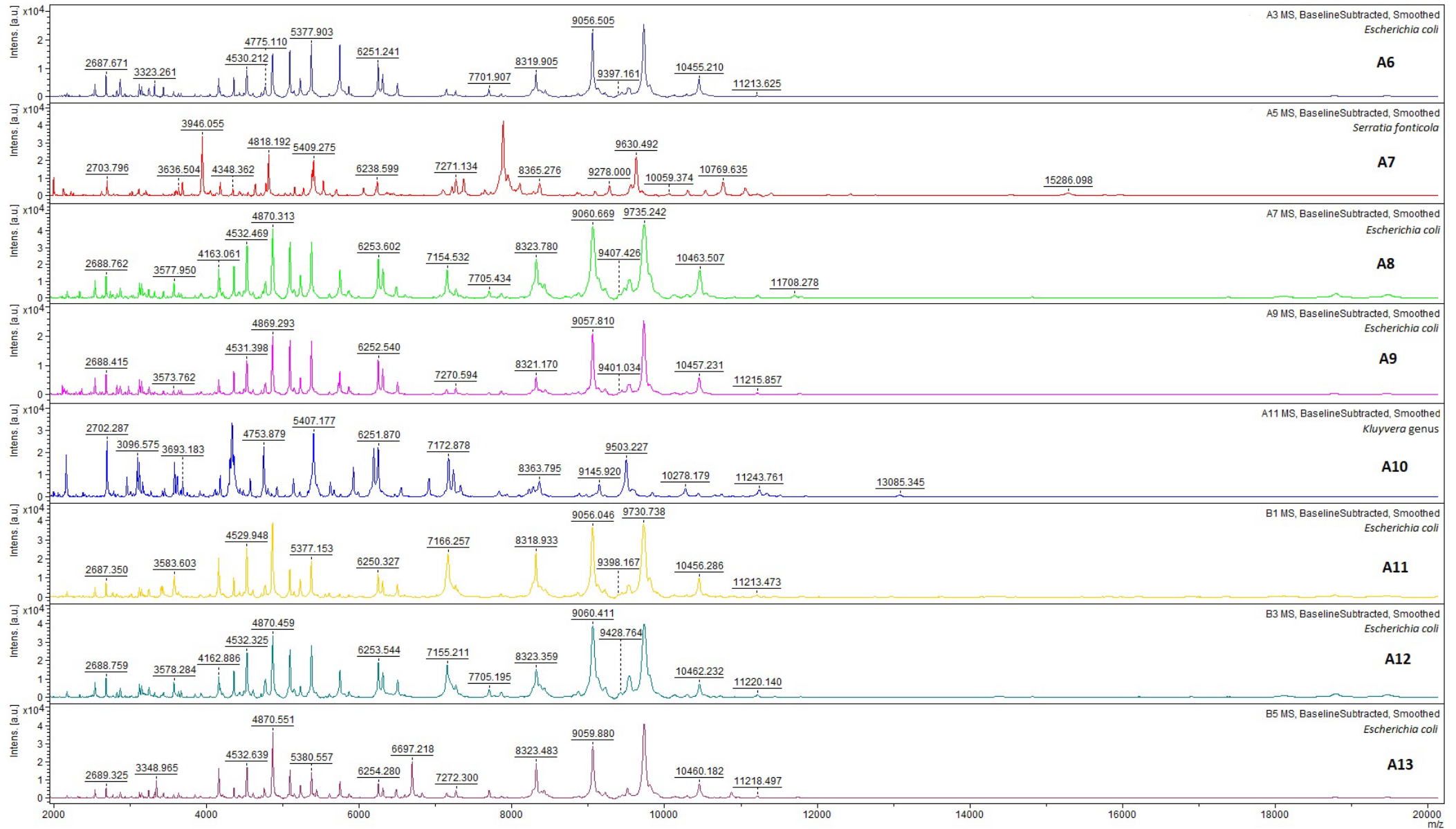
Figures A6-A13 MALDI-TOF spectra of individual organisms detected from Objective 3.2

CHAPTER 4

INVESTIGATING UV-C IRRADIATION TREATMENT EFFECTS ON MICROBIAL INDICATORS AND PATHOGENS PRESENT IN PLANKENBURG, EERSTE, FRANSCHHOEK AND MOSSELBANK RIVERS IN THE WESTERN CAPE

4.1 Abstract

This research chapter served as a follow-up study to work performed by Bursey (2020). Bursey (2020) reported the growth of Heterotrophic Plate Count (HPC) colonies after certain UV doses were applied as part of a UV disinfection step. Bursey (2020) determined the reduction of indicator bacteria after UV irradiation, however, these UV resistant bacteria were not characterised and identified. As part of this study, the research gap was addressed by investigating the UV-C irradiation effect on characterised microorganisms from four Western Cape rivers (Plankenburg, Eerste, Franschhoek and Mosselbank river) regularly used as irrigation water.

The microorganisms tested included indicators such HPC, Total Psychrotrophic Aerobic Bacteria Count (TPAC), (*E. coli),* and pathogens such as Shiga toxin-producing *E. coli* (STEC) and Extended spectrum beta-lactamase (ESBL) – producing *Enterobacteriaceae.* Each of the microorganisms were exposed to a 20, 40 and 60 mJ.cm^{-2} UV dose produced by a lab-scale low-pressure UV system. During this study, physico-chemical characteristics such as ultraviolet transmission (UVT), total dissolved solids (TDS), total soluble solids (TSS), chemical oxygen demand (COD), pH and electrical conductivity (EC) were tested.

Each of the four rivers selected (sampled during January-March 2021) illustrated their own water quality profile, in terms of microbial and physico-chemical characteristics. The results indicated that HPC and TPAC populations included various microbial species, such as *Bacillus cereus*, *Aeromonas hydrophilia*, *Bacillus megaterium* and the *Exiguobacterium* genus, each species with their distinct response to UV-C exposure. With regards to river profiles, it was observed that the initial microbial levels can be independent of the dose response or UV sensitivity of a particular populations. The results show that the higher the initial counts, the higher the chance of surviving UV-C radiation. However, population composition and resistance to a specified UV-C dosage should be considered as well. Furthermore, STEC and ESBL-producing organisms were also confirmed from the four rivers with potential exposure to point and non-point sources of faecal contamination. Ultraviolet radiation proved to eliminate most STEC and ESBL strains, however, one environmental *E. coli* strain survived the lowest UV dose once, stressing that these organisms could still pose a risk after UV disinfection.

These findings may perhaps imply that UV as a disinfection method was effective in reducing indicator populations and eliminating most STEC and ESBL. Nevertheless, the physico-chemical profile

of different sources may differ, which may affect the UV disinfection efficacy. Consequently, an effective pre-treatment and correct UV dose contact time should be monitored closely for best results.

4.2 Introduction

The Heterotrophic plate count (HPC) is used as an indicator of food quality, when evaluating the degree of bacterial regrowth and the presence of biofilm formation (Oguma *et al*., 2018). HPC growth refers to the heterotrophic bacteria able to grow in conditions with limited nutrient sources (Oguma *et al*., 2018). In addition, HPC include opportunistic bacterial species such as *Pseudomonas* and *Acinetobacter* which can be found in the food production environment, potentially causing infection in immunocompromised humans (Tokajian *et al*., 2005). With regards to water environments, Oguma et al. (2018) reported that there is a high regrowth potential of heterotrophic bacteria in water distribution networks and storages. This suggests that it is important to implement a treatment strategy such as UV disinfection at the end of such a water supply to address bacterial regrowth. In addition, total psychrotrophic aerobic bacteria count (TPAC) are also food indicators and comparable to HPC, as they share the same environment, however, psychrotrophic aerobic bacteria can grow better under colder conditions (Holvoet *et al*., 2014; Przemieniecki *et al*., 2014).

With regards to the UV dose response of HPC bacteria, limited research has been performed. Mofidi et al. (2004) studied the ability of low- and medium pressure UV lamps to eliminate heterotrophic bacteria and reported that HPC populations present downstream from a water treatment facility could be eliminated with a UV dose of up to 17 mJ.cm^{-2}. It should be noted that the physico-chemical characteristics measured by Mofidi et al. (2004) indicated UVT% and turbidity values ranging from 79 to 95% and 0.01 to 0.13, respectively. When considering the physico-chemical characteristics of the Plankenburg river in Chapter 3, the UVT% was significantly lower with high turbidity levels. Bursey (2020) also studied the physico-chemical profiles of four selected rivers in the Western Cape and reported significantly poorer physico-chemical profiles (including lower UVT %s), compared to the river profile specified by Mofidi et al. (2004). With regards to UV disinfection, water profiles with lower UVT% result in poorer UV irradiation, if the UV dose is not adjusted. Furthermore, poor water quality can ultimately result in less microorganisms being exposed to UV light, which could result in elevated levels of surviving bacterial species.

Furthermore, the HPC represents uncharacterised and mixed bacterial populations where each population may potentially exhibit their own UV dose-response characteristics (Mofidi *et al*., 2004). Mofidi et al. (2004) concluded a UV dose of 17 mJ.cm^{-2} was required to achieved counts lower than 10 CFU.mL^{-1} on a consistent basis.

Holvoet et al. (2014) studied the correlation between various hygiene-indicator bacteria and enteric bacterial pathogens in soil, produce and irrigation water. Their results indicated that there was

no significant difference between *E. coli* and TPAC in soil and fresh produce samples (Holvoet *et al.,* 2014). In addition, it was noted that both *E. coli* and TPAC counts were significantly higher when pathogens were present in water samples (Holvoet *et al*., 2014). A key characteristic of psychrotrophic bacteria is their versatility to grow at varying temperatures (Przemieniecki *et al*., 2014). Pyschrotrophic bacteria can replicate in cold environments, close to 0°C, and thrive in environments with temperatures between 15-30°C (Pzremieniecki *et al*., 2014). In addition, Kahn et al. (2005) reported that psychrotrophic bacteria can grow between 0° and 35°C, indicating that numerous temperatures and time periods are suggested for the enumeration of these bacteria. The statement made by Kahn et al. (2005) suggests that incubation temperatures of 30°C for HPC population enumeration can promote the growth of TPAC colonies in this study as well.

Economou et al. (2012) investigated indicator organisms in environmental and fresh produce samples such as water and lettuce, respectively. Economou et al. (2012) reported that TPAC cannot be considered as a good indicator of overall sanitary quality and faecal contamination, which is in line with the conclusion made by Holvoet et al. (2014).

The presence of HPC populations have been confirmed before in the Plankenburg, Eerste, Franschhoek and Mosselbank rivers of the Western Cape during (August - November) (Bursey, 2020). Bursey (2020) reported the growth of HPC populations after certain UV doses were applied as part of a UV disinfection step. Although the levels of these bacteria have been determined pre- and post-UV disinfection, Bursey (2020) did not characterise and identify these indicator organisms. As mentioned during Chapter 3, various pathogens such as STEC can be transferred from contaminated river water via irrigation systems to fresh produce, ultimately increasing the health risk to consumers (Isik *et al*., 2020). In addition, effluents from domestic and agricultural wastewater in river water systems have been widely studied for the presence of antibiotic resistant bacteria, such as ESBL- producing *Enterobacteriaceae* (Almakki *et al*., 2019). It is therefore important to continue with the work of Bursey (2020) to identify indicator populations such as HPC and TPAC, as there is a potential risk of unidentified HPC survivors. Furthermore, it is necessary to understand the variability of physico-chemical characteristics between rivers and the possible impact these parameters could have on the efficacy of UV disinfection methods.

This study follows on the work of Bursey (2020) and aims to address the questions raised by her study. With this in mind, the experimental design of this study was structured around three main objectives. Firstly, follow-up Bursey's (2020) findings by characterising the four selected rivers (Plankenburg, Eerste, Franschhoek and Mosselbank river) in terms of physico-chemical characteristics a year after the previous analysis (January – March 2021). Secondly, studying the impact of UV treatment (using three UV doses of 20, 40 and 60 mJ.cm^{-2}) on the TPAC and HPC populations present

in the four rivers. These results could also provide more information regarding the UV dose response of these indicator organisms in the four rivers. The last objective was to investigate the occurrence and UV dose response of STEC and ESBL - producing *Enterobacteriaceae*.

4.3 Materials and Methods

4.3.1 Research Design

Previous research has reported the growth of indicator organisms such as HPC populations after UV doses of 20, 40 and 60 mJ.cm^{-2} from the Plankenburg, Eerste, Franschhoek and Mosselbank river (Bursey, 2020). Water from each of the four selected rivers are used directly or indirectly for irrigation purposes during the summer irrigation period in the Western Cape, South Africa (Bursey, 2020). The experimental design of this study was structured around three main objectives.

Firstly, follow-up Bursey's (2020) findings by characterising the four selected rivers (Plankenburg, Eerste, Franschhoek & Mosselbank river) in terms of physico-chemical characteristics a year after the previous physico-chemical analysis was performed. The physico-chemical analysis consists of testing the total dissolved solids (TDS), total suspended solids (TSS), pH, turbidity, electrical conductivity (EC), alkalinity, ultraviolet transmission (UVT%) and the chemical oxygen demand (COD) of the sampled river water. Furthermore, the analysis is performed in duplicate within 24 hours after the river water was sampled.

Secondly, studying the impact of UV treatment (using three UV doses of 20, 40 and 60 mJ.cm^{-2}) on the TPAC and HPC populations present in the four rivers, ultimately characterising the UV dose response of the four rivers. The procedure included a serial dilution of each river water sample, in duplicate, after the exposure to UV radiation. Post-UV radiation water samples were plated on Plate Count Agar (PCA) and incubated at two different temperatures (22°C and 30°C), suggesting the growth of HPC populations and psychrotropic aerobic bacterial counts, respectively. Growth observed after UV radiation was isolated and identified using MALDI-TOF.

The last objective was to investigate the occurrence of (STEC) and (ESBL) - producing *Enterobacteriaceae*, thereby contributing to a more in-depth study of the microbiological characteristics of each river. For STEC detection the Pall GeneDisc real-time PCR system was used, whereas the ESBL testing included a selective enrichment procedure and MALDI-TOF identification. Both STEC and ESBL – producing *Enterobacteriaceae* was also exposed to the three UV doses of 20, 40 and 60 mJ.cm^{-2}, indicating the UV dose response of each.

All three objectives were evaluated on three separate sampling occasions between the summer irrigation months of January 2021 – March 2021. The Irrigation Water Guidelines (1996a) served as reference for results interpretation, in order to determine if water were safe and acceptable for agricultural irrigation usage. In Figure 1 a schematic diagram is presented that summarises the post-sampling analyses conducted per river to achieve the three objectives mentioned
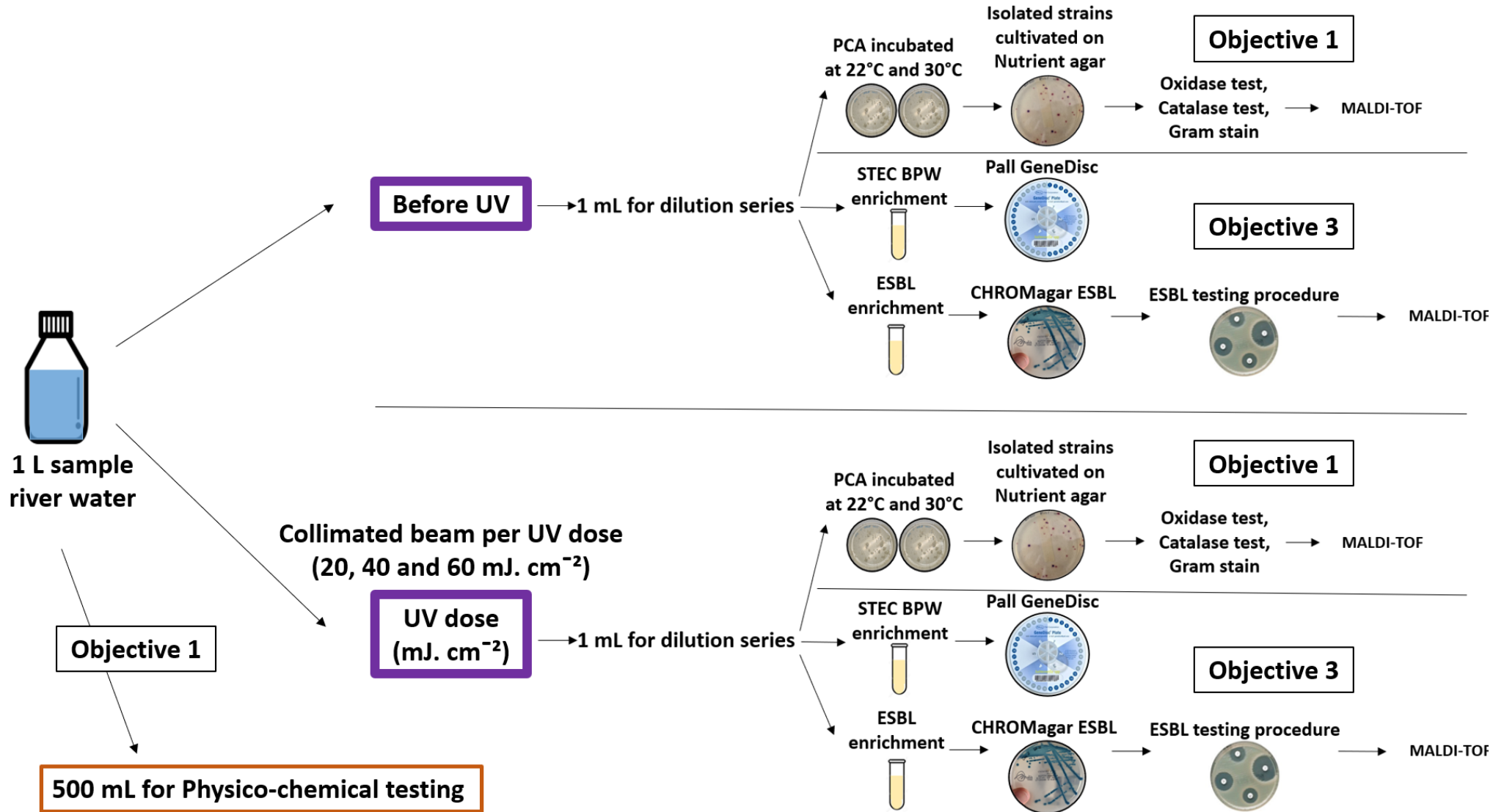
Figure 4.1 Schematic diagram of the post-sampling experimental procedures used in this study to achieve all three objectives

4.4 General materials and methods

4.4.1 Site selection

Four specific rivers (Plankenburg, Eerste, Mosselbank and Franschhoek river) were selected based on their direct or indirect use as irrigation water sources. The Plankenburg river, which is located in Stellenbosch, (33°55'58.50" S 18°51'06.80" E) acted as a study control site as previous studies from various authors have indicated very high microbial loads at this site (Sivhute, 2019; Bursey, 2020). In addition, this site is situated downstream from non-point pollution sources, such as effluents from informal settlements and exposure to wastewater from an industrial area. The Eerste River (33°56'36.10" S 18°50'42.00" E), which is further downstream of the Plankenburg River, is consistently used for the irrigation of fruit and fresh produce by surrounding commercial farmers (Bursey, 2020). The Mosselbank River is in the Kraaifontein area (33°49'11.00" S 18°42'10.6" E) situated downstream of a wastewater treatment works which merges with a storm water run-off channel. This river acts as the irrigation water source for large-scale commercial farmers further downstream (Bursey, 2020). The last river, the Franschhoek River in Franschhoek (33°53'56.80" S 19°05'35.30" E), was sampled at the merging point of two rivers, the Berg River and the Stiebeuel River, respectively. Vineyards and large-scale commercial tomato farmers make use the Franschhoek River as irrigation water source (Bursey, 2020). These four rivers were specifically chosen for this study as all are located in the Western Cape of South Africa and serve as direct or indirect irrigation water sources for farmers. Some of these rivers have been previously studied and illustrated concerning levels of microbial loads (Olivier, 2015; Loff *et al*., 2014; Bursey, 2020). Table 1 provides a short description and the coordinates for the four rivers.

Table 4.1 Five river sampling location names and descriptions of this study

River sample location	Location description	Coordinates
Plankenburg river	Located in Stellenbosch and situated downstream of informal settlements and industrial effluents	33°55'58.50" S 18°51'06.80" E
Eerste river	Located downstream of Plankenburg river and regularly used as irrigation water for fresh fruit and produce	33°56'36.10" S 18°50'42.00" E
Franschhoek river	Located in Franschhoek and regularly used as irrigation water for vineyards and large-scale farmers	33°53'56.80" S 19°05'35.30" E
Mosselbank river	Located in Kraaifontein and situated downstream of a WWTP and regularly used for large-scale farmers	33°49'11.00" S 18°42'10.6" E

4.4.2 Sample collection method

Each of the four river locations were sampled on three separate rounds, with the use of a sampling rod containing a sterile 1 L Schott bottle before being transported to the Department of Food Science for analysis. The South African National Standards (SANS) method 5667-6 for water sampling (SANS, 2006) was followed for each sampling occasion. Microbiological tests were performed within six hours of sampling. Physico-chemical tests were performed within 24 hours of the sampling. The water sample were stored in a fridge at 5°C for 24 hours.

4.5 Physico-chemical analysis of river water samples

4.5.1 Ultraviolet Transmission percentage (UVT %)

To determine the UVT% of the water sample a Sense T254 UV Transmission (%) Photometer was used according to the manufacturer's instructions (Berson, Netherlands). Distilled water was used for the calibration of the photometer as it represented a UVT% of 100%. The analysis was performed in duplicate for each sample, after which the average values was determined.

4.5.2 Total Dissolved Solids (TDS)

A (TDS)-3 meter (HM Digital) was used to the determine the total dissolved solids (TDS) of the water sample. To determine the TDS measurement, the sum of mobile charged ions is measured by the handheld device, expressed in parts per million (ppm) which corresponds to a reading in $mg.L^{-1}$. The mobile charged ions are also directly proportional to the sample's electrical conductivity. The analysis was performed in duplicate for each sample, after which the average values was determined.

4.5.3 Total Suspended Solids (TSS)

The total suspended solids content of each water sample was determined by following the instructions set out by Standards Methods (APHA, 2005). The procedure was performed by filtering the river water sample through a glass microfiber filter (Munktell, Sweden). The clean filter was weighed before filtering the water sample. After filtration the filter was heated in a crucible for 2 hours at 105°C. After heating, the crucible was cooled in a desiccator and weighed again. The weight before filtration and after heating was used to determine the TSS measurement, expressed in $mg.L^{-1}$. The analysis was performed in duplicate for each sample, after which the average values was determined.

4.5.4 Chemical Oxygen Demand (COD)

A Spectroquant Nova 60 COD cell test (Merck Millipore, South Africa) was used to photometrically measure the COD of each river water sample. Chemical oxygen demand (COD) refers to the amount of oxygen available in a solution for oxidative reactions to consume. As per the standard testing procedure, three millilitres of each water sample were transferred to a COD cell test (Merck, South

Africa). The test cell was thoroughly vortexed and placed in a thermal reactor (Hach, USA) to digest for 2 hours at 148°C. After digestion, samples were cooled to room temperature, followed by measuring the COD values with the Spectroquant NOVA 60 Spectrophotometer (Merck Millipore, South Africa). COD values were expressed in units of mg $O_2.L^{-1}$. The analysis was performed in duplicate for each sample, after which the average values was determined.

4.5.5 Turbidity

An Orion AQ3010 Turbidity Meter (Thermo Scientific, USA) was utilised to measure the turbidity of the water samples. The turbidity meter was calibrated with control samples with known turbidity values. Turbidity is expressed in Nephelometric Turbidity Units (NTU). The analysis was performed in duplicate for each sample, after which the average values was determined.

4.5.6 pH

A portable pH meter (WTW, Germany) was used to measure the pH of water samples, according to the manufacturer's instructions. Prior to testing, the pH meter (WTW, Germany) was calibrated by using standard pH solutions of pH 7, pH 4 and pH 10. The analysis was performed in duplicate for each sample.

4.5.7 Alkalinity

The alkalinity of the water samples was measured according to Standard Methods (APHA, 2005). A solution of 0.1 N H_2SO_4 was prepared and transferred to a glass burette. The solution was titrated into a glass beaker containing 50 mL of a water sample and a pH probe. The titration was performed until a pH of 4.3 was reached. The volume of H_2SO_4 needed to reach a pH 4.3 was used to determine the alkalinity with the use of a standard calculation. Alkalinity is expressed in units of mg $CaCO_3.L^{-1}$. The analysis was performed in duplicate for each sample, after which the average values was determined.

$$\text{Calculation:} \quad \frac{50\,000 \; x \; 0.1}{20 \; x \; amount \; used \; in \; titration} = x \; mg \; CaCO_3.L$$

4.5.8 Electrical Conductivity (EC)

A portable HI 8733 Conductivity Meter (Hanna Instruments, USA) was used to determine the electrical conductivity (EC) of the water samples. The instrument measured the quantity of dissolved salts in the samples to obtain a value in $mS.m^{-1}$. The analysis was performed in duplicate for each sample, after which the average values was determined.

Table 4.2 below indicates the suggested limits for physico-chemical characteristics of irrigation water, as set out the South African Water Quality Guidelines (DWAF, 1996a).

Table 4.2 Suggested limits for physico-chemical characteristics of irrigation water (DWAF, 1996a)

Water quality characteristics	Irrigation water limit
TDS	260 mg.L^{-1}
TSS	50 mg.L^{-1}
pH	6.5 – 8.4
Turbidity [1]	10 NTU
EC	40 mS.m^{-1}
Alkalinity [2]	< 120 mg CaCO$_3$.L^{-1}
UVT%	Not stipulated
COD [3]	< 75 mg O$_2$.L^{-1}.

[1] No turbidity limits are stipulated for irrigation water (DWAF, 1996a), however the Water Quality Guidelines for Domestic Use (DWAF, 1996c) stated that water with turbidity values > 10 NTU, can potentially carry an associated health risk of disease. Therefore, this guideline was used as reference limit in this study

[2] No alkalinity limits are stipulated for irrigation water (DWAF, 1996a). An alkalinity value of < 120 mg CaCO$_3$.L^{-1} was selected as guideline limit in this study from the Industrial Water Guidelines (DWAF, 1996b) as crop quality can be affected by alkalinity values above 120 mg CaCO$_3$.L^{-1}.

[3] No COD limits are stipulated for irrigation water used in agriculture (DWAF, 1996a). The Industrial Water Guidelines (DWAF, 1996b) states that acceptable COD levels for land irrigation should be < 75 mg O$_2$. L^{-1}. This guideline limit was used as reference limit in this study.

4.6 Collimated beam UV-C irradiation procedure

At laboratory-scale, water samples were treated with specific UV doses by using a collimated beam UV system (Berson, The Netherlands). The UV system emits a monochromatic wavelength at the germicidal range of 254 nm, ultimately damaging the genetic material of the microorganism (Bolton & Cotton, 2011).

Every time before the collimated beam UV system (Berson, The Netherlands) was used in this study, the light was allowed to warm up for 10 minutes. After warmup and prior to UV irradiation, the UVT% of each water sample was measured with the Sense T254 UV Transmission (%) Photometer (Berson, The Netherlands). Furthermore, a ILT1400 radiometer (International Light Technologies,

USA), which is coupled with a XRL140T254 detector (International Light Technologies, USA), was used to measure the UV lamp's light intensity. Both the intensity (Iavg,λ) and the UVT% (UVT) were used to determine UV exposure times needed for each individual water sample. Equation 1 below indicates the calculation used to determine the average UV light intensity. The value calculated from Equation 1 was used in Equation 2, which calculates the required exposure time to achieve a specific UV dose (Hallmich & Gehr, 2010).

$$I\text{avg,λ } (mW.cm^{-2}) = I0λ \, [1 - ed\ln(\text{UVT}(λ)) - d\ln(\text{UVT}(λ))] \ \ldots \ [1]$$

$$Desired \ dose \ (mJ.cm^{-2}) = Average \ intensity \ (mW.cm^{-2}) \times Exposure \ time \ (s) \ldots \ [2]$$

During this study, the effects of UV doses of 20, 40 and 60 mJ.cm^{-2} were studied. These UV doses were applied by subjecting a 600 mL glass beaker with 153 mL river water to UV irradiation for different exposure times, before removing specific volumes of water needed for microbial analysis after each of the three UV doses were applied. Importantly, as the water sample depth in the beaker decreased after each dose and subsequent test sample removal, the exposure time needed for the next UV dose also decreased. This resulted in calculating an exposure time for each new sample depth. Figure 4.2 below represents a simplified design of the collimated UV system (Berson, The Netherlands) used in this study. This system has also been used previously (Olivier, 2015; Sivhute, 2019). In this study, the glass beaker was placed on a magnetic stirrer in the UV chamber, and agitated during UV irradiation
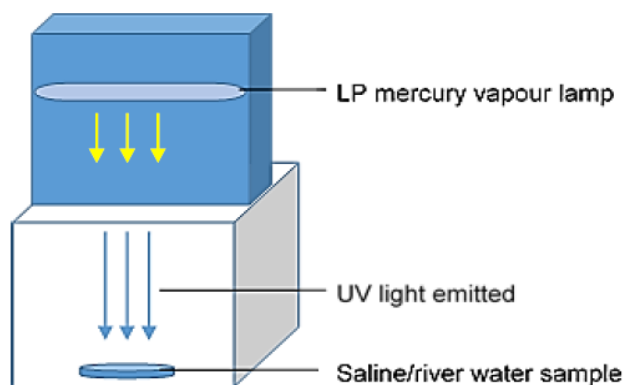
Figure 4.2 Simplified design of the collimated beam UV system (Berson, The Netherlands) used (Olivier, 2015).

4.7 Microbiological analysis of water samples

4.7.1 HPC and TPAC

The HPC was determined using Plate Count Agar (PCA) (Oxoid, South Africa) according to the SANS method 5221 (SANS, 2011). A dilution series was prepared before and after UV radiation, in duplicate. Furthermore, the dilution series was plated, in duplicate, on PCA using the pour plate method. Plates were incubated at 30°C for 72 hours, where straw-coloured colonies between 30 and 300 were counted based on recommendations of SANS 5221 (SANS, 2011) in all samples before UV treatment.

It is acknowledged that using a lower limit of 30 CFU per plate provides the most statistically reliable precision-based count of the actual microbial load at that particular dilution. However, it was accepted in this study that any colonies present at the lowest dilution (10^{-1}) after UV treatment could be indicative of the survival of UV resistant bacteria, even if the exact number or bacterial load can't be statistically determined based on the standard's limits (SANS, 2011). Considering this, all CFU below 300 that were present after UV at the lowest dilution plated (10^{-1}) were counted and indicated in the results.

TPACs were also analysed using PCA (Oxoid, South Africa). The method was followed according to the standard SANS method 5221 (SANS, 2011), with the exception that the plates were incubated at 22°C for 72 hours, instead of the standard 30°C (Holvoet *et al*., 2014). A dilution series was prepared before and after UV radiation, in duplicate. Furthermore, the dilution series were plated, in duplicate, on PCA using the pour plate method. Plates were incubated at 22°C for 72 hours, where straw-coloured colonies between 30 and 300 were counted.

4.7.2 Detection and isolation of presumptive positive ESBL colonies

The ESBL isolation method used in this study was adapted from Zurfluh et al. (2013 & 2015). A 100 mL river water sample was filtered through sterile cellulose nitrate membrane filter with a pore size of 0.45 μm and diameter of 47 mm (Milipore, South Africa). The membrane was then transferred with sterile forceps to 20 mL of sterile BPW and incubated at 37°C for 2 hours. After incubation, 1 mL of the BPW were transferred into 9 mL of EE broth (Oxoid, South Africa), and further incubated for 24 hours at 37°C. After incubation, a loopful suspension of the EE broth was streaked onto selective CHROMagar ESBL plates (MediaMage, South Africa) and incubated at 37°C for 24 hours. Presumptive positive ESBL colonies (pink colour) were picked and streaked onto other CHROMagar ESBL plates (MediaMage, South Africa) to ensure purity of colonies, which were further incubated at 37°C for 24 hours. Pink coloured colonies were then picked and streaked onto VRBGA agar (Oxoid, South Africa) and incubated at 37°C for 24 hours. Purple/pink colonies, indicating presumptive positive *Enterobacteriaceae*, were picked and streaked on non-selective Nutrient Agar, followed by incubation at 37°C for 24 hours. After incubation, straw colour colonies from Nutrient Agar plates were picked and transferred to 5 mL of sterile Tryptic Soy Broth, thoroughly mixed with a vortex, and incubated at 37°C for 24 hours. After incubation, 800 μL of the TSB suspension was transferred to 800 μL of sterile 50% (v.v^{-1}) glycerol solution in a sterile Cryo.s^{TM} Freezing tube (Greiner Bio-one, Austria) and thoroughly mixed. The Cryo.s^{TM} Freezing tube contained a final glycerol stock solution concentration of 25% (v.v^{-1}), which was stored at -80°C until further analysis.

4.7.3 Preparation of presumptive ESBL strains for ESBL confirmation testing

The bacterial stock isolate was removed from the -80°C and defrosted. After reaching room temperature, 20 μL inoculum was suspended in five mL of sterile TSB and incubated at 37°C for 24 hours. After incubation, the TSB-bacterial suspension was streaked onto CHROMagar ESBL plates (MediaMage, South Africa) for phenotypic confirmation. Following confirmation on the CHROMagar ESBL plates, presumptive positive strains were streaked on non-selected Nutrient Agar (Oxoid, South Africa) and incubated for 24 hours at 37°C, inversely. A single colony from each Nutrient Agar plate was transferred to a MacCartney bottle with 25 mL sterile distilled H_2O ($_dH_2O$), to reach an approximate cell density similar to the 0.5 McFarland Standard (BioMèrieux, South Africa). A Spectroquant Prove 600 Spectrophotometer (Merck, South Africa) was zeroed with sterile distilled water before measuring the absorbance of each suspension at 600 nm. Suspensions were adjusted to obtain a final absorbance reading of 0.2 for all suspensions prior to ESBL testing.

4.7.4 ESBL confirmation testing procedure

To test whether *Enterobacteriaceae* strains were ESBL producers, the EUCAST (2021) disc diffusion testing procedure was followed. Mueller-Hinton agar (Oxoid, South Africa) was prepared and inoculated with suspensions of the isolated *Enterobacteriaceae* strains, in duplicate. This was done using a sterile cotton swab, which had been soaked in the respective bacterial suspensions, prior to inoculation. Excess fluid was removed from the swab by pressing it against the sides of the swab tube provided.

According to the inoculation procedure set out by EUCAST (2021), the swab was moved in horizontal, vertical, and diagonal directions to ensure that the surface of the agar plate was completely covered with the inoculum. Following the inoculation procedure, a disc dispenser (Thermo Scientific, South Africa) was used to dispense discs onto each plate, in duplicate.

The discs of ceftazidime [30 μg], cefotaxime [30 μg] and cefepime [30 μg], each individually and in combination with clavulanic acid [10 μg] were used for each strain (Davies Diagnostics, South Africa). Each plate was then inversely incubated at 37°C for 24 hours. After incubation, the zone diameters were measured. EUCAST (2021) indicates that if the inhibition zone diameter of discs containing clavulanic acid are ≥ 5 mm larger than discs with without the clavulanic acid, a strain can be considered an ESBL producer.

4.7.5 Characterisation of bacterial strains using the Oxidase- and Catalase test

The oxidase reaction test was used for the characterisation of bacterial isolates. Although there are many method variations to the oxidase test, the Filter Paper Spot Method was used for this study. The oxidase reagents used was 1% tetra-methyl-*p*-phenylenediamine dihydrochloride (g. V^{-1}), in water. A

disposable loop was used to pick a well-isolated, fresh colony (18-to-24-hour culture) from Nutrient Agar plates (Oxoid, South Africa). The picked colony was rubbed onto filter paper, followed by one to two drops of 1% tetra-methyl-*p*-phenylenediamine dihydrochloride on the bacterial smear. Oxidase positive microorganisms changed to a dark purple colour in about 10 seconds, compared to oxidase negative microorganisms which showed no colour changed.

The catalase test detects the presence of the catalase enzyme, differentiating catalase-positive from catalase-negative microorganisms. The catalase test reagent used was hydrogen peroxide (H_2O_2), as H_2O_2 is broken down by the catalase enzyme, resulting in the formation of bubbles (Ripolles-Avila *et al*., 2018). The Slide (drop) Method (Sharon *et al*., 1978) was used for this study. A well-isolated, fresh colony (18-to-24-hour culture) from Nutrient Agar plates (Oxoid, South Africa) was picked and transferred to a microscope slide with a disposable loop. Using a sterile dropper, one drop of the 3% H_2O_2 was placed on the bacterial smear. The petri dish was covered with the petri dish lid and kept still. For catalase-positive microorganisms immediate bubble formation was observed, compared to catalase-negative microorganism that showed no bubble formation.

4.7.6 Characterisation of bacterial strains using Gram staining

Gram staining is fundamental to the phenotypic characterisation of bacteria. The staining procedure differentiates bacteria according to their cell wall structure. Gram-positive bacteria have a thick peptidoglycan layer which stains dark purple. Moreover, gram-negative bacteria stain pink as they have a thin peptidoglycan layer. The gram stain procedure was followed according to the protocol of Smith & Hussey (2005). A heat fixed smear was prepared by mixing a small amount of a bacterial colony with a drop of water on a microscope slide, followed by heating the microscope slide over a Bunsen burner for two seconds. The slide was flooded with crystal violet staining reagent for one minute, followed by rinsing with tap water for five seconds. The slide was then flooded with Gram's Iodine for another one minute, followed by rinsing for five seconds. The slide was then flooded with a decolorising agent for 15 seconds, followed by rinsing for five seconds. Lastly, the slide was flooded with a counterstain, safranin, for one minute, followed by rinsing. The slide was blotted dry with absorbent paper and observed with a microscope under oil immersion with a 100x magnification. As mentioned, gram-positive bacteria stained dark purple and gram-negative bacteria stained pink.

4.7.7 Identification of bacterial strains using MALDI-TOF

Following the isolation and characterisation procedures, pure environmental strains were prepared for MALDI-TOF analysis. Extracts were prepared with sterile distilled water and HPLC-grade ethanol according to standard procedures (Zvezdanova *et al*., 2020). After preparation, samples were identified with the MicroFlex LT Matrix-Assisted Laser Desorption/Ionisation Time of Flight (MALDI-

TOF) mass spectrometer (Bruker Daltonics, Germany; Zulu, Z. 2020, Researcher, Faculty of Natural and Agricultural Sciences, University of Pretoria, Pretoria).

MALDI-TOF is a technique used to detect and characterise organic molecules based on their mass. In addition, the technique uses a laser to disperse and ionise the sample into different molecules, which results in the molecules moving through an electric field vacuum before being detected by a membrane (Feucherolles *et al*., 2019). Feucherolles et al. (2019) further reported that the time of flight of molecules depends on their electrical charge and mass, which results in specific spectra for each organism. The spectra obtained from the samples were compared to spectra of reference strains in the database of the MALDI Biotyper 3.0 software, to determine each isolate's identity. For each isolate a logarithmic score was obtained that indicated similarity between a reference strain and tested isolate (Zulu, Z. 2020, Researcher, Faculty of Natural and Agricultural Sciences, University of Pretoria, Pretoria).

According to the manufacturer's guidelines, these logarithmic scores are classified into four value ranges that serve as indications of the reliability of the MALDI-TOF identification (Table 4.3) (Zvezdanova *et al*., 2020). A logarithmic score lower than 1.700 would for instance indicate a mixed culture or the absence of reference spectra on the database for the tested isolate. Scores above 2.3 indicate identification is reliable up to species level.

Table 4.3 Logarithmic score interpretations of MALDI-TOF results (Zvezdanova *et al*., 2020)

Log value range	Logarithmic score interpretation
<1.700	No identification possible
1.700 – 1.999	Only genus identification
>2.000	Probable species identification
>2.300	Species identification with high accuracy

4.7.8 Screening for Shiga toxins with Pall GeneDisc STEC Top 7 test

The Pall GeneDisc STEC Top 7 system enables the detection of DNA from Shiga-toxin *E. coli* (STEC) belonging to O-serogroups O26, O103, O111, O145, O45, O121 and O157 using real time polymerase chain reaction analysis. The wells of the disc are preloaded with specific reagents for PCR analysis including internal inhibition controls and specific primers and probes for the detection of virulence genes and *E. coli* Top 7 serogroups (O26, O103, O111, O145, O45, O121 and O157*).* Water samples were enriched in BPW, prior to further testing. Furthermore, non-selective BPW suspensions were

stored in glycerol solution (25% v.v⁻¹) at -80°C, similar to strategy 1 of Chapter 3. These suspensions were resuscitated by defrosting to room temperature, followed by transferring 100 µL of glycerol suspension to 5 mL of sterile BPW (Oxoid, South Africa) tubes. The BPW tubes were then incubated at 37°C for 24 hours.

After incubation, 50 µL of BPW enrichment were transferred into lysis tube (Pall Corporation, France). The lysis tubes were then incubated in a heating block at 102°C ± 2°C. Extracted samples were placed in a fridge at 5°C ± 3°C, until PCR analysis. For storage longer than 6 hours, samples were frozen at -20°C ± 2°C. Figure 3 shows a schematic diagram of the post-sampling process
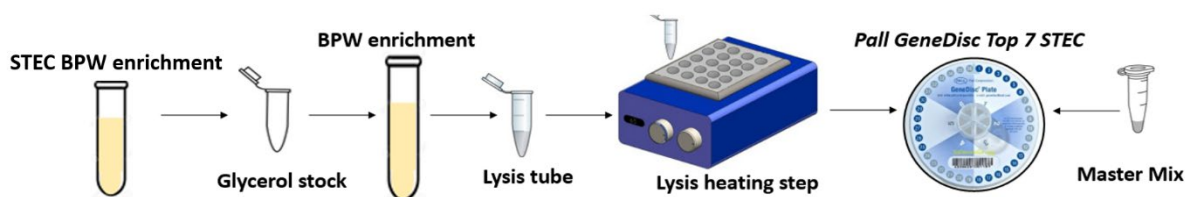
Figure 4.3 Schematic diagram of STEC screening procedure (chapter 3 optimisation)

4.7.9 Statistical analysis

Statistical analysis was performed by using Statistica software (13.5.0). Significant differences ($p<0.05$) were determined by utilising a 95% confidence interval. Statistica was used for the calculation of means, standard deviations and Pearson correlation coefficients. Microsoft Excel 2013 were used for table and graph construction in figures.

4.8 **Results and Discussion**

4.8.1 Objective 1: Characterising the physico-chemical profile of the four selected rivers

During this part of the study, the physico-chemical profile for each of the four selected rivers were determined during three separate sampling occasions. The physico-chemical profile of a river is an indication of the river quality in terms of physical and chemical characteristics, such as pH, TDS, TSS, electrical conductivity and UVT%. In this study, results were compared to the existing Water Quality Guidelines (Table 2) to determine whether the physico-chemical characteristics observed, were within acceptable limits for agricultural irrigation. All the physico-chemical characteristics analysed in this study are recognized indicators of water quality and could also have a significant influence on UV disinfection efficacy. The results of the physico-chemical characteristics are presented in Table 4.4.

As stressed during Chapter 3, the TDS level of water is considered as one of the most important parameters in terms of water quality. Irrigation water with high levels of TDS have shown to negatively affect the water uptake of root systems (Saiyood *et al*., 2012), which could result in fresh produce to perish. Furthermore, TDS provides an indication of other physico-chemical characteristics, as it is directly correlated with turbidity and electrical conductivity (EC) (Kothari *et al*., 2021). This correlation (Kothari *et al*., 2020) was also observed in this study.

As for the results presented in Table 4.4, both the Eerste and Mosselbank river samples exceeded the guideline limit for TDS (Table 4.2) with averages of 341 mg.L^{-1} and 792 mg.L^{-1}, respectively. Even though both exceeded the limit, there was a significant difference (p<0.05) between the Eerste and Mosselbank river samples, as the Mosselbank results were significantly higher. Bursey (2020) has also investigated the TDS values of these two rivers across five sampling rounds and reported average TDS values of 283 mg.L^{-1} and 786 mg.L^{-1} for the Eerste and Mosselbank river, respectively. Bursey's findings (Bursey, 2020) are in line with the findings of this study, indicating the state of these rivers have not changed remarkably over a year. The significantly higher TDS levels observed for the Mosselbank river (between 696 - 865 mg.L^{-1}) could possibly be due to effluents from the WWTP, further upstream from the sampling site. Oraeki et al. (2018) also observed TDS values in the range of 541- 709.5 mg.L^{-1} from wastewater effluent, which is in line with the values observed for the Mosselbank river.

Physico-chemical results

Table 4.4 Physico-chemical characteristics of four selected rivers over three sampling occasions during the summer irrigation period (January 2021 – March 2021)

Characterisitcs	Plankenburg River				Eerste River				Franschhoek River				Mosselbank River			
	1	2	3	Avg. SD	1	2	3	Avg. SD	1	2	3	Avg. SD	1	2	3	Avg. SD
UVT%	71.2	77	71	73.1 2.8	63	69,3	69	67.10 2.90	69.1	73	72.4	71.5 1,7	31.1	39.3	49.1	39.8 7.4
TDS (mg.L^{-1})	98	110	154	120.7 24.1	370	355	298	341 31.02	174	153	202	176.3 20.1	865	815	696	792 70.9
TSS (mg.L^{-1})	8.3	8	12	9.4 1.8	11	7	15	11 3.27	11	7	9	9.0 1.6	21	18	28	22.3 4.2
COD (mg O$_2$.L^{-1})	19	10	32	20.3 9	29	22	30	27 3.56	10	10	13	11.0 1.4	60	51	43	51.3 6.9
pH	7.32	7.55	6.92		7.69	7.44	7.4		7.23	7.09	7.06		7.32	7.47	7.12	
Turbidity (NTU)	2.9	2.3	3.1	2.8 0.34	3.3	3.0	3.0	3.10 0.14	2.8	3.1	2.8	2.9 0.1	11.1	10.2	9.7	10.3 0.6
EC (mS.m^{-1})	0.29	0.16	0.24	0.23 0.05	0.24	0.31	0.32	0.29 0.04	0.15	0.11	0.21	0.16 0.04	0.55	0.74	0.68	0.7 0.1
Alkalinity (mg CaCO$_3$.L^{-1})	46	60	64	55 7.72	120	111	91	107.3 12.12	95	105	120	106.7 10.27	305	290	219	270 37.5

*UVT – ultraviolet Transmittance *TDS – Total Dissolved Solids *TSS – Total Suspended Solids *COD – Chemical Oxygen Demand *EC – Electrical Conductivity

For additional interpretation of the results in Table 4.4, a PCA biplot (Statistica) was produced. As seen below in Figure 4.4, the PCA biplot (Statistica) indicates a positive or negative correlation between the variables of UVT%, TDS, Alkalinity, COD, EC, turbidity and TSS. The PCA biplot suggests a strong positive correlation between the variables of TDS, Alkalinity, COD, EC, Turbidity and TSS. The variable of UVT% shows a strong negative correlation with the other physico-chemical characteristics.
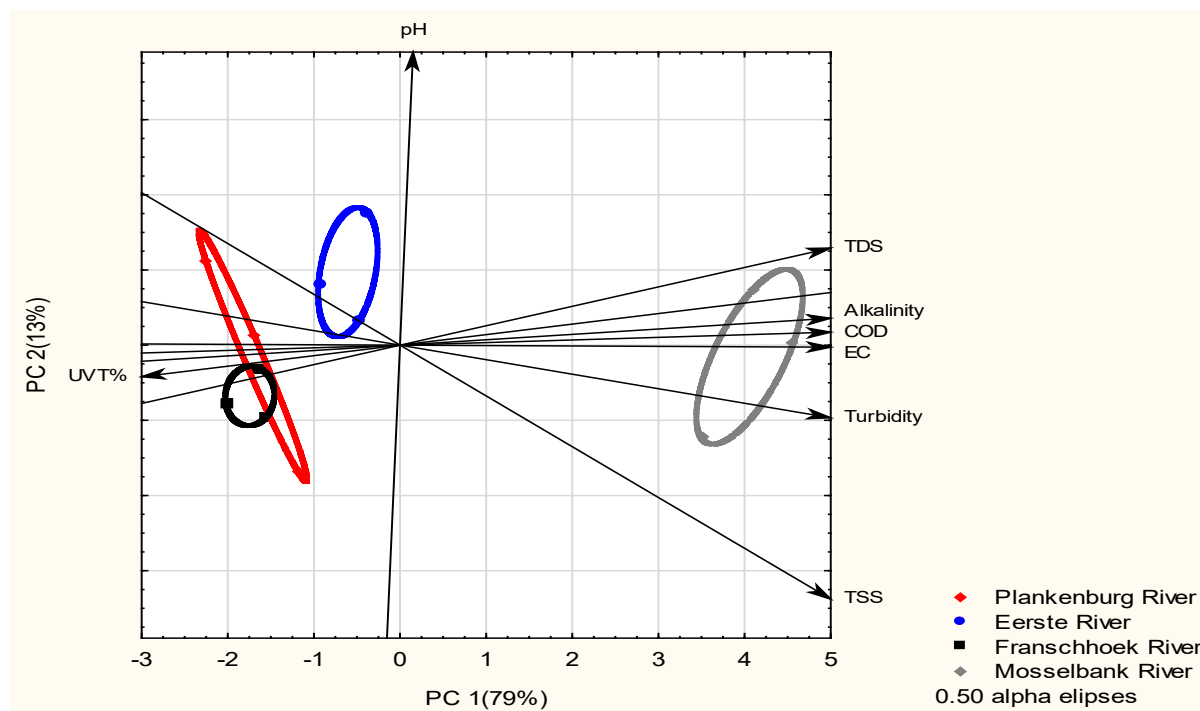
Figure 4.4 PCA biplot (Statistica software 13.5.0) for all physico-chemical characteristics of the four chosen rivers.

The total suspended solids (TSS) content was determined by filtering a specific amount of river water through a 0.6µm glass fibre filter paper after which it is dried for 2 hours at 105°C. As mentioned before, the procedure follows the American Public Health Association (APHA, 2005) standard method for TSS content. The final TSS value represents both suspended inorganic and organic material present in the water sample. The inorganic material present can be a mixture of mineral precipitates, sand and clay (Butler & Ford, 2018).

The Mosselbank river had the highest average TSS, with a value of 22.3 mg.L^{-1} (Table 4.4). The TSS values of Mosselbank river were significantly higher ($p<0.05$) than the other three rivers. There were no significant differences ($p>0.05$) observed between Plankenburg, Eerste and Franschhoek rivers. These findings indicate that water from the Mosselbank river consists of high levels of both suspended and dissolved solids, when compared to the other three river profiles. Of the three remaining rivers, the Eerste river had elevated levels of TDS, although TSS levels were in line with that of Plankenburg and Franschhoek rivers. The Eerste river also had higher alkalinity values when

compared to that of Plankenburg and Franschhoek rivers. This could suggest that the Eerste river's water carried a higher concentration of dissolved salt and minerals, compared to suspended particles.

Although each of the four rivers fall within the TSS limits (Table 4.2), it is generally advisable to lower TSS levels as much as possible before the application of UV technologies. This can be done in larger installations using an in-line bag filter as pre-treatment. In terms of UV disinfection, the presence of suspended solids in water decreases the process efficiency of UV plants (Herraiz-Carboné *et al*., 2021). In addition, it may suggest that microorganisms present in water could be protected as the suspended particles provide shielding from the incoming UV light, resulting in an ineffective treatment.

Turbidity is one of the most used parameters for measuring water quality prior to UV disinfection (Farrell *et al*., 2018). Turbidity causing materials (TCMs) impact UV inactivation through various mechanisms, such as absorption and scattering of incident UV light, and through the shielding of microorganisms (Huber *et al.,* 2011). Johnson et al. (2010) reported that there is a positive correlation between TSS and turbidity, suggesting that increased levels of TSS would indicate higher levels of turbidity. When considering the values of TSS and turbidity, both indicated a positive correlation during this study (Table 4.4). The TSS and turbidity values of Mosselbank river were significantly higher ($p < 0.05$) than the other three rivers. On the other hand, there were no significant difference ($p > 0.05$) between the TSS and turbidity values of Plankenburg, Eerste and Franschhoek river.

The average turbidity value observed at Mosselbank was 10.3 NTU (Table 4.4), exceeding the suggested limit of irrigation water (DWAF, 1996a) of 10 NTU. All other river sites were considerably lower. With regards to UV disinfection efficacy, it is very important to reduce turbidity as much as possible. Liu et al. (2016) indicated that a 33% reduction in the initial UV dose will take place when turbidity is increased from 1-10 NTU. In addition, the scatter of incident UV light is directly proportional to the turbidity measured (Farrell *et al*., 2018). As Mosselbank illustrated the highest turbidity, TDS and TSS values, it could be expected that these values might impact UV irradiation efficacy as some bacteria may be shielded during treatment.

Another important consideration is that low levels of turbidity cannot be linked to low levels of microbial counts (Clark & Bettin, 2006). The Plankenburg river showed relatively low turbidity levels, but it should not be assumed the river water from this sampling site had low microbial loads. It has been previously reported that this sampling site has, in fact, elevated levels of microorganisms (Bursey, 2020: Sivhute, 2019; Britz *et al*., 2013).

UVT% is also considered as one of the most important parameters of water quality, as it measures the amount of light (in percentage) passing through a specific water sample at 254 nm. As

observed for the results collected in Chapter 3, there was a high negative correlation between turbidity and UVT% in this study. The results presented in Table 4 indicated that the UVT% values of Mosselbank river were significantly (p<0.05) lower than the other three river sites. Furthermore, at a 95.0% confidence interval, there were no significant difference (p>0.05) in UVT% between Plankenburg, Eerste and Franschhoek river. This finding can possibly be attributed to the high TDS, TSS and turbidity levels observed at the Mosselbank river, which could have contributed to a significantly decreased UVT%. In support of this observation, Xie & Hung (2020) reported that UVT% will reduce with the increase in TSS and turbidity. In addition, as indicated in Chapter 3, a very high negative correlation was observed between UVT% with TSS and turbidity (Figure 4.4).

As UVT% is often used as the only measurement with regards to water quality for UV treatment optimisation, it was important to investigate the correlation between UVT% and other physico-chemical characteristics. Investigating these correlations will provide valuable information regarding other physico-chemical characteristics when UVT% is the only measurement. With the use of Statistica, a Pearson correlation coefficient were set up between UVT% and the other characteristics, which include TDS, TSS, COD, pH, turbidity, EC, and alkalinity, and the results are presented in Table 4.5. Overall, all physico-chemical characteristics illustrated a negative correlation coefficient, meaning that if UVT% decreases, the other characteristics will increase, and vice versa. These results are in line with the findings of Shahawy et al. (2018).

Table 4.5 Pearson correlation coefficients (Statistica software 13.5.0) between UVT% and other physico-chemical characteristics

Variable 1	Variable 2	Pearson correlation coefficient
UVT%	TDS	-0.97
UVT%	TSS	-0.79
UVT%	COD	-0.93
UVT%	pH	-0.13
UVT%	Turbidity	-0.97
UVT%	EC	-0.87
UVT%	Alkalinity	-0.97

The parameters TDS, COD, turbidity, EC, and alkalinity indicated very strong negative correlations to UVT%, with Pearson correlation coefficients lower than -0.87 (Table 4.5). In addition, TSS and UVT% indicated a Pearson correlation coefficient of -0.79, resembling a relatively strong negative correlation. Furthermore, a very weak negative correlation was observed between UVT% and pH, resulting in a Pearson correlation coefficient of -0.13. The correlation strength was interpreted

according to the article by Akoglu (2018), which provides the guide to correlation coefficients between different variables.

The COD test is based on the principle of a strong oxidising agent under acidic conditions, that fully oxidises all organic compounds in one litre of water to carbon dioxide (Wu *et al*., 2011). The COD value ultimately provides an indication of the available oxygen present in the water sample. The guideline limits for industrial use stated that the COD limit for water should be < 75 mg $O_2.L^{-1}$ (DWAF, 1996b), to be acceptable for use. In this study the Industrial guideline limits were consulted as there is no guideline limits for COD in agricultural irrigation water.

The COD values of the four rivers ranged from 10 to 60 mg $O_2.L^{-1}$ (Table 4.4), indicating notable variation between the different river profiles. Mosselbank river reported the highest single COD value of 60 mg $O_2.L^{-1}$, however, all of the measurements recorded from the four rivers were acceptable, based on the limit chosen for this study (DWAF, 1996b). Furthermore, the COD values of Mosselbank river were significantly higher ($p<0.05$) when compared to the other three rivers. These findings are supported by the observations of Park et al. (2013) that indicated that sites with increased COD concentrations often receive inputs from domestic and industrial wastewaters. The COD values of Plankenburg river were not significantly higher ($p>0.05$) when compared to that of the Franschhoek river. The slight increase in COD levels observed for the Plankenburg river could possibly be attributed to the river also being exposed to untreated domestic wastewater effluents. These effluents could possibly be from the informal settlement and industrial area further upstream. Over the three sampling occasions, the Franschhoek river showed the lowest average COD values, with an average reading of 11 mg $O_2.L^{-1}$. These finding correlated with the results reported by Bursey (2020), which also indicated the that of the four rivers studied, the lowest COD values were observed at the Franschhoek river in 2020.

According to the Irrigation Water Guidelines (DWAF, 1996a), the alkalinity content should be <120 mg $CaCO_3.L^{-1}$ to be acceptable for the use in agricultural irrigation. The alkalinity values measured for the four rivers, over three sampling rounds, ranged from 46 to 305 mg $CaCO_3.L^{-1}$, indicating significantly different alkalinity profiles. The alkalinity values measured at Mosselbank river all exceeded the recommended guideline limit, with an average value of 270 mg $CaCO_3.L^{-1}$ (Table 4.4). The Mosselbank river were significantly higher ($p<0.05$) than any of the other three water sites. As for Eerste and Franschhoek river, there were no significant difference ($p>0.05$) observed over the three sampling rounds, with both rivers having alkalinity values below the guideline limit, on average. Furthermore, Plankenburg river reported the lowest overall alkalinity values, with an average of 55 mg $CaCO_3.L^{-1}$. A positive correlation could be observed between alkalinity and TDS values (Table 4.4), as was also reported by Bursey (2020) and Shroff et al. (2015). With regards to UV disinfection, high

alkalinity content can accumulate on the sleeve of the UV lamp, resulting in intensity fluctuations of the UV lamp (USEPA, 2003).

The pH values obtained during the course of this study ranged from 6.92 to 7.69, indicating minor differences between the four rivers. The Irrigation Water Guideline limit (DWAF, 1996a) for pH, ranges between 6.5 – 8.4, indicating that all four rivers are deemed acceptable for use. As for EC, the Irrigation Water limit (DWAF, 1996a) is 40 mS.m^{-1}, which indicated that the EC values of all water samples (Table 4.4) were well within the guideline limit for irrigation water.

Overall, the results shown in Table 4.4 indicated that the Mosselbank river always showed the poorest water quality across the three sampling occasions. Water from the Mosselbank river had the lowest UVT%, ranging from 31.1 to 49.1%. These low UVT% could probably be attributed to the high TDS, turbidity and alkalinity content recorded at this sampling site. A strong negative correlation was observed between UVT% and TDS, which may indicate that TDS is the main contributor of the low UVT%. In addition, previous studies have indicated the detrimental effects of low UVT% on the UV disinfection efficacy of river water (Olivier, 2015; Bursey, 2020). Furthermore, in terms of UVT%, TDS and alkalinity values, the Plankenburg river proved to have the best profile. In addition, the Franschhoek river provided similar results in terms of UVT% and TSS values, when compared to the Plankenburg. The Franschhoek river indicated the lowest COD values, suggesting the lowest biological matter of the four rivers, possibly suggesting the lowest microbial loads of the four rivers. These observations are in line with the conclusions made by Bursey (2021) regarding the profiles of the same four rivers a year earlier.

4.8.2 Objective 2: Investigating the UV dose response of HPC and TPAC populations from the four different rivers

In this study, microbiological loads were determined of the four selected rivers during the same three sampling rounds. The HPC and TPAC populations present in river water were determined before and after UV disinfection using three different UV doses. The river water samples were exposed to a UV light source through a monochromatic laboratory-scale collimated-beam UV device. As mentioned before, the UV system emits UV light at wavelength in the germicidal range of 254 nm, ultimately damaging the genetic material of the microorganism (Bolton & Cotton, 2011). The three different UV doses applied were 20, 40 and 60 mJ.cm^{-2}. The microbial loads indicated below were determined by preparing standard dilution series (10^{-1} to 10^{-6}), followed with a standard plating method. Microbial plates were incubated at two different temperatures of 22°C and 30°C, to determine the growth of TPAC and HPC populations, respectively. After incubation, colony counts were obtained for each of the river water samples.

The figures 4.5 A – H, indicate the HPC and TPAC populations before and after UV treatment using three separate UV doses and incubation temperatures at 30°C and 22°C, respectively. As indicated in methods, after UV, all CFU's below 300 were counted at the lowest dilution plated (10^{-1}) as an indicator of UV resistant colonies. Although it is acknowledged that statistically reliable counts could only be determined if a lower limit of 30 CFU is adhered to (SANS, 2011), it is also logical to assume that the consistent presence of surviving colonies (between 1- 15 CFU at 10^{-1} dilution) still indicates the limits of UV treatment. In addition, Table 6 below indicate the average log reductions for both HPC and TPAC populations, between the before counts to after 20 mJ.cm^{-2} UV counts.

Fig. 4.5 A   Fig. 4.5 B

148
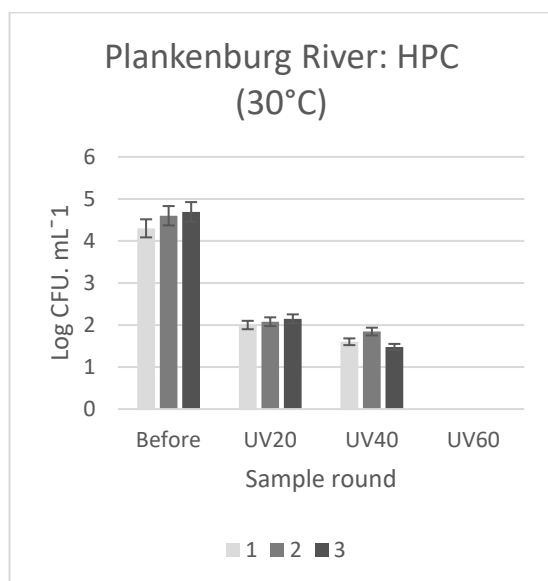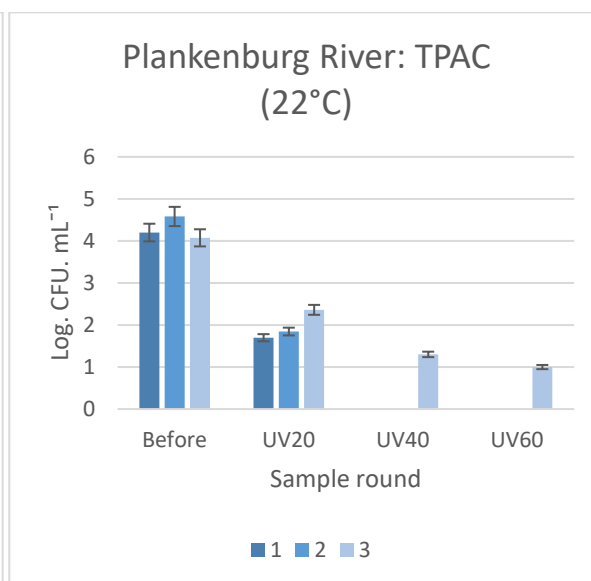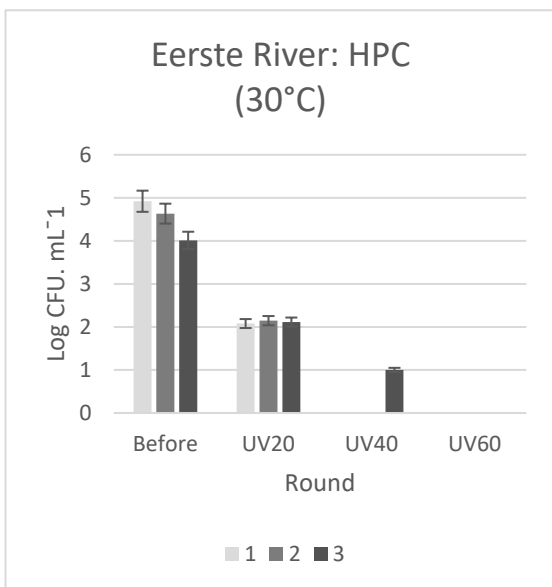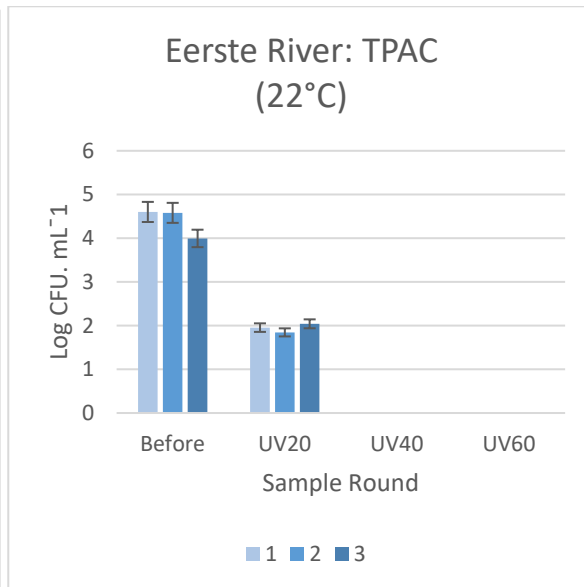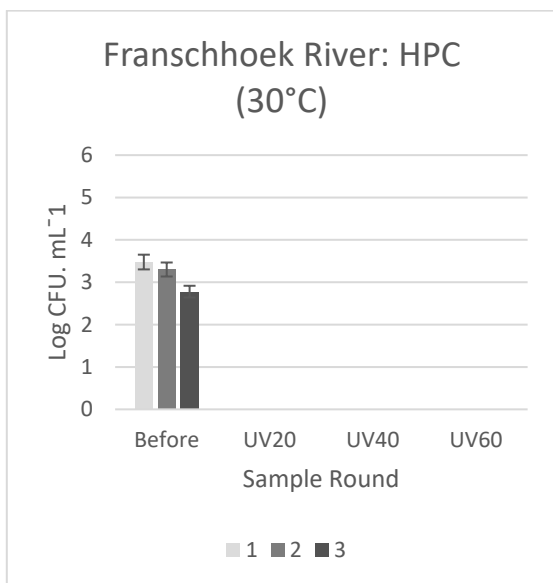
Fig. 4.5 C

Fig. 4.5 D

Fig. 4.5 E
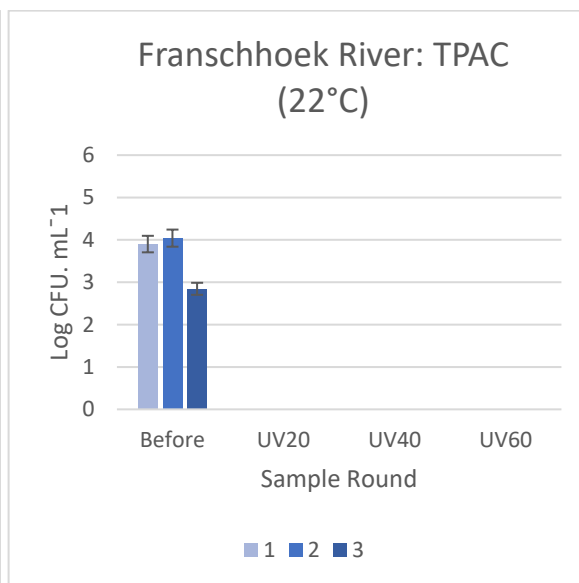
Fig. 4.5 F
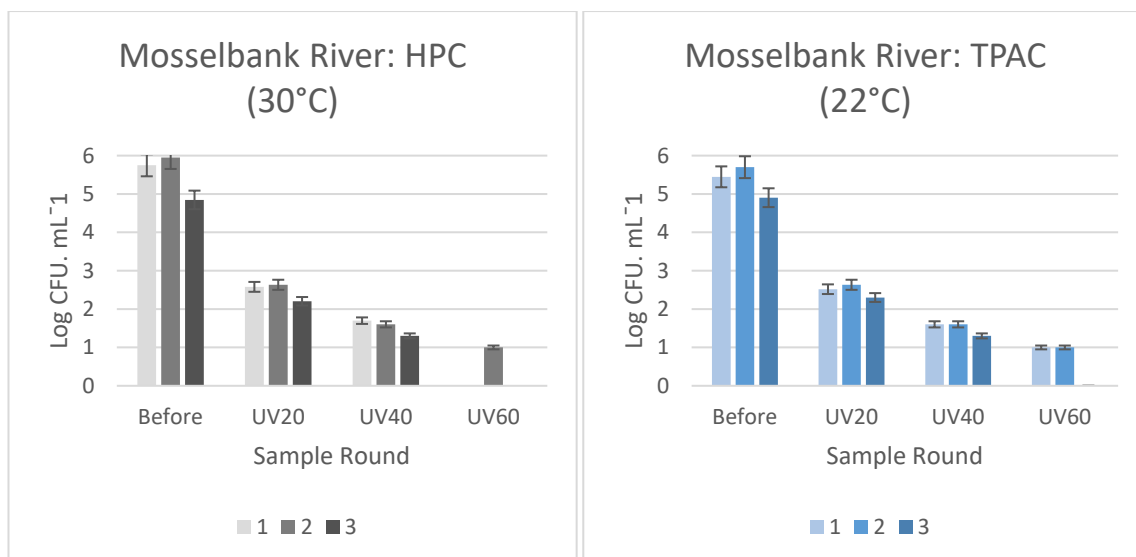
Fig. 4.5 G   Fig. 4.5 H

Figure 4.5 A-H Results of HPC and TPAC population remaining after the exposure to three different UV doses (UV20, 40 & 60 mJ.cm⁻²) and incubation temperatures 30°C & 22°C, respectively. All results expressed as log. CFU.mL⁻¹, with standard deviation error bars included. The detection limit for this method after UV is 1.0 log CFU.mL⁻¹, since all colonies below 300 CFU were counted at the lowest dilution, as motivated in methods.

Table 4.6 Average log reductions illustrated for both the HPC and TPAC populations from the three sampling rounds, between the initial counts to after the first UV dose of 20 mJ.cm⁻², expressed in log CFU. mL⁻¹

Colony counts (Log CFU.mL⁻¹)	River location							
	Plankenburg		Eerste		Franschhoek		Mosselbank	
	HPC	TPAC	HPC	TPAC	HPCA	TPAC	HPC	TPAC
Log reduction Before-After (UV20)	2,41	2,45	2,41	2,45	0	0	3,04	2,87

Bursey (2020) has previously reported that HPC populations from these four selected rivers, survived UV doses of 20 and 40 mJ.cm⁻². The results and findings of the study performed by Bursey (2020) was used as a reference for this study. Bursey's study were also performed approximately 12 months earlier, which added an extra dimension to the current study, as annual fluctuations and the river quality state could be investigated.

When considering the results of this study, it can be noted that the initial HPC and TPAC population loads differ between the four rivers. With regards to the UV radiation applied, reduction in HPC and TPAC population loads were also observed after each of the three UV doses. As seen in Table 4.6, certain log reductions were more gradual than other, it could suggest that the UV dose response differ between microbial populations. Sivhute (2019) noted that the degree of log

reductions can be linked to the microbial population's sensitivity to UV radiation. With regards to the UV treatment efficacy and microbial inactivation, the Franschhoek river proved to be the best-case scenario, compared to the Mosselbank river, which proved to be the worst-case scenario. This supports Bursey's (2020) findings who established that the Franschhoek river indicated the best-case scenario in terms of water quality and UV dose efficacy against the HPC population. In addition, the Mosselbank river also proved to be the worst-case scenario river as HPC populations survived UV doses of 20 mJ.cm⁻² and 40 mJ.cm⁻² frequently (Bursey, 2020).

The highest initial levels of both the HPC and TPAC populations were observed in the Mosselbank river prior to UV disinfection (Fig 4.5G & 4.5H). Not surprisingly, the Mosselbank river also had the highest HPC and TPAC population loads after all three UV doses were applied. In addition, the second round of the Mosselbank river illustrated a log reduction of 3.32, resulting in the biggest reduction from the initial colony count to after a UV dose of 20 mJ.cm⁻². In support of this statement, Bursey (2020) reported that the higher the initial microbial loads were, the higher the UV log reduction may be. Furthermore, the high initial loads could possibly be attributed to the high TDS, TSS, COD, alkalinity and turbidity measurements, as seen in Table 4.4. As mentioned before, TCMs could have impacted UV inactivation of the Mosselbank river through various mechanisms, such as absorptions and scattering of incident UV light along with the shielding of microbial populations (Huber *et al.,* 2011). However, apart from the fact that physico-chemical components can shield the microorganisms from the initial UV dose, the composition and characteristics of the microbial population present in a river should also be considered. Previous studies have not yet considered the composition and characteristics of indicator organisms such as HPC and TPAC populations from these four rivers after UV treatment.

With regards to the other three rivers, the Plankenburg river results showed the growth of a single psychrotroph (TPAC) colony at a 1:10 dilution, after a UV dose of 40 and 60 mJ.cm⁻² (Fig 4.5A & 4.5B). Furthermore, it was observed that at the Franschhoek river, with the lowest initial loads, had no HPC and TPAC populations could be detected after the lowest UV dose of 20 mJ.cm⁻² (Fig 4.5E & 4.5F). As for the Eerste river, some of HPC and TPAC colonies survived a UV dose of UV dose of 20 mJ.cm⁻² (Fig 4.5C & 4.5D). In addition, during three sampling rounds, there was only a single occasion where HPC populations were still observed after a UV dose of 40 mJ.cm⁻² (Fig 4.5C). All these results support the observation that the HPC and TPAC populations differ between the four rivers, and even between different sampling rounds in the same river. In addition, these findings could indicate HPC and TPAC populations have different degrees of UV resistance.

A Least Squares (LS) mean graph (Statistica software 13.5.0) was done to investigate the interaction between log reductions and different UV doses for each of the HPC and TPAC loads of the

four rivers. The LS graphs for HPC and TPAC population loads are presented in Figures 6 and 7, respectively.
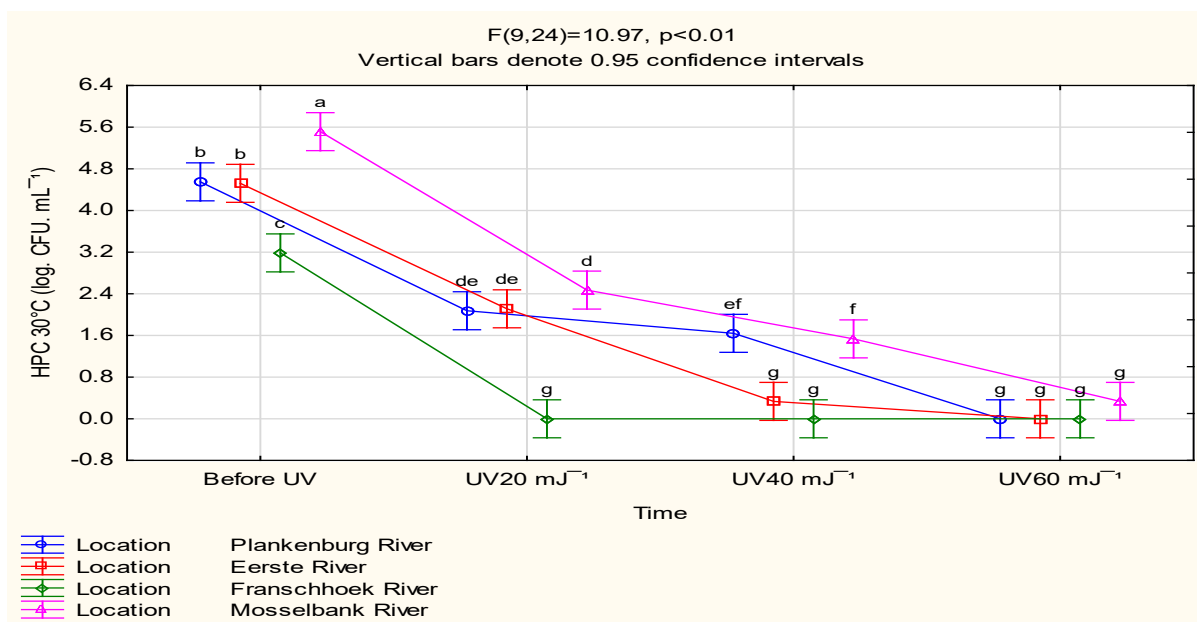
Figure 4.6 LS mean graph (Statistica software 13.5.0) indicating the interaction between log reductions and different UV doses for each of the HPC population loads of the four selected rivers
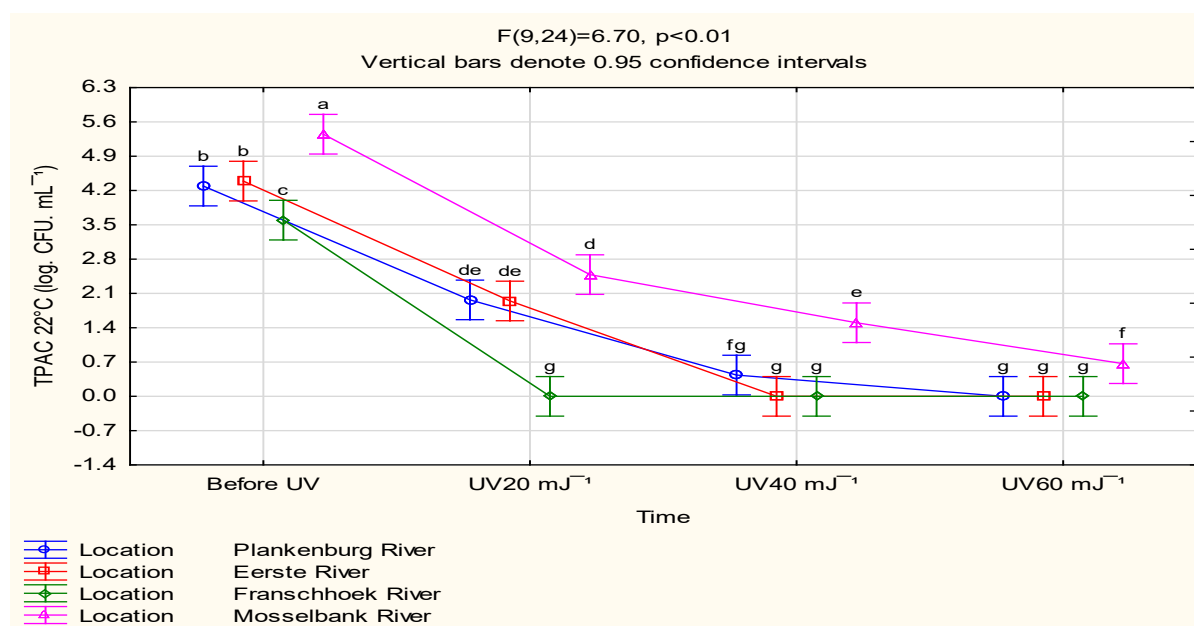
Figure 4.7 LS mean graph (Statistica software 13.5.0) indicating the interaction between log reductions and different UV doses for each of the TPAC population loads of the four selected rivers

There was no significant difference (p>0.05) between the initial HPC and TPAC loads present in the Plankenburg and Eerste rivers (Fig 4.6 & 4.7). The initial HPC and TPAC loads of Mosselbank river were significantly higher (p<0.05) than any other river (Fig 4.6 & 4.7). This observation could possibly suggest why HPC and TPAC populations were still detected after all three UV doses. The Franschhoek

results indicted significantly lower (p<0.05) HPC and TPAC loads (Fig 4.6 & 4.7), when compared to the other three rivers. This observation could possibly suggest why no populations survived after the UV doses, due to loads being significantly lower prior to UV disinfection.

There was no significant difference (p>0.05) in the reductions of HPC and TPAC populations after a UV dose of 20 mJ.cm^{-2} for water samples obtained from the Plankenburg, Eerste and Mosselbank rivers (Fig 4.6 & 4.7). Taking into account the results (Fig 4.6 & 4.7), along with average log reductions (Table 4.6), these findings could suggest that although the Mosselbank river had higher initial numbers, the populations seem to be more sensitive to UV when compared to Plankenburg and Eerste river. In addition, these findings could suggest that the initial levels can be independent of the dose response or UV sensitivity of a particular populations. It can be noted that the higher the initial microbial counts, the higher the chance of survival after a given UV dose. However, population composition and resistance to a certain dose should be considered as well.

Furthermore, with regards to UV dose of 40 mJ.cm^{-2} there were no significant difference in HPC levels for the Mosselbank and Plankenburg river (Fig 4.5A & 4.5G). As for the TPAC populations observed after 40 mJ.cm^{-2}, there were significantly higher (p<0.05) loads measured at the water site of Mosselbank (Fig 4.5H), compared to the levels of the other three rivers (Fig 4.5B, 4.5D & 4.5F). These findings could support the conclusion that the HPC and TPAC populations show great microorganism diversity, each with their unique response to UV radiation.

As mentioned in the previous section, low levels of turbidity do not automatically imply that low microbial loads are present (Clark & Bettin, 2006). This statement can be supported by the findings of this study. Although the Plankenburg river showed relatively low turbidity levels (Table 4.4), ranging from 2.3 to 3.1 NTU, it still had average initial microbial loads higher than 4 log CFU.mL^{-1}.

In addition, these results indicate that the physico-chemical profile of water is important prior to UV disinfection. The Mosselbank river had the worst physico-chemical profile of the four rivers (Table 4.4). Furthermore, the Mosselbank river also had the worst microbial profile of the four rivers (Fig 4.5G & 4.5H). With regards to the initial UV treatment efficacy, log reductions of indicator organisms still occurred.

In this study HPC and TPAC populations were reduced with UV disinfection. All three these rivers reported growth after the lowest UV dose of 20 mJ.cm^{-2}. Franschhoek river, on the other hand, reported no growth after any UV doses and would be considered safe for use after a UV disinfection treatment. It is well established that certain HPC isolates from these four rivers, can withstand specific UV doses (Bursey, 2020). However, with regards to the HPC and TPAC colonies that did survive various UV doses, no research thus far has been performed to identify these microorganisms. Moreover, characteristics of HPC and TPAC colonies obtained after specific UV doses of this study, were isolated

and stored for identification purposes. During objective three of this study, the isolates were characterised and identified.

4.8.3 Objective 3: Characterisation and identification of HPC and TPAC populations and other important pathogens after specific UV doses from the four rivers

In the previous section (Objective 2) it was well established that some members of the HPC and TPAC populations demonstrated UV resistance. The occurrence of UV-resistant HPC populations in these four rivers were in line with the findings of previous research (Olivier, 2015). Plate count agar (PCA) (Oxoid, South Africa), a non-selective agar, was used to determine the total HPC and TPAC populations of river water samples. This testing procedure has its limitations, as HPC testing does not provide information whether the water samples contain pathogenic or non-pathogenic bacteria. Bursey (2020) also stressed this limitation with relation to the HPC findings of her study. From a food safety point, identifying unknown HPC and TPAC colonies that survive UV treatment are crucial to determine the possible health risks associated with treated irrigation water.

Considering these limitations, isolates that illustrated UV resistance during this study (Objective 2), were isolated and further characterised and identified as part of the first section (Objective 3.1) of this objective. The second section of this objective (Objective 3.2) involved the testing of river water samples for the presence of STEC and ESBL- producing organisms before and after UV irradiation.

4.8.3.1 Objective 3.1: Characterisation and identification of HPC and TPAC populations after specific UV doses from the four rivers

For this objective, the HPC and TPAC isolates (n=20) that were previously isolated as part of Objective 2, were resuscitated from glycerol stock solutions stored at -80°C, and re-streaked onto PCA (Oxoid, South Africa), followed by Gram staining. After staining, bacterial slides were observed with a microscope under oil immersion with a 100x magnification to distinguish between Gram-positive (violet cells) and Gram-negative (red cells). In addition, the bacterial strains were tested for oxidase production according to the method of Tarrand & Gröschel (1982). Oxidase positive microorganisms changed to a dark purple colour, compared to oxidase negative microorganisms which showed no colour changes. The bacterial strains were also tested for catalase production using the method of Chester (1979). This testing procedure clearly differentiated catalase-positive from catalase-negative microorganisms. After these microbial characterisation tests, the pure strains were identified with the MALDI Biotyper® system that involves the use of a MicroFlex LT Matrix-Assisted Laser Desorption/Ionisation Time of Flight (MALDI-TOF) mass spectrometer (Bruker Daltonics, Germany) to obtain genus or species identification. The results for all characterisation and identification tests are summarised in Table 4.7, with MALDI-TOF spectra for individual organisms in Appendix B.

Table 4.7 Summary of characterisation and MALDI-TOF results, indicating the microbial species isolated after specified UV doses from HPC and TPAC populations

River location	UV dose (mJ.cm⁻²)	Incubation temperature (22°C/30°C)	Gram staining (+/-)	Catalase test (+/-)	Oxidase test (+/-)	MALDI-TOF Score[a]	Identification	Appendix B spectra
Mosselbank	20	30	+	+	+	1.76	*Exiguobacterium genus*	B6
Eerste	20	22	+	+	+	1.71	*Exiguobacterium genus*	B7
Eerste	20	30	+	+	+	1.85	*Exiguobacterium genus*	B8
Mosselbank	20	22	+	+	+	1.81	*Exiguobacterium genus*	B9
Mosselbank	20	30	+	+	-	1.82	*Bacillus megaterium*	B13
Plankenburg	20	30	+	+	+	1.97	*Exiguobacterium genus*	B10
Plankenburg	20	22	+	+	-	2.11[b]	*Bacillus cereus*	B11
Plankenburg	20	22	+	+	-	2.03	*Bacillus cereus*	B12
Mosselbank	40	30	+	+	-	2.31	*Bacillus megaterium*	B14
Eerste	40	22	-	-	-	2.34[c]	*Aeromonas hydrophila*	B22
Plankenburg	40	22	-	-	-	2.20	*Aeromonas hydrophila*	B23
Mosselbank	40	22	+	+	-	2.32	*Bacillus megaterium*	B15
Plankenburg	40	22	-	-	-	2.32	*Aeromonas hydrophila*	B24
Plankenburg	40	30	-	-	-	2.17	*Aeromonas hydrophila*	B25
Mosselbank	60	22	+	+	-	2.28[d]	*Bacillus megaterium*	B16
Mosselbank	60	30	+	+	-	2.26	*Bacillus megaterium*	B17
Plankenburg	60	22	+	+	-	1.83	*Bacillus megaterium*	B18
Plankenburg	60	22	+	+	-	1.92	*Bacillus megaterium*	B19
Mosselbank	60	22	+	+	-	1.75	*Bacillus megaterium*	B20
Mosselbank	60	30	+	+	-	1.73	*Bacillus megaterium*	B21

[a]MALDI-TOF Score. A logarithmic score lower than 1.70 would indicate a mixed culture or the absence of reference spectra on the database for the tested isolate, score between 1.70 – 1.99 indicate low-confidence identification score between 2.00 – 2.30 indicate high probability species identification and score between 2.30 – 3.00 indicate high confidence species identification.

[b]*Bacillus cereus*. The quality of the spectra (score) depends on the degree of sporulation.

[c]*Aeromonas hydrophila.* Species of this genus have very similar patterns, therefore, distinguishing their species is difficult.

[d]*Bacillus megaterium*. The quality of the spectra (score) depends on the degree of sporulation.

The results presented in Table 4.7 suggest that the HPC and TPAC populations represent a broad field of aerobic microorganisms, which include both Gram-positive and Gram-negative strains alike. It is safe to say that the surviving isolates at the highest UV dose include various *Bacillus* spp., although only one species was identified with high confidence during this study: *Bacillus megaterium. Bacillus* is a spore-forming genus and is commonly found in soil and on plant material across various environments (Mendes *et al.*, 2013).

Nascimento et al. (2020) reported the isolation of several spore-forming *Bacillus* spp. strains from stressful environments. Among these strains was *B. megaterium,* which indicated resistance to heavy metal concentrations and elevated levels of salinity (Nascimento *et al.*, 2020). As for this study, the strains were isolated from the Mosselbank and Plankenburg river, both of which are downstream of industrial and domestic wastewater effluents. This statement can be supported by the site description in Table 4.1, indicating the potential non-point pollution sources these rivers are exposed to.

With regards to physico-chemical characteristics, the EC of water is a function of its chemical salt composition and total salt concentration, ultimately providing a measurement of water salinity (Corwin & Yemoto, 2020). The results obtained from the Mosselbank river showed the highest alkalinity levels (Table 4.4), suggesting high levels of salinity. The results from the Plankenburg river also indicated elevated EC levels (Table 4.4), however, not as high as Mosselbank river. These findings could also possibly support the abundance of these strains at these river water sites, as the stress factors present in these effluents could have contributed to environmental selection pressures in favour of these populations. In addition, Nascimento et al. (2020) indicated that *B. megaterium* can grow at temperatures between 7°C and 45°C. This finding could explain why the strain was detected at both incubation temperatures of 22°C and 30°C for this study.

There is very limited research regarding the UV resistance of *B. megaterium*. It is, however, a well-established fact that spore-forming bacteria in general should undergo significantly longer UV exposure to be deactivated (Clair *et al.*, 2020).

Apart from *B. megaterium,* another spore-forming organism known as *Bacillus cereus* has been identified with a high probability (MALDI-TOF score 2.00-2.3) during this study (Table 4.7). Even though *B. cereus* was only detected in river water from the Plankenburg river after the lowest UV dose of 20 mJ.cm^{-2} was applied, the potential presence of this foodborne pathogen can be important. The pathogenic *B. cereus* can produce toxins in food, resulting in food poisoning (Begyn *et al.*, 2020). Fiedler et al. (2017) reported that *B. cereus* are frequently isolated from various food crops and plants. In addition, Frentzel et al. (2018) noted that vegetables such as carrots, lettuce, cucumbers and salad leaves indicated to be the main carrier of *B. cereus*.

The attachment of *B. cereus* to fresh produce alone would not cause severe illness, although there is a food safety and health risk associated if endospores germinate and produce toxins in food, and survive in the stomach passage (Wijnands *et al.*, 2009). Berthold-Pluta et al. (2015) reported that the ingestion and growth of *B. cereus* in the intestines would not be the main requirement to cause diarrheal infection in humans. Berthold-Pluta et al. (2015) further stressed that the concentrated production and growth of enterotoxins in the area surrounding the epithelial cells (human mucus layer), would be the main cause of the diarrheal type, opposed to the ingestion of the toxin cereulide (intoxication). With regards to the diarrheal type, it is established that a healthy intestinal microbiota significantly prevents vegetative *B. cereus* cells from growing (Berthold-Pluta *et al.*, 2015).

With regards to *Bacillus* spores, it has been shown to be between 20 and 50 times more resistant to UV radiation, compared to vegetative cells (Setlow, 2014). Spores have evolved with various UV resistant characteristics such as dedicated DNA repair mechanisms, modifications in the DNA's UV photochemistry due to specialised proteins and pigments on the outer layer (Begyn *et al.*, 2020; Setlow, 2014).

Furthermore, Begyn et al. (2020) studied the UV-C resistance of *B. cereus* endospores specifically. He reported that if these endospores were repeatedly exposed to UV exposure, the UV-C stress would result in the selection of mutants, yielding more UV-C resistance. Begyn et al. (2020) noted that the exposure of endospores to UV-C (UV dose of 20 mJ.cm^{-2}) for very short periods of a few minutes resulted in increased resistance. Considering that the spores took a short time to gain UV-C resistance, it can be argued that *B. cereus* spores could possibly gain UV resistance after a UV disinfection technique, such as the collimated beam procedure in this study. Furthermore, Begyn et al. (2020) showed endospores can survive UV-C doses of between 96 mJ.cm^{-2} and 107 mJ.cm^{-2}. These findings could suggest that even though *B. cereus* was only isolated after a dose of 20 mJ.cm^{-2} during this study, it has potential to survive higher doses such as UV dose 60 mJ.cm^{-2}.

Another well-known Gram-negative bacterium, *Aeromonas hydrophila,* were detected in the Eerste and Plankenburg river water after UV dose of 40 mJ.cm^{-2} (Table 4.7). As the sampling site of the Eerste is situated downstream of the Plankenburg site, it could possibly explain the occurrence of *A. hydrophila* at both sites. The detection of *A. hydrophila* is in line with previous research, as it has commonly been found in soil and various aquatic environments before (Liu *et al.*, 2016). With regards to the UV resistance of this organism, very limited research has been documented before. However, Colejo et al. (2018) has studied the UV-C inactivation of various Gram-positive and Gram-negative organisms, which included *A. hydrophila.* Colejo et al. (2018) reported 0.19 and 0.24 log$_{10}$ cycles of inactivation for *Aeromonas hydrophila,* after UV doses of 20 mJ.cm^{-2} and 50 mJ.cm^{-2}, respectively.

Very limited information is available with regards to how *Aeromonas hydrophila* express resistance to UV radiation.

MALDI-TOF identification also indicated the presence of another bacterial genus known as *Exiguobacterium* in water from all three rivers after the lowest UV dose of 20 mJ.cm^{-2} (Table 4.7). *Exiguobacterium* is a Gram-positive member of the *Firmicutes* phyla of bacteria (White *et al.*, 2019). Furthermore, *Exiguobacterium* represents two major clades, clade I, which is cold-adapted strains, and clade II, which included strains that range from temperate to hot environments (Gutiérrez-Preciado *et al.,* 2017). The results obtained could suggest that the strains isolated during this study proved to belong to both clade I and II, as growth were observed after both incubation temperatures of 22°C and 30°C, respectively. As the results only identified the isolates to genus level, it could suggest that the isolates include various species within the *Exiguobacterium* genus. In previous studies, isolates of *Exiguobacterium* spp have shown resistance to significant temperature changes (White *et al.*, 2019) along with resistance to high heavy metal levels (Ordonez *et al.*, 2013).

As *Exiguobacterium* indicated resistance to environmental factors such as temperature and heavy metal levels, it could suggest that this genus shows other environmental resistance as well. In addition, Chen et al. (2020) studied the revival characteristics of microorganisms in water, after UV disinfection. During this study, Chen et al. (2020) reported that the Gram-positive *Exiguobacterium* were one of the predominant genera in the effluent water samples after UV radiation. This is in line with the findings of this study, as *Exiguobacterium* were the predominant genera of the river isolates after UV 20 mJ.cm^{-2}.

Very limited research has been done regarding the mechanisms *Exiguobacterium* follow to restore damaged cells. However, Ordonez et al. (2013) stated that *Exiguobacterium* illustrated UV resistance due to powerful enzyme contenders within the bacteria. These enzymes assist with photo-enzymatic repair of the damaged cells (Gutiérrez-Preciado *et al.,* 2017). In addition, Lionard et al. (2012) reported that the carotenoids found in *Exiguobacterium* may shield some of the incoming UV light penetrating the organism. With regards to the UV dose response of *Exiguobacterium*, very limited research has been done. However, Chen et al. (2020) reported that the genus *Exiguobacterium* survived UV doses of up to 26.10 mJ.cm^{-2} but remained in a non-culturable state. Furthermore, it was also shown that the regrowth of *Exiguobacterium* after UV disinfection is significant in the dark phase. White et al. (2019) performed various biochemical tests on selected strains of *Exiguobacterium* and reported that this genus is catalase and oxidase positive. These findings are in line with the results of this study (Table 4.4). From a food safety perspective, the occurrence of *Exiguobacterium* in water is not a concern, however, elevated levels of these microorganisms in disinfected water could be unwanted.

With regards to the MALDI-TOF scores, it was observed that the scores measured for the *Exiguobacterium* genus were remarkably lower than the scores of the three other species detected (Table 4.7). The higher the MALDI-TOF score, the higher the accuracy of species identification of the test (Zvezdanova *et al*., 2020). This indicated that the identification of *B. cereus, B. megaterium* and *A. hydrophila* using MALDI-TOF were more reliable.

In conclusion, it was established that the HPC and TPAC populations include various microbial species, each with their unique response to UV radiation. Furthermore, the proportion of Gram-positive bacteria were significantly higher compared to Gram-negative bacteria. The results obtained, indicated that 80% of the strains isolated after UV disinfection were Gram-positive bacteria. This finding can be supported by previous reports of Chen et al. (2020), who suggested that the bacterial cell wall has an influence on UV resistance. Gram-positive bacteria have one cytoplasmic membrane with a peptidoglycan polymer in multi layers along with a thicker cell wall than Gram-negative strains (Chen *et al*., 2020), making it more resistant to UV radiation.

4.8.3.2 Objective 3.2: UV dose response of STEC and ESBL- producing organisms from four rivers

The aim of this section was to screen water from the four different river sites for the presence of other important organisms such as STEC (Objective 3.2.1) and ESBL-producing *Enterobacteriaceae* in (Objective 3.2.2). The presence of these organisms was also determined before and after river samples were exposed to the same three UV doses (20 mJ.cm⁻², 40 mJ.cm⁻² and 60 mJ.cm⁻²) as applied in Objective 2.

As mentioned in previous chapters, STEC and ESBL-producing *Enterobacteriaceae* are considered important pathogens, which can severely impact consumer health (Isik *et al*., 2020; Pitout, 2012). For these two objectives, detection was based on presence-absence testing that followed separate enrichment protocols (as described in Methods section). A selective chromogenic agar (CHROMagar ESBL) was used for the detection of presumptive ESBL-producing *Enterobacteriaceae*. As concluded in Chapter 3, the STEC isolation strategy using CHROMagar STEC plate (MediaMage, South Africa) was not reliable in the identification of STEC strains. Based on this finding, only the non-selective isolation strategy was followed, (Chapter 3, STEC Strategy 2) in this study, which included the Pall GeneDisc STEC Top 7 test (Figure 4.3).

4.8.3.2.1 Objective 3.2.1: UV dose response of STEC from four rivers

The importance of STEC has been mentioned in the previous chapters. To reiterate, the presence of STEC has been associated with food-borne disease-outbreaks and severe hospitalisation, which is of major public health importance (Paletta *et al*., 2020). Important pathogens such as STEC have been transferred from river water via irrigation systems to fresh produce, ultimately increasing the risk of contamination, influencing fresh produce safety and consumer health (Isik *et al*., 2020).

With regards to the primary sources of STEC strains, literature has reported that farm animals such as cattle, goats and pigs are the main carriers of these organisms. The animal faeces are then spread through agricultural soils and water runoff, ultimately ending in nearby river systems (Iwu *et al*., 2021). From a food safety perspective, this is an important cycle, as contaminated water could be transferred to fresh fruit and vegetables via irrigation. While most major foodborne outbreaks are associated to the O157 serogroup, other important non-O157 strains have also demonstrated the ability to cause serious diseases, such as thrombotic thrombocytopenic purpura, haemolytic-uremic syndrome, and haemorrhagic colitis (Kintz *et al*., 2020). According to Strachan et al. (2002), only a small number of STEC bacteria, less than 100 organisms, can cause illness.

Water from the four sites was screened for the presence of STEC using the Pall Genedisc STEC Top 7 test and a summary of the results, presented in Table 8, indicates the presence and absence of targeted groups (o-serotype, *stx* genes and virulence gene) prior to UV disinfection (0 mJ.cm⁻²). Included in Table 8 are also the results from UV dose of 20 mJ.cm⁻² of Eerste river. The results obtained after UV doses 40 mJ.cm⁻² and 60 mJ.cm⁻² of the four rivers were not included in Table 4.8 as none of the targeted gene groups were detected after UV treatment. The fluorescence spectra obtained during GeneDisc analysis for each of the four rivers (Figures B1-B4) are attached in Appendix B, at the end of the chapter.

Table 4.8 Summary of results indicating the presence/absence of targeted genes (o-serotype, *stx* genes and virulence gene) from the four rivers prior to UV disinfection along with results from UV dose of 20 mJ. cm^{-2} of Eerste river

Targets	UV dose				
	0 mJ.cm^{-2}				20 mJ.cm^{-2}
	Plankenburg (Figure B1)	Eerste (Figure B2)	Franschhoek (Figure B3)	Mosselbank (Figure B4)	Eerste (Figure B5)
O103	Presence	Presence	Presence	Presence	Presence
O111	Presence	Presence	Presence	Presence	Not detected
O145	Presence	Presence	Presence	Presence	Not detected
O157	Presence	Presence	Not detected	Presence	Not detected
O26	Presence	Presence	Not detected	Presence	Not detected
O45-O121	Presence	Presence	Presence	Presence	Not detected
stx1: stx2	Presence	Presence	Not detected	Presence	Not detected
Vir. O111	Presence	Presence	Presence	Presence	Not detected
Vir. O145-O157	Not detected	Presence	Not detected	Not detected	Not detected
Vir. O26	Presence	Presence	Presence	Presence	Not detected
Vir. O45-O103-O121	Presence	Presence	Presence	Presence	Not detected
STEC presence (Yes/No)	Yes	Yes	No	Yes	No

As for the results obtained, presumptive STEC was detected in Plankenburg, Eerste and Mosselbank river water before UV treatment. The results from the Franschhoek river indicated the absence of STEC. The BPW-suspensions before UV treatment of the Plankenburg, Eerste and Mosselbank river showed the presence of STEC strains (*stx*1: *stx*2), whereas the Franschhoek river showed the absence of STEC. Furthermore, the results from the Eerste river, after a UV dose of 20 mJ.cm^{-2}, showed the presence of a non-STEC *E. coli* strain, O103 (Table 4.8). This finding suggests that some *E. coli* strains present in the Eerste river, showed resistance to UV irradiation. The results from Plankenburg, Franschhoek and Mosselbank river did not indicate the presence of any of the seven O-chain specific *E. coli* or STEC strains after a UV dose of 20 mJ.cm^{-2} during this study.

Analysis of the Plankenburg river water indicated the presence of *stx* genes and virulence genes in the presence of O-serogroup sequences related to O26, O111, O45-O121, O145, O157 and O103 (Appendix B, Figure B1). The O-serogroups of O145 and O157 were present without their corresponding virulence gene of Vir. O145-O157 (Table 4.8). Farfan et al. (2012) noted that *E. coli*

strains carrying the *stx* gene alone cannot cause severe illness without the presence of their virulence gene (Vir. O145-O157). This further suggest that O-serogroups of O145 and O157 detected from this sampling site would potentially not cause severe illness.

Even though most common foodborne outbreaks are linked to the O157 serogroup, other important non-O157 strains have also demonstrated the ability to cause serious diseases (Kintz *et al.*, 2020). Therefore, the presence of non-O157 Shiga-producing strains are also important in terms of water quality and food safety. The Plankenburg river results corresponded with the results observed at Plank 2 during the previous chapter. This is not surprising, as the locations were identical, but it also points to the long-term presence of non-O157 STEC at this site, as there was roughly a four-month period between studies.

Water from the Eerste river tested positive for the presence of all targeted genes as all O-serogroups, including O157, were detected, along with all the respective O-serogroup virulence factors and the presence of *stx* genes (Table 4.8 & Fig. B2 in Appendix B). These results are in line with the results obtained at Plank 4 in Chapter three, which is further upstream of the Eerste river site in this study. These findings could be supported by the fact that the Eerste river is exposed to effluents from farm activities and possible exposure to a WWTPs far upstream from the sampling site. In general, WWTP's have been previously identified as potential sources of STEC strains (Mughini-Gras *et al.*, 2018, Pires *et al.*, 2019).

Furthermore, water analyses of the Mosselbank river, which is situated very near a WWTP, have also shown the presence of all O-serogroups and *stx* genes (Table 4.8 & Fig B4). All other virulence genes, apart from O145-O157, have also been detected (Table 4.8). This finding is similar to the Plankenburg river, where O-chain-serogroups of O145 and O157 were also present without their virulence gene. Analysis of the Mosselbank river has consistently shown it to have a very poor physico-chemical and microbial profile (Bursey, 2020). The results obtained in this study are in line with previous findings regarding the microbial profile of this river water site.

In terms of the potential influence that wastewater treatment effluent can have on the microbial quality of rivers, it is interesting to note previous research that reported that most treatment plants can not completely eliminate STEC and other pathogenic *E. coli* strains (Ayaz *et al.*, 2014). Furthermore, in some WWTPs the mixing of animal and human wastewater occurs, resulting in opportunities for various *E. coli* populations to exchange genes (Bibbal *et al.*, 2018).

In contrast to the other test sites, the results from Franschhoek river revealed that some *E. coli* strains did carry *stx*-genes (Table 4.8 & Fig. B3). During the course of the study, the Franschhoek river consistently had the best physico-chemical and microbial profiles. As seen in Objective 2, the Franschhoek site had the lowest HPC and TPAC population counts (Fig. 5E & 5F), compared to the

other three rivers. The Franschhoek river was sampled at the merging point of two rivers, the Berg river and the Stiebeuel river. These rivers are not directly downstream of WWTP or informal settlements as the rivers originates in Franschhoek mountain range.

4.8.3.2.2 Objective 3.2.2: UV dose response of ESBL- producing Enterobacteriaceae from four rivers

The excessive use of antibiotics (ABs) in the animal husbandry and agriculture in past decades, caused a global spread of antibiotic resistance (Almakki *et al*., 2019). The detection of ESBL-producing *Enterobacteriaceae* in river water is of high importance as the increase of antibiotic resistant microorganisms is documented (Almakki *et al*., 2019). Environmental microorganisms illustrating antibiotic resistance are a major concern as these organisms can potentially enter the food chain through contaminated irrigation water used on fresh produce. ESBL-producing bacteria have been widely detected in waters which are downstream of animal farms and WWTPs (Liu *et al*., 2016). Moreover, animal farms may be a possible contributor of ESBL-producing microorganisms in river systems through the farm sewage system (Kar *et al*., 2015).

Effluents from domestic wastewater that enter river water systems have been widely studied for the presence of antibiotic resistant bacteria (ARB) and antibiotic resistance genes (ARGs) (Almakki *et al*., 2019). The disposal of various wastewaters in rivers acts as a contact and exchange location for environmental and human microorganisms, pathogenic and non-pathogenic (Välitalo *et al*., 2017). The mixing of different chemical compounds and microorganisms imposes selection pressure which favours the emergence of antibiotic resistance (Amos *et al*., 2018).

In this study, the presence of ESBL-producing *Enterobacteriaceae* was determined on a presence/absence basis. Table 9 indicates the presumptive positive ESBL-producing *E. coli* and other ESBL-producing *Enterobacteriaceae* colonies isolated during sampling round three of this study, before and after specific UV doses were applied.

Table 4.9 Growth observed on CHROMagar ESBL (MediaMage, South Africa) for presumptive positive ESBL *E. coli* and other *ESBL Enterobacteriaceae* on sample round three for each of the three given UV doses, for all four river locations during January – March 2021. Growth is indicated by '+' and no growth by '-'

Organism	Plankenburg				Eerste				Franschhoek				Mosselbank			
	UV dose (mJ. cm⁻²)				UV dose (mJ. cm⁻²)				UV dose (mJ. cm⁻²)				UV dose (mJ. cm⁻²)			
	0	20	40	60	0	20	40	60	0	20	40	60	0	20	40	60
ESBL *E. coli*	+	-	-	-	-	-	-	-	+	-	-	-	+	-	-	-
ESBL *Enterobacteriaceae*	+	-	-	-	-	-	-	-	+	-	-	-	+	-	-	-

Both ESBL-producing *E. coli* and *Enterobacteriaceae* were phenotypically detected in water from the Plankenburg, Franschhoek and Mosselbank rivers. In contrast, no ESBL-producing strains were observed in water from the Eerste river site. As seen above, none of the initial presumptive ESBL strains survived the specified UV doses, as all organisms were eliminated by the lowest UV dose of 20 mJ.cm⁻². The results obtained from the Eerste river differs from the ESBL *E. coli* and ESBL *Enterobacteriaceae* results on the same river system in the previous chapter. These results could suggest that microbial fluctuations can occur in one river system.

The presumptive positive ESBL-producing *E. coli* and *Enterobacteriaceae* strains isolated from the four river sampling sites were prepared for MALDI-TOF analyses. This analysis was aimed at species confirmation, as the strains demonstrated the correct phenotypical morphologies on CHROMagar ESBL (MediaMage, South Africa). Table 4.10 indicate the species identification results after the MALDI-TOF analyses, with individual spectra presented in Appendix B (B6-B30). In addition, for this study only presumptive *E. coli* colonies were prepared for MALDI-TOF.

As all isolated strains were confirmed to be part of the *Enterobacteriaceae* family, each of the strains were prepared for the ESBL testing procedure. As was discussed in Chapter three, the test involved the microorganism being exposed to β-lactamases inhibitors such as clavulanic acid (EUCAST, 2021) after which inhibition zones were measured to determine whether an isolate was an ESBL producer. Table 4.11 show the results of five river water isolates (pre-UV treatment), after the ESBL testing procedure (EUCAST, 2021). EUCAST (2021) indicates that if the inhibition zone diameter is ≥ 5 mm larger with discs containing the clavulanic acid (CV) alongside disc without the clavulanic acid,

strains are considered ESBL producers. A positive control, *Klebsiella pneumoniae ATCC 700603* and a negative control, *E. coli ATCC 25922*, were also included for each testing round to ensure accuracy of the test. All three river water strains isolated from the Plankenburg, Franschhoek and Mosselbank river were confirmed as ESBL producers.

Table 4.10 Presumptive positive ESBL-producing *Enterobacteriaceae* isolated from the CHROMagar ESBL plates during the third sampling round, were identified using MALDI-TOF analyses

River location	UV dose (mJ. cm^{-2})	Incubation temperature	MALDI-TOF score[a]	Identification	Appendix B spectra
Plankenburg	0	37°C	2.19	*Escherichia coli*[b]	B26
Plankenburg	0	37°C	2.18	*Escherichia coli*	B27
Franschhoek	0	37°C	2.40	*Escherichia coli*	B28
Mosselbank	0	37°C	2.26	*Escherichia coli*	B29
Mosselbank	0	37°C	2.28	*Escherichia coli*	B30

[a] MALDI-TOF Score. A logarithmic score lower than 1.70 would indicate a mixed culture or the absence of reference spectra on the database for the tested isolate, score between 1.70 – 1.99 indicate low-confidence identification score between 2.00 – 2.30 indicate high probability species identification and score between 2.30 – 3.00 indicate high confidence species identification.

[b]*Escherichia coli* spectra is closely related to *Shigella/ Escherichia fergusonii* and not definitely distinguishable at the moment.

Table 4.11 Results obtained with the ESBL testing procedures (EUCAST, 2021) of all *E. coli* isolates pre-UV treatment

River location	Organism	Zone diameter (mm)												ESBL producer
		CPM		CPM/CV		CTX		CTX/ CV		CAZ		CAZ/ CV		
		1	2	1	2	1	2	1	2	1	2	1	2	(Yes/No)
Plankenburg	*Escherichia coli*	19	19	27	24	13	15	20	21	24	23	29	28	Yes
Plankenburg	*Escherichia coli*	17	16	25	21	15	14	20	21	19	19	24	24	Yes
Franschhoek	*Escherichia coli*	14	14	21	19	12	17	24	23	23	19	28	26	Yes
Mosselbank	*Escherichia coli*	22	20	28	28	14	15	21	22	15	21	21	27	Yes
Mosselbank	*Escherichia coli*	24	23	32	29	26	21	32	29	19	30	19	24	Yes
	Klebsiella pneumoniae ATCC 700603	22	21	27	26	20	21	26	26	17	17	24	23	Yes
	Escherichia coli ATCC 25922	32	33	32	33	31	32	32	32	30	31	30	30	No

*CPM – Cefepime, CTX – Cefotaxime, CAZ – Ceftazidime, CV – Clavulanic Acid

The fact that all *E. coli* isolates tested during this study were identified as ESBL producers is concerning. Stoesser et al. (2016) stressed that bacterial species in the environment, that are antibiotic resistant, can be a public health threat. According to the WHO (2019), it is estimated that between 2015 and 2050, 2.4 million people from various countries may die due to diseases caused by antibiotic resistant bacteria. With regards to South Africa, this is a concerning matter, as inadequate infrastructure and sanitation facilities may contribute to the spread of antibiotic resistance and raise the risk of antibiotic resistant infections. However, the lowest UV dose of 20 mJ.cm^{-2} has inactivated ESBL producers detected during this study.

4.9 Conclusion

Overall, it was noted that physico-chemical characteristics vary, as the Plankenburg, Eerste, Franschhoek and Mosselbank river has its own unique profile. The results (Table 4) showed that the Mosselbank river consistently had the poorest water quality across the three sampling occasions. Furthermore, a strong negative correlation was observed between UVT% and TDS (Figure 4.4 & Table 4.5), which may indicate that TDS is the main contributor of the low UVT%. Previous studies have indicated the detrimental effects of low UVT% on the UV disinfection efficacy of river water (Olivier, 2015; Bursey, 2021). These observations are in line with the conclusions made by Bursey (2021) regarding the profiles of the same four rivers.

With regards to Objective two, it is well established that certain HPC isolates from these four rivers, can withstand specific UV doses (Bursey, 2021). The findings made by Bursey (2021) could be supported by the results obtained from this study as part of Objective 2, indicating that HPC populations do show resistance to UV doses up to 60 mJ.cm^{-2}. In addition, TPAC populations also illustrated resistance to UV radiation up to 60 mJ.cm^{-2}, as seen in Objective 2. Furthermore, there was no significant difference between HPC and TPAC populations regarding initial loads and overall UV dose responses.

During Objective three it was clearly recognised that the HPC and TPAC populations include various microbial species, each species with their unique response to UV radiation. Isolates were successfully characterised and identified by MALDI-TOF. The species identified during this study included *B. cereus*, *A. hydrophilia*, *B. megaterium* and *Exiguobacterium* genus (Table 4.7). In addition to these findings, it was noted that the proportion of Gram-positive bacteria were significantly higher compared to Gram-negative bacteria (Table 4.7). The results obtained, indicated that 80% of the isolates found after UV disinfection were Gram-positive bacteria (Table 4.7). These findings were supported by literature as Chen et al. (2020) indicated that UV disinfection response can be influenced by the presence of a cell wall.

Lastly, STEC strains were detected in the Plankenburg, Eerste and Mosselbank rivers during this study, whereas the results from Franschhoek river indicated the absence of STEC (Table 4.8). The presence of STEC is important as it suggests that faecal contamination from animals, predominantly cattle, along with wastewater exposure could have occurred upstream of these river sites. In addition, as these rivers are used as irrigation water sources for fresh produce, it may increase food safety risks, as STEC can possibly be transferred from contaminated river water to fresh fruits and vegetables. There is a food safety risk associated with *E. coli* and STEC, however, UV irradiation of 20 mJ.cm^{-2} during this study proved to eliminate all STEC in rivers, except one 0103 in Eerste River. But during UV dose response tests, the results indicated that some *E. coli* strains survived the lowest UV dose of 20 mJ.cm^{-2}, suggesting STEC strains, in this case from environmental origin, still poses a risk for UV disinfection. Overall, it has been regularly documented that STEC and other *Enterobacteriaceae* were frequently detected and isolated together from river water (Gu *et al*., 2013, Johnson *et al*., 2003 and Straw *et al*., 2013). The findings of this study can support these statements, as presumptive positive STEC and other *Enterobacteriaceae* were regularly reported from the same river water.

Furthermore, results from Plankenburg, Franschhoek and Mosselbank rivers also indicated the presence of ESBL-producing *E. coli* before UV irradiation (Table 4.11). The frequent and misuse of antibiotics in the animal husbandry and agriculture the past decades, caused a global spread of antibiotic resistance among bacteria (Almakki *et al*., 2019). This statement could be supported by the results of this study, as a number of *E. coli* isolates were confirmed as ESBL producers. The detection of ESBL-producing organisms in river water is of high importance since the increase of antibiotic resistant microorganisms has become a serious threat to public health because the ability to treat infectious diseases is compromised (Almakki *et al.,* 2019). In addition, no ESBL-producing organisms survived the three given UV doses (20, 40 & 60 mJ.cm^{-2}) (Table 4.9 & 4.11), similar to the STEC results observed.

These findings could suggest that UV as a disinfection method of STEC and ESBL is effective. However, the physico-chemical profile of different sources may differ, suggesting that UV optimisation in terms of effective pre-treatment and correct UV dose contact time should monitored closely.

4.10 References

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Appendix B

Fig. B1
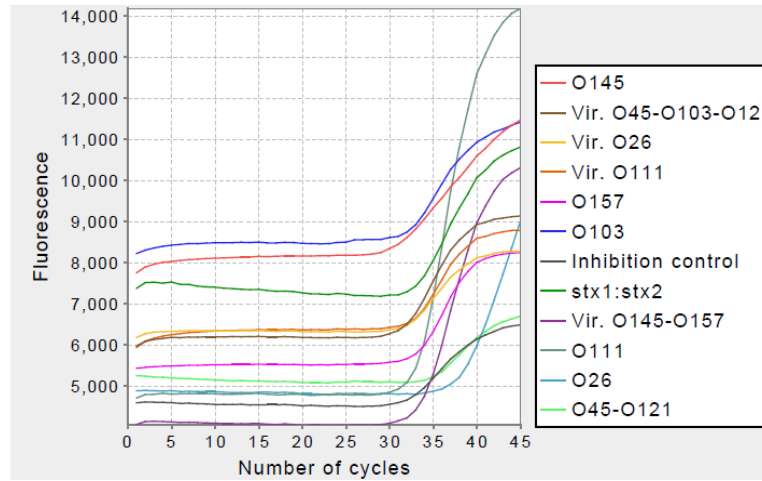
Fig. B2
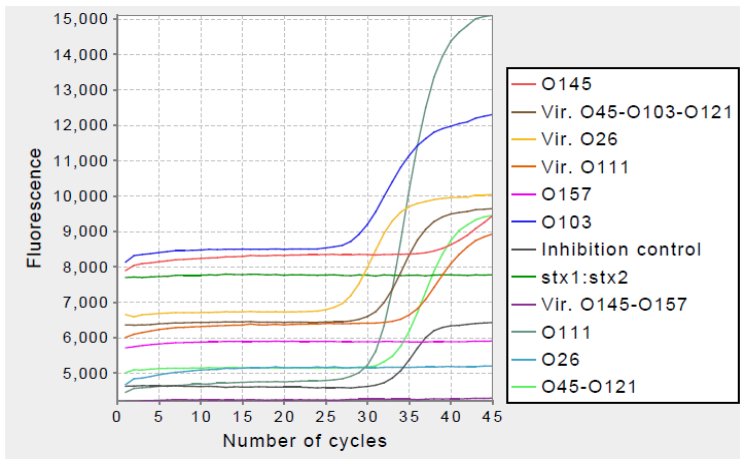
Fig. B3
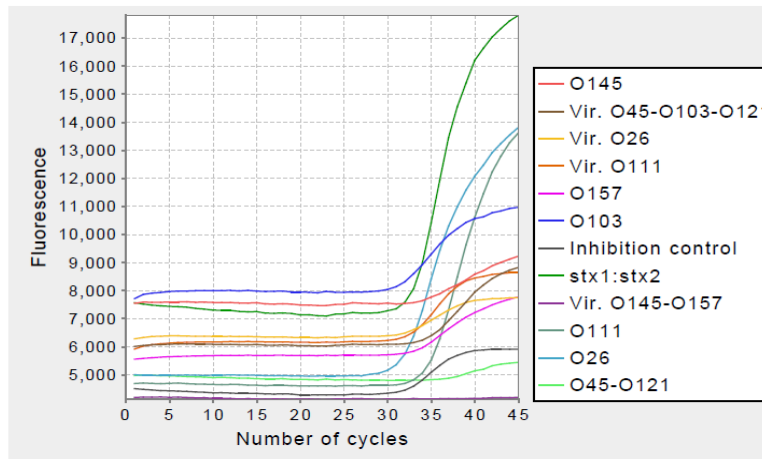
Fig. B4
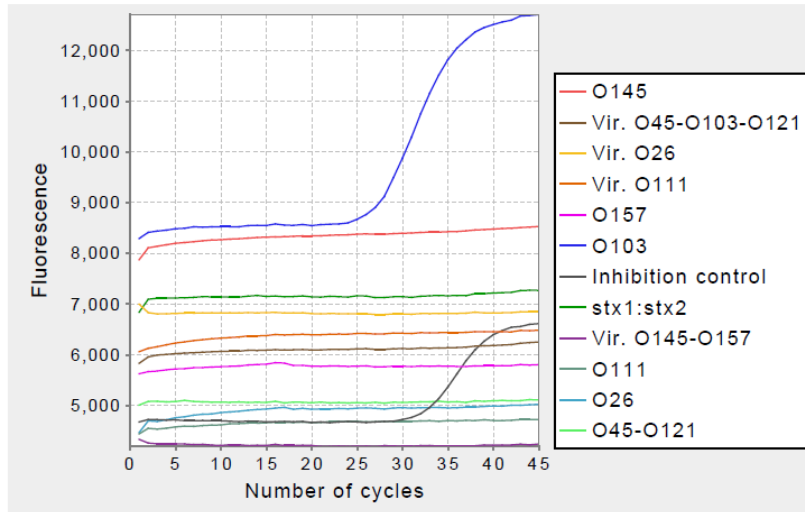
Fig. B5

Figures B1-B5 Representation of the spectra obtained from the Pall GeneDisc STEC Top 7 test results (objective 3.2.1)
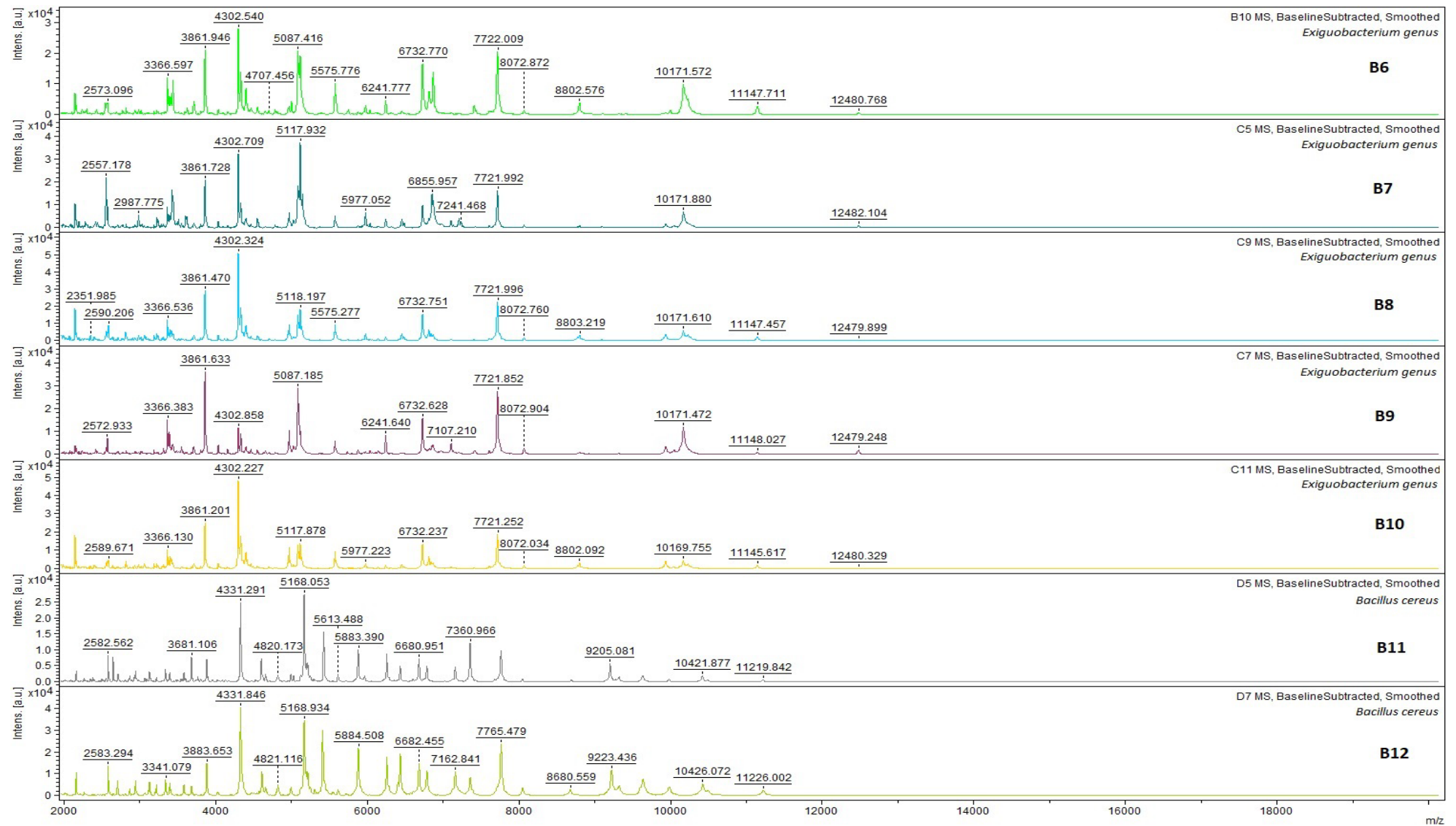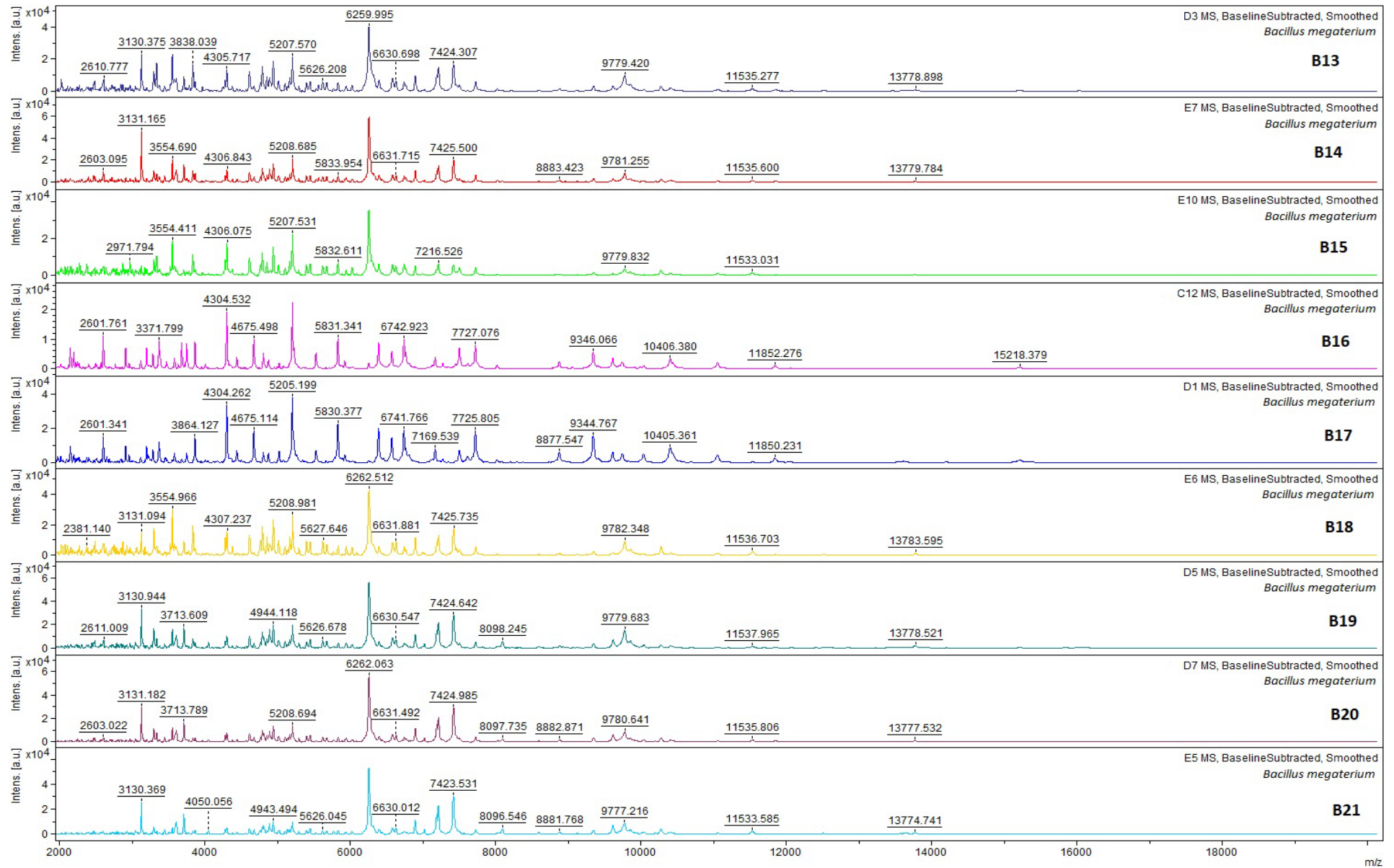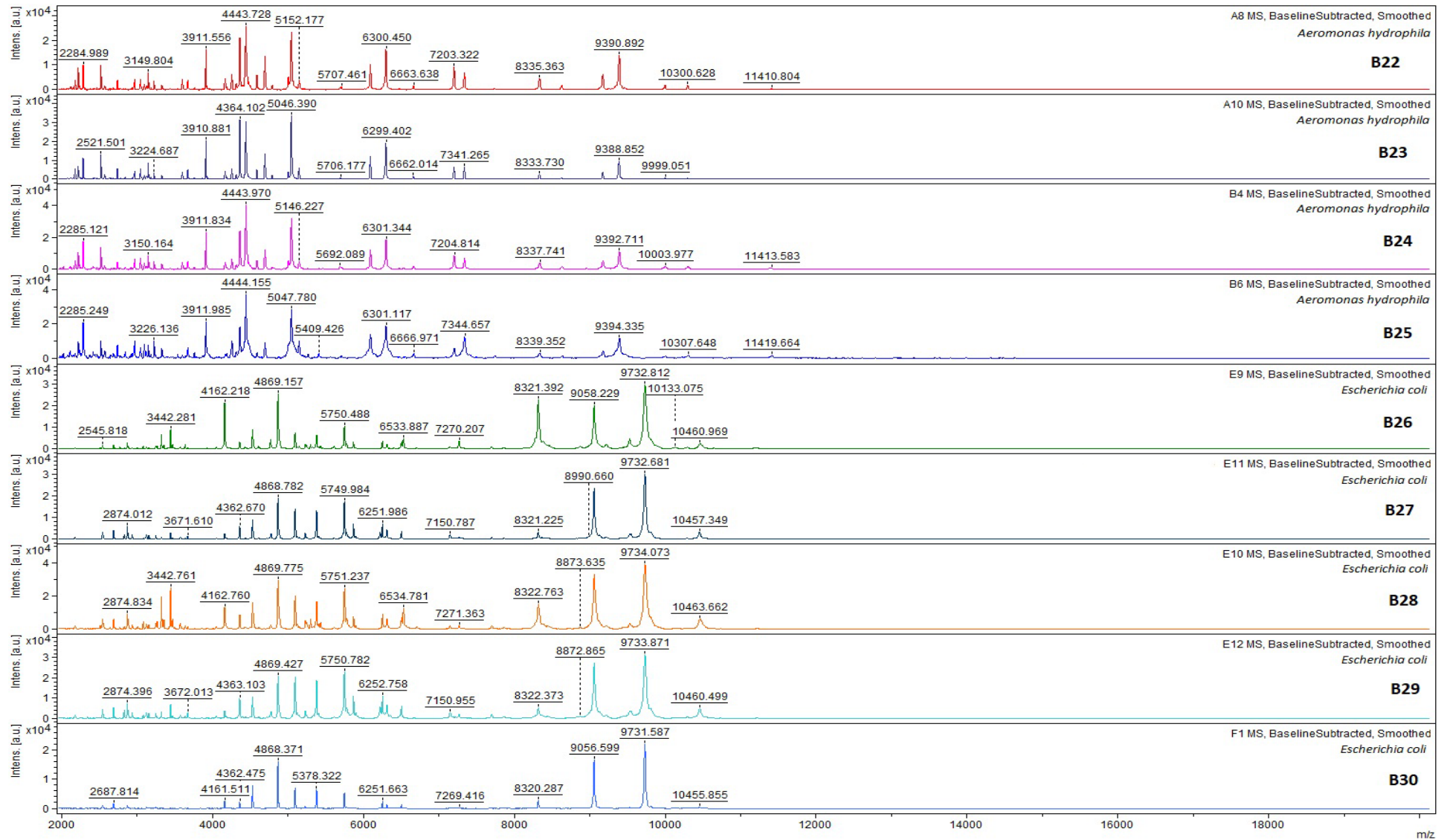
Figures B6-B30 MALDI-TOF spectra of individual organisms detected during Objective 3

CHAPTER 5

THE OPTIMISATION OF A MEDIUM-PRESSURE UV DISINFECTION SYSTEM AT PILOT-SCALE TO TREAT THREE DIFFERENT RIVERS USED AS IRRIGATION WATER SOURCES

5.1 Abstract

This research chapter was performed to optimise a UV disinfection system with a pre-treatment step at pilot-scale, by treating large volumes of rivers (Plankenburg, Franschhoek and Mosselbank river) often used as irrigation water. Four different bag filters (5, 20, 50 & 100 µm) was investigated as a pre-treatment step, prior to medium-pressure UV disinfection, on a pilot plant with the purpose of improving the water quality in terms of physico-chemical and microbial characteristics. The bag filter study was part of a follow-up study, by including a different river profile, to establish the effect of bag filtration on various rivers from a 1 000 L river water sample.

In addition, the effect of a medium-pressure UV system at pilot-scale on the microbiological characteristics were investigated in three different rivers with a 1 000 L river water sample. This study serves as an introduction to the transition from low-pressure, lab-scale UV radiation (Chapter 4) to a medium-pressure, pilot-scale UV system. The microbiological characteristics included the testing for Heterotrophic Plate Count (HPC), *Escherichia coli* (*E. coli*), and coliforms along with the screening of Shiga toxin-producing *Escherichia coli* (STEC) and Extended spectrum beta-lactamase (ESBL) producing *Enterobacteriaceae*. The physico-chemical tests included ultraviolet transmission (UVT), total dissolved solids (TDS), total soluble solids (TSS), chemical oxygen demand (COD), pH and electrical conductivity (EC), along with an anion and cation analysis.

Results from the bag filter study showed slight improvements in the physico-chemical characteristics, specifically with the insoluble characteristics such as the suspended solids. Most dissolved physico-chemical characteristics such as total dissolved solids, pH and electrical conductivity had very little reduction. The improving of ultraviolet transmission percentage (UVT %) was also not remarkable. As for microbiological characteristics, reductions were not expected due to the size of microorganisms, however, when reductions occurred, it was possibly attributed to microbial attachment to larger suspended solids. However, considering the slight improvements associated with the four bag filter options, the results indicated that the smallest pore size of 5 µm was the best option.

As for UV radiation, it was noted that the medium-pressure UV system at pilot-scale is highly dependent on the water quality of the river, especially the physico-chemical profile of the river water sample. The medium-pressure UV system indicated some limitations regarding achievable UV doses with certain river water profiles. A single 20 mJ.cm^{-2} UV dose was applied, followed by a second UV dose of 20 mJ.cm^{-2} for the three rivers. The results showed that *E. coli*, coliforms, STEC, ESBLs and

Listeria monocytogenes were eliminated by 20 mJ.cm^{-2}. The HPC populations showed resistance to both UV doses, and certain surviving Gram-positive and Gram-negative microorganisms were identified. These results and findings would suggest that a follow-up study is performed in order to further optimise a medium-pressure UV system at pilot-scale level.

5.2 Introduction

Contaminated river water used as an irrigation water source for fresh produce on-farm can pose a health risk to consumers and food safety, as there is a risk of microbial transfer. It is therefore important that polluted river water should undergo treatment, such as UV radiation, ultimately reducing the risk of microbial transfer and improving food safety. Various practical factors should be considered when transitioning from a laboratory-scale low-pressure UV system to pilot-scale medium-pressure UV plant. Mahon & Gill (2018) reported that the transition from lab-scale research to pilot-scale for water treatment, such as UV disinfection, remains a challenging procedure.

Along with the transition from lab-scale volumes to pilot-scale volumes, the implementation of medium-pressured UV lamps instead of low-pressure UV lamps might add additional treatment variability (Zimmer-Thomas, 2007). Firstly, the low-pressure UV system at lab-scale uses a monochromatic wavelength, emitting waves of only 253.7 nm (Zimmer-Thomas, 2007). The medium-pressure UV makes use of polychromatic wavelengths, with a broader range from 185 to 1 367 nm, when compared to low-pressure UV (Mofidi *et al*., 2002). Ijpelaar et al. (2010) stated that medium-pressure UV may be more advantageous for a pilot-scale water disinfection system as the output energy and power density is significantly higher, enabling the construction of a compact system. In addition, the broader wavelength range may possibly result in targeting more microbial structures and micro pollutants, resulting in irreversible physiological changes in microorganisms (Zimmer & Slawson, 2002).

Apart from the difference between medium-pressure and low-pressure UV systems, is the effect that water quality may have on UV disinfection efficacy. As Farrell et al. (2018) reported, the ability of the UV treatment to inactivate microorganisms will mainly depend on the quality of the water before the disinfection process commences. The parameters that are commonly associated with water quality include ultraviolet transmission (UVT), total soluble solids (TSS), total dissolved solids (TDS), turbidity, chemical oxygen demand (COD) and particle size (Brahmi *et al*., 2010). When implementing a pilot-scale plant, filtration is required to reduce or eliminate suspended solids, as a preliminary treatment, before proceeding with UV disinfection (Ong *et al*., 2018). Apart from the upscaling factor, the inclusion of a pre-treatment step is important to ensure the most effective UV dose can be applied, by reducing suspended physico-chemical and microbial particles prior to disinfection (Adhikari *et al*., 2019; Farrell *et al*., 2018).

In addition to the effect that water quality may have on the UV efficacy, the operation of the pilot-scale plant itself is also critical. The size of UV plants differs along with the change in pump sizes and flow capacities, resulting in variation of the UV disinfection effectiveness. AbdelRahim et al. (2013) stated UV doses can be increased by reducing the water flow rate, therefore, increasing the contact time for possible pathogens in the water. When considering the flow of water through the pilot plant, lower flow rates may result in more laminar flow which causes less mixing of the water sample, resulting in better disinfection (AbdelRahim *et al*., 2013). In contrast to this statement, Crittenden *et al*. (2003) noted that a reduced water flow rate causes a reduction in UV disinfection as the microorganisms are not appropriately exposed to incident UV rays. With these findings in mind, the correct flow rate and UV dose should be verified when the pilot-scale UV system is investigated, ultimately to achieve the maximum UV exposure with the lowest water flow rate.

The aim of this study was to evaluate the efficacy of a pilot plant UV system to reduce microbial loads in river water to acceptable levels for fresh produce irrigation. This study had two main objectives. Objective 1 was to investigate the effect of a bag filter on the physico-chemical and microbiological characteristics of water from the Plankenburg river. And Objective 2 investigated the effect of a medium-pressure pilot-scale UV system on the microbiological characteristics of three different river profiles.

Objective 1 was a follow-up study to work performed by Bursey (2020). Bursey (2020) previously studied the effect of bag filtration on the Mosselbank river, which is known for its very poor physico-chemical characteristics. During this study, the effect of bag filtration (through 5, 20, 50 and 100 µm pore sized filters) was further explored on the Plankenburg river, which had very poor microbiological characteristics during the course of this study.

For further investigation on the transition from the lab-scale collimated beam system (Chapter 4) to pilot-scale, the implementation of a medium-pressure UV system was studied as part of Objective 2 during this chapter. For this objective, three rivers from the Western Cape with different physico-chemical and microbial characteristics were chosen, to test the UV system's efficacy to reduce microbial levels in water samples with different profiles.

5.3 Research design

During this study, the aim was to optimise the UV disinfection system at pilot-scale, studying the effect that a medium-pressure UV system has on the microbial profiles of three different river sources. The experimental design of this study was structured around two main objectives.

The first objective was to investigate the effect of a bag filter, as pre-treatment to UV disinfection, on water from the Plankenburg river. This objective is a follow-up study to work done by Bursey (2020), which also investigated the effect of bag filtration, but on a different river (Mosselbank). The water quality of the Mosselbank river is of great concern, as some of the worst physico-chemical profiles have been measured at this site (Bursey, 2020). For this follow-up study, the Plankenburg river was chosen as it shows a better physico-chemical profile, when compared to the Mosselbank river (Bursey, 2020). Previous studies have however indicated that the Plankenburg river consistently had high microbial levels, with counts often exceeding recommended limits (DWAF, 1996) (Bursey, 2020; Britz *et al*., 2013, Olivier, 2015). Repeating the bag filtration study on a different river, such as the Plankenburg river, may provide more insight to the effectiveness of bag filtration. In this study four different bag filter pore sizes (5, 20, 50 & 100 µm) were tested, using a 1 000 L volume of the Plankenburg river water, on three different occasions, measuring improvements in the overall physico-chemical and microbial characteristics. Based on the results, one bag filter pore size was chosen for Objective 2.

Secondly, as part of Objective 2, we investigated the disinfection efficacy of a pilot-scale medium-pressure UV system by studying the effect on the microbiological characteristics of three different rivers (Plankenburg river – poorest microbial characteristics, Mosselbank river – poorest physico-chemical characteristics and Franschhoek river – best microbial and physico-chemical characteristics). The choice of these rivers was based on results in previous chapters and work performed by Bursey (2020). Prior to UV radiation, a 1 000 L water sample from each river was pumped through a bag filter (with chosen pore size determined based on the results of Objective 1). After filtration, a UV dose of 20 mJ.cm⁻², first as a single (1 x 20 mJ.cm⁻²) and later as a double dose (2 x 20 mJ.cm⁻²), was applied by the medium-pressure UV system (Berson, The Netherlands) on river water.

As part of Objective 2, standard physico-chemical analysis was performed on samples of all three rivers after bag filter filtration before UV treatment only. The microbiological tests were performed before and after UV radiation and included Heterotrophic Plate Count (HPC), *E. coli*, coliforms, *Listeria monocytogenes* and *Salmonella* species. In addition, microbial colonies that survived UV radiation were identified by MALDI-TOF analysis. Screening for the presence of Shiga

toxin-producing *Escherichia coli* (STEC) and Extended spectrum beta-lactamases (ESBL) before and after UV radiation were also done.

5.4 Materials and Methods

5.4.1 General materials and methods

5.4.1.1 Site selection

Three specific rivers in the Western Cape were selected based on their use for irrigation and known information of the microbial and physico-chemical status that was established in previous chapters of this thesis. Plankenburg river, which is located in Stellenbosch, (33°55'58.50" S 18°51'06.80" E) acts as a control as previous studies have indicated very high microbial loads (Bursey, 2020; Sivhute, 2019). The Plankenburg river has previously been referred to as the worst-case scenario, in terms of microbiological characteristics (Bursey, 2020).

The Mosselbank river site is in the Kraaifontein area (33°49'11.00" S 18°42'10.6" E) situated after a wastewater treatment works (WWTW). This river acts as the irrigation water source for large-scale commercial farmers further downstream (Bursey, 2020). When compared to the Plankenburg river, the Mosselbank river has previously been referred as the worst-case scenario, in terms of physico-chemical characteristics (Bursey, 2020).

The last river, the Franschhoek river in Franschhoek (33°53'56.80" S 19°05'35.30" E), is sampled at the merging point of two rivers, the Berg river and the Stiebeuel river, respectively. Vineyards and large-scale commercial tomato farmers make use the Franschhoek river as irrigation water source (Bursey, 2020). Previous analyses of the Franschhoek have indicated that this river has some of the best physico-chemical and microbial characteristics, compared to three other rivers investigated in the Western Cape (Bursey, 2020). Table 5.1 provides a short description, coordinates for the three river sampling sites, and where additional data regarding these three river sites can be found in the previous chapters of this thesis.

Table 5.1 Three river sampling location names and descriptions of this study

River location	Location description	Coordinates	Additional data about river location (this thesis)
Plankenburg river	Located in Stellenbosch and is downstream of potential non-point pollution sources, including industrial and agricultural activities and effluents from informal settlements	33°55'58.50" S 18°51'06.80" E	Chapter 3 (Table 4, 10, 13 and Figure 6 & 7), Chapter 4 (Table 4,7,8, 10 & 11)
Franschhoek river	Located in Franschhoek and regularly used as irrigation water for vineyards and large-scale farmers	33°53'56.80" S 19°05'35.30" E	Chapter 4 (Table 4, 8, 10 &11)
Mosselbank river	Located in Kraaifontein and situated downstream of a WWTP and regularly used for large-scale farmers	33°49'11.00" S 18°42'10.6" E	Chapter 4 (Table 4, 8, 10 & 11

5.4.1.2 Sample collection method

A utility vehicle with a 1 000 L mobile water tank was used to collect a 1 000 L river water per occasion. The river water was pumped with the use of a Honda WL20XH centrifugal water pump and water pipes with port diameters of 50 mm into the 1 000 L mobile tank.

As part of the first objective, the Plankenburg river was sampled on three separate occasions during August – September 2020 with duplicate samples taken for the before bag filter sample and after each of the four bag filter water samples. For Objective 2, each of the three rivers (Mosselbank, Franschhoek and Plankenburg river) was sampled on one occasion, with 1 L water samples been taken in triplicate at the pilot plant setup, before UV disinfection, after the first UV dose and after the second UV dose, respectively. Furthermore, microbiological tests were performed on the same day of sampling, within six hours of sampling. Physico-chemicals tests were performed the next day, within 24 hours of the sampling.

5.4.1.3 Bag filtration

As mentioned before, bag filtration is used to reduce or eliminate suspended solids in water, as a pre-treatment option for UV disinfection systems (Ong *et al*., 2018). During this study four different pore sized bag filters were investigated (5, 20, 50 and 100 µm) (Darlly Total Filtration Solutions). The bag filter mount is located at the start of the pilot-scale plant, where water from the mobile unit is pumped through the plant. A water sample was taken prior to bag filtration, acting as the 'before' sample. Furthermore, each of the four different bag filters were individually inserted into the system and water was pumped through each filter for a set time, before collecting a water sample after filtration. The water samples (1 x before and 4 x after) were analysed in duplicate for microbial characteristics along with physico-chemical characteristics, with the whole sampling procedure repeated twice for each 1 000L batch of water sampled.

5.4.1.4 Pilot plant operating procedure and UV dosing

In Figure 5.1 an illustration of the pilot plant system is presented, which incorporates both the bag filtration (Objective 1) and UV radiation (Objective 2) steps of river water. As described, 1 000 L of river water was collected from each site with the use of a utility vehicle and a 1 000 L mobile water tank. Upon arrival at the pilot plant, the river water was pumped through a bag filter with a pore size of 5 µm. After filtration, the filtered water flowed into a fixed 1 000 L holding tank at the pilot plant site. This was the first step of the pilot plant UV treatment procedure. The filtered river water was stored overnight before undergoing the UV disinfection the next day.

For the disinfection step, the UV chamber was filled with river water from the holding tank, prior to start up. Once the chamber was filled with water, the UV system was switched on to prevent overheating of the UV lamp. Adjustments were made to the system to set the desired UV dose in

mJ.cm^{-2} and flow rate in m^3.h^{-1}, respectively. Once the UV lamp was warmed up and the desired UV dose was set, the river water was pumped through the UV chamber to facilitate UV radiation of the river water. The UV system has its own instrument measuring the UV dose applied to the water, which measures the UV dose (mJ^{-2}) applied in real-time and indicates this reading on the display screen. As the user sets the system to the required dose (20 mJ.cm^{-2} UV dose was chosen for this study), the system's reading could therefore be used for verification purposes (as the actual dose applied might be different from the dose setting, depending on the water quality and the water flow). In addition, the water flow could be adjusted by the flow control valve on the UV system. Water samples intended for further analyses were taken with sterile 1L Schott bottles at taps situated directly before and after the UV chamber. Triplicate water samples were taken before and after UV radiation, for each 1 000 L batch of river water collected.

As for UV dosing, during Objective 2 of this study it was decided to apply a single and double UV dose of 20 mJ.cm^{-2}. As mentioned, the maximum UV dose that can be applied at a time can be affected by the state of the river water, especially the physico-chemical profile. The Berson EC tronic 'Ω medium-pressure UV system may thus not be able to produce a certain output energy and UV dose with all river water profiles. The inability to provide a once-off high UV dose to water with a low UVT% is a limitation of the Berson EC tronic 'Ω medium-pressure pilot-scale UV system used in this study. To ensure proper dosing, however, lower doses were repeated (e.g., 2 x 20 mJ.cm^{-2} = 40 mJ.cm^{-2}) instead of applying only one high dose (e.g., 1 x 40 mJ.cm^{-2}).
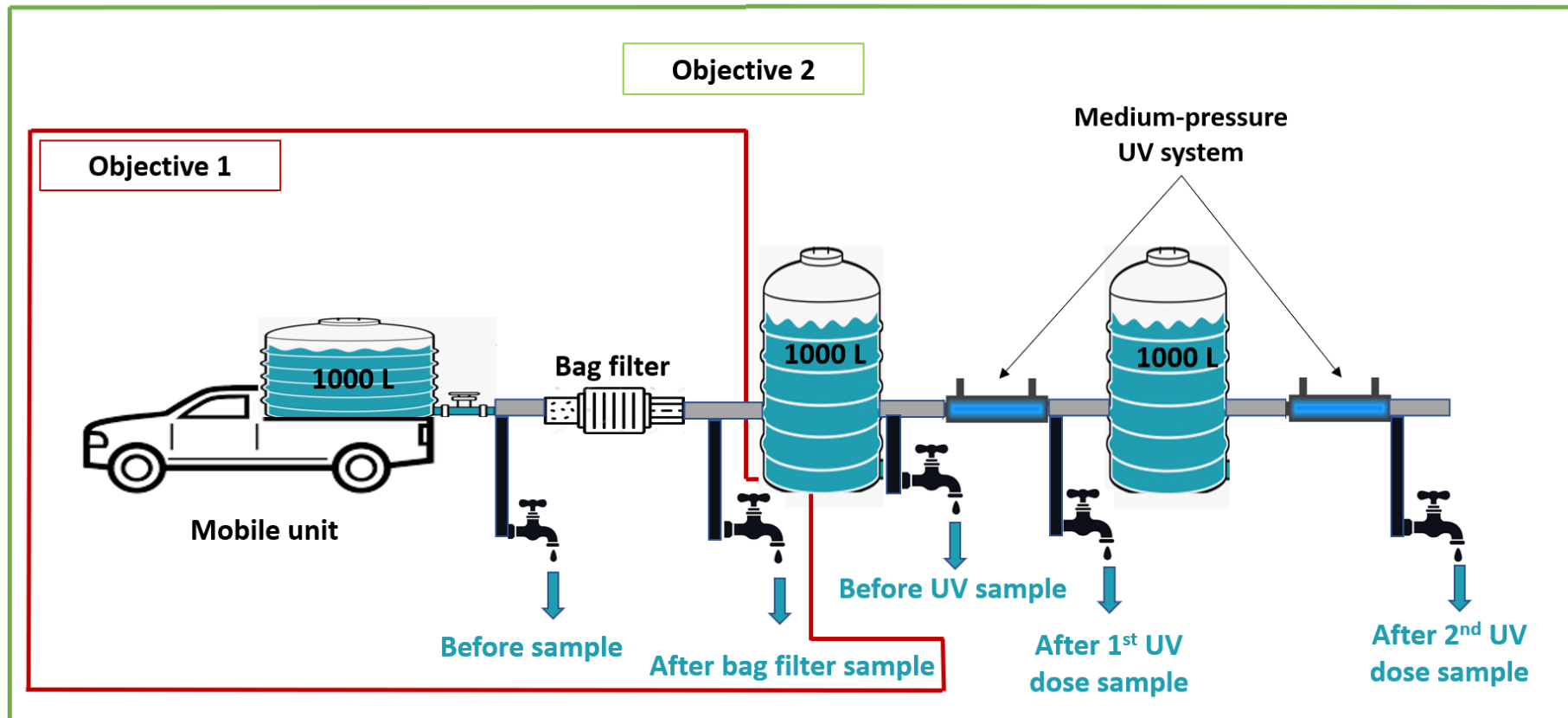
Figure 5.1 Visual illustration of the pilot plant system, with objective one covering the bag filtration and objective two covering both the filtered water and UV disinfection

5.4.1.5 Pilot plant cleaning procedure

After use, the pilot plant procedure, the mobile unit along with all holding tanks and water pipes were disinfected with the use of chlorine dioxide disinfectant (Stericlear, South Africa) with a concentration of 100 ppm. The disinfectant was sprayed along the inside of the tanks and pipes after a sampling round, with the use of 5 L pressure sprayer. The disinfectant was left to dissociate as the chemical (Stericlear, 2021. [Internet Document] URL https://www.stericlear.co.za/oxyfect8500/. Accessed 19/11/2021).

5.4.2 Physico-chemical analysis of river water samples

The procedures mentioned below are similar to the analyses described in the previous two chapters, with the addition of the anion and cation analysis for this research chapter. Table 2 below indicates the suggested limits for physico-chemical characteristics of irrigation water, as set out the South African Water Quality Guidelines (DWAF, 1996a).

5.4.2.1 Ultraviolet Transmission percentage (UVT %)

To determine the UVT% of the water sample a Sense T254 UV Transmission (%) Photometer was used according to the manufacturer's instructions (Berson, Netherlands). Distilled water was used for the calibration of the photometer as it represented a UVT% of 100%. The analysis was performed in duplicate for each sample, after which the average values was determined.

5.4.2.2 Total Dissolved Solids (TDS)

A (TDS)-3 meter (HM Digital) was used to the determine the total dissolved solids (TDS) of the water sample. To determine the TDS measurement, the sum of mobile charged ions is measured by the handheld device, expressed in parts per million (ppm) which corresponds to a reading in $mg.L^{-1}$. The mobile charged ions are also directly proportional to the sample's electrical conductivity. The analysis was performed in duplicate for each sample, after which the average values was determined.

5.4.2.3 Total Suspended Solids (TSS)

The total suspended solids content of each water sample was determined by following the instructions set out by Standards Methods (APHA, 2005). The procedure was performed by filtering the river water sample through a glass microfiber filter (Munktell, Sweden). The clean filter was weighed before filtering the water sample. After filtration the filter was heated in a crucible for 2 hours at 105°C. After heating, the crucible was cooled in a desiccator and weighed again. The weight before filtration and after heating was used to determine the TSS measurement, expressed in $mg.L^{-1}$. The analysis was performed in duplicate for each sample, after which the average values was determined.

5.4.2.4 Chemical Oxygen Demand (COD)

A Spectroquant Nova 60 COD cell test (Merck Millipore, South Africa) was used to photometrically measure the COD of each river water sample. Chemical oxygen demand (COD) refers to the amount of oxygen available in a solution for oxidative reactions to consume. As per the standard testing procedure, three millilitres of each water sample were transferred to a COD cell test (Merck, South Africa). The test cell was thoroughly vortexed and placed in a thermal reactor (Hach, USA) to digest for 2 hours at 148°C. After digestion, samples were cooled to room temperature, followed by measuring the COD values with the Spectroquant NOVA 60 Spectrophotometer (Merck Millipore, South Africa). COD values were expressed in units of mg $O_2.L^{-1}$. The analysis was performed in duplicate for each sample, after which the average values was determined.

5.4.2.5 Turbidity

An Orion AQ3010 Turbidity Meter (Thermo Scientific, USA) was utilised to measure the turbidity of the water samples. The turbidity meter was calibrated with control samples with known turbidity values. Turbidity is expressed in Nephelometric Turbidity Units (NTU). The analysis was performed in duplicate for each sample, after which the average values was determined.

5.4.2.6 pH

A portable pH meter (WTW, Germany) was used to measure the pH of water samples, according to the manufacturer's instructions. Prior to testing, the pH meter (WTW, Germany) was calibrated by using standard pH solutions of pH 7, pH 4 and pH 10. The analysis was performed in duplicate for each sample.

5.4.2.7 Alkalinity

The alkalinity of the water samples was measured according to Standard Methods (APHA, 2005). A solution of 0.1 N H_2SO_4 was prepared and transferred to a glass burette. The solution was titrated into a glass beaker containing 50 mL of a water sample and a pH probe. The titration was performed until a pH of 4.3 was reached. The volume of H_2SO_4 needed to reach a pH 4.3 was used to determine the alkalinity with the use of a standard calculation. Alkalinity is expressed in units of mg $CaCO_3.L^{-1}$. The analysis was performed in duplicate for each sample, after which the average values was determined.

$$\text{Calculation:} \quad \frac{50\,000\ x\ 0.1}{20\ x\ amount\ used\ in\ titration} = x\ mg\ CaCO_3.L$$

5.4.2.8 Electrical Conductivity (EC)

A portable HI 8733 Conductivity Meter (Hanna Instruments, USA) was used to determine the electrical conductivity (EC) of the water samples. The instrument measured the quantity of dissolved salts in the samples to obtain a value in $mS.m^{-1}$. The analysis was performed in duplicate for each sample, after which the average values was determined.

5.4.2.9 Anion and cation analysis

Following the sampling procedure, water samples were prepared and transported to the BIOGRIP Node for Soil and Water Analysis (Stellenbosch University, Stellenbosch), for anion and cation analysis The analysis was performed on a Metrohm 930 Compact Ion Chromatography Flex ove/SES/PP/DEG (Metrohm, Switzerland) (Dr Colling, J. 2021, Manager of Vibrational Spectroscopy, BIOGRIP Node for Soil and Water Analysis, Central Analytical Facility, Stellenbosch University, Stellenbosch).

Table 5.2 Suggested limits for physico-chemical characteristics of irrigation water (DWAF, 1996a)

Water quality characteristics	Irrigation water limit
TDS	260 $mg.L^{-1}$
TSS	50 $mg.L^{-1}$
pH	6.5 – 8.4
Turbidity [1]	10 NTU
EC	40 $mS.m^{-1}$
Alkalinity [2]	< 120 mg $CaCO_3.L^{-1}$
UVT%	Not stipulated
COD [3]	< 75 mg $O_2.L^{-1}$.

[1] No turbidity limits are stipulated for irrigation water (DWAF, 1996a), however the Water Quality Guidelines for Domestic Use (DWAF, 1996c) stated that water with turbidity values > 10 NTU, can potentially carry an associated health risk of disease. Therefore, this guideline was used as reference limit in this study

[2] No alkalinity limits are stipulated for irrigation water (DWAF, 1996a). An alkalinity value of < 120 mg $CaCO_3.L^{-1}$ was selected as guideline limit in this study from the Industrial Water Guidelines (DWAF, 1996b) as crop quality can be affected by alkalinity values above 120 mg $CaCO_3.L^{-1}$.

[3] No COD limits are stipulated for irrigation water used in agriculture (DWAF, 1996a). The Industrial Water Guidelines (DWAF, 1996b) states that acceptable COD levels for land irrigation should be < 75 mg $O_2.L^{-1}$. This guideline limit was used as reference limit in this study.

5.4.3 Microbiological analysis of water samples

As part of this study, the microbiological analysis included monitoring the *E. coli*/ coliform and HPC population counts. For each 1 000 L of river water collected, 3x 'before UV' samples, 3x 'after first UV' samples, and 3x 'after second UV' 1 L samples were taken for microbiological analysis. Furthermore, one dilution series (10^{-1} to 10^{-6}) was prepared per water sample, where each dilution was plated out twice.

In addition, the presence of *Salmonella* species, *Listeria monocytogenes*, STEC and ESBL-producing organisms were also determined before UV, after the first UV dose and after the second UV dose. Here the triplicate water samples, as mentioned above, were pooled together per water sampled, as these tests were based on a presence-absence basis. The procedures included in this chapter are also similar to those previously described in Chapters 3 and 4.

5.4.3.1 Enumeration and identification of *E. coli* and coliforms

Escherichia coli and coliforms was determined using Brilliance *E. coli*/ coliform selective agar (Oxoid, South Africa) according to ISO method 16654. For the dilution series, all test tubes contained sterile buffered peptone water (BPW) (Oxoid, South Africa), as according to SANS method 6887-1 (SANS, 1999). Furthermore, following ISO method 16654 (ISO, 2001), the standard pour plate technique was used using Brilliance *E. coli*/ coliform agar (Oxoid, South Africa), after which plates were incubated at 37°C for 24 hours. Following incubation, presumptive *E. coli* colonies (purple) could be observed from other coliforms (pink colonies). The ISO method 16654 (ISO, 2001) stated colonies between 10 and 30 CFU should be counted, as a limit of 10 CFU per plate will provide the most statistically reliable counts of the actual plate, at the specific dilution. During this study it was accepted that all colonies below 300, and at the lowest dilution (10^{-1}) before and after UV treatment would be counted, as potential UV resistant bacteria can fall below the limit of 10 CFU.

5.4.3.2 Enumeration of Heterotrophic Plate Count (HPC)

The HPC was determined using Plate Count Agar (PCA) (Oxoid, South Africa) according to the SANS method 5221 (SANS, 2011a). A standard dilution series was prepared, followed by plating on PCA using the pour plate method. Plates were incubated at 30°C for 72 hours, where straw-coloured colonies between 30 and 300 were counted based on recommendations of SANS 5221 (SANS, 2011) in all samples before and after UV treatment. The SANS 5221 (SANS, 2011) method stated that using a lower limit of 30 CFU per plate will provide the most statistically reliable precision-based count of the actual microbial load, at that particular dilution. It was however accepted in this study that any colonies present at the lowest dilution (10^{-1}) after UV treatment, could be an indication of UV resistant bacteria, even though the exact number or bacterial load can't be statistically determined based on

the standard's limits (SANS, 2011). With regards to this, it was decided that all CFU below 300, that were present after UV at the lowest dilution plated (10^{-1}), were counted and indicated in the results.

5.4.3.3 Identification of *Listeria monocytogenes*

The presence or absence of *L. monocytogenes* was determined using Rapid'*L.mono* agar plates according to ISO method 11290-1 (2017). Water samples, in duplicate, was enriched in Half-Fraser enrichment broth (Oxoid, South Africa), with the addition of *Listeria* Selective supplement (BioRad, South Africa), and incubated at 30°C for 24 hours. Following incubation, a loopful suspension was streaked onto Rapid'*L.mono* plates (BioRad, South Africa) and further incubated at 35°C for 24 hours. After incubation, presumptive positive colonies were presented as black colonies on the red Rapid'*L.mono* plates. As this test is a presence-absence method, no colonies counts were obtained.

5.4.3.4 Enumeration and identification of *Salmonella* species

The presence or absence of *Salmonella* species was determined using two selective agars, Xylose Lysine Deoxycholate (XLD) agar (Oxoid, South Africa) and Hektoen Enteric agar (Oxoid, South Africa), respectively. The SANS method 19250 (SANS, 2011b) was used for *Salomonella* species enrichment. A 25 mL sample of each water sample (before and after UV radiation) was incubated in sterile BPW (Oxoid, South Africa) at 35°C for 24 hours. After incubation, 0.1 mL of the BPW suspension was transferred to 10 mL sterile, autoclaved Rappaport-Vassiliadis soya peptone (RVS) broth (Oxoid, South Africa and incubated at 42°C for 24 hours. After incubation, a loopful suspension was streaked onto the XLD (Oxoid, South Africa) agar and Hektoen Enteric (Oxoid, South Africa) agar, followed by further incubation at 37°C for 24 hours. After incubation, presumptive positive colonies were observed as red colonies with black centres on the red XLD plates. As for Hektoen Enteric agar, presumptive positive colonies presented as blue-green colonies with black centres. As this test is a presence-absence method, no colonies counts were obtained.

5.4.3.5 Detection and isolation of presumptive positive ESBL colonies

The ESBL isolation method used in this study was adapted from Zurfluh et al. (2013 & 2015). A 100 mL river water sample was filtered through sterile cellulose nitrate membrane filter with a pore size of 0.45 μm and diameter of 47 mm (Milipore, South Africa). The membrane was then transferred with sterile forceps to 20 mL of sterile BPW and incubated at 37°C for 2 hours. After incubation, 1 mL of the BPW were transferred into 9 mL of EE broth (Oxoid, South Africa), and further incubated for 24 hours at 37°C. After incubation, a loopful suspension of the EE broth was streaked onto selective CHROMagar ESBL plates (MediaMage, South Africa) and incubated at 37°C for 24 hours. Presumptive positive ESBL colonies (pink colour) were picked and streaked onto new CHROMagar ESBL plates (MediaMage, South Africa) to ensure purity of colonies, which were further incubated at 37°C for 24

hours. Pink coloured colonies were then picked and streaked onto VRBGA agar (Oxoid, South Africa) and incubated at 37°C for 24 hours. Purple/pink colonies, indicating presumptive positive *Enterobacteriaceae*, were picked and streaked on non-selective Nutrient Agar, followed by incubation at 37°C for 24 hours. After incubation, straw colour colonies from Nutrient Agar plates were picked and transferred to 5 mL of sterile Tryptic Soy Broth, mixed, and incubated at 37°C for 24 hours. After incubation, 800 µL of the TSB suspension was transferred to 800 µL of sterile 50% (v.v⁻¹) glycerol solution in a sterile Cryo.s™ Freezing tube (Greiner Bio-one, Austria) and thoroughly mixed. The Cryo.s™ Freezing tube contained a final glycerol concentration of 25% (v.v⁻¹) and was stored at -80°C until further analysis.

5.4.3.6 Preparation of presumptive ESBL strains for ESBL confirmation testing

Each bacterial stock isolate was individually removed from -80°C and defrosted. After reaching room temperature, 20 µL inoculum was suspended in five mL of sterile TSB and incubated at 37°C for 24 hours. After incubation, the TSB-bacterial suspension was streaked onto CHROMagar ESBL plates (MediaMage, South Africa) for phenotypic confirmation. Following confirmation on the CHROMagar ESBL plates, presumptive positive strains were streaked on non-selected Nutrient Agar (Oxoid, South Africa) and incubated for 24 hours at 37°C, inversely. An inoculum was prepared by transferring a single colony from each Nutrient Agar plate to a MacCartney bottle with 25 mL sterile distilled H_2O ($_dH_2O$), to reach an approximate cell density similar to the 0.5 McFarland Standard (BioMèrieux, South Africa). A Spectroquant Prove 600 Spectrophotometer (Merck, South Africa) was zeroed with distilled water before measuring the absorbance of each suspension at 600 nm. Suspensions were adjusted to obtain a final absorbance reading of 0.2 for all suspensions prior to ESBL testing.

5.4.3.7 ESBL confirmation testing procedure

To confirm whether *Enterobacteriaceae* strains were ESBL producers, the EUCAST (2021) disc diffusion testing procedure was followed. Mueller-Hinton agar plates (Oxoid, South Africa) was prepared and inoculated with suspensions of the isolated *Enterobacteriaceae* strains, in duplicate. This was done using a sterile cotton swab, which had been soaked in the respective bacterial suspensions, prior to inoculation. Excess fluid was removed from the swab by pressing it against the sides of the swab tube provided.

According to the inoculation procedure set out by EUCAST (2021), the swab was moved in horizontal, vertical, and diagonal directions to ensure that the surface of the agar plate was completely covered with the inoculum. Following the inoculation procedure, a disc dispenser (Thermo Scientific, South Africa) was used to dispense discs onto each plate, in duplicate.

The discs of ceftazidime [30 µg], cefotaxime [30 µg] and cefepime [30 µg], each individually and in combination with clavulanic acid [10 µg] were used for each strain (Davies Diagnostics, South

Africa). Each plate was then inversely incubated at 37°C for 24 hours. After incubation, the zone diameters were measured. EUCAST (2021) indicates that if the inhibition zone diameter of discs containing clavulanic acid are ≥ 5 mm larger than discs with without the clavulanic acid, a strain can be considered an ESBL producer.

5.4.3.8 Characterisation of bacterial strains using the Oxidase- and Catalase test

The oxidase reaction test was used for the characterisation of bacterial isolates. Although there are many method variations to the oxidase test, the Filter Paper Spot Method was used for this study according to procedure performed by Tarrand & Gröschel (1982). The oxidase reagents used was 1% tetra-methyl-*p*-phenylenediamine dihydrochloride (g.v⁻¹), in water. A disposable loop was used to pick a well-isolated, fresh colony (18-to-24-hour culture) from Nutrient Agar plates (Oxoid, South Africa). The picked colony was rubbed onto filter paper, followed by one to two drops of 1% tetra-methyl-*p*-phenylenediamine dihydrochloride on the bacterial smear. Oxidase positive microorganisms changed to a dark purple colour in about 10 seconds, compared to oxidase negative microorganisms which showed no colour changed.

The catalase test detects the presence of the catalase enzyme, differentiating catalase-positive from catalase-negative microorganisms. The catalase test reagent used was 3% hydrogen peroxide (H_2O_2), as H_2O_2 is broken down by the catalase enzyme, resulting in the formation of bubbles (Ripolles-Avila *et al*., 2018). The slide (drop) method (Sharon *et al*., 1978) was used for this study.

5.4.3.9 Characterisation of bacterial strains using Gram staining

Gram staining is fundamental to the phenotypic characterisation of bacteria. The staining procedure differentiates bacteria according to their cell wall structure. Gram-positive bacteria have a thick peptidoglycan layer which stains dark purple. Moreover, gram-negative bacteria stain pink as they have a thin peptidoglycan layer. In this study the Gram stain procedure was followed according to the protocol of Smith & Hussey (2005).

5.4.3.10 Identification of bacterial strains using MALDI-TOF

Following the isolation and characterisation procedures, pure environmental strains were prepared for MALDI-TOF analysis. Extracts were prepared with sterile distilled water and HPLC-grade ethanol according to standard procedures (Zvezdanova *et al*., 2020). After preparation, samples were identified with the MicroFlex LT Matrix-Assisted Laser Desorption/Ionisation Time of Flight (MALDI-TOF) mass spectrometer (Bruker Daltonics, Germany; Zulu, Z. 2020, Researcher, Faculty of Natural and Agricultural Sciences, University of Pretoria, Pretoria).

MALDI-TOF is a technique that uses a laser to disperse and ionise an organic sample into different molecules, which results in the molecules moving through an electric field vacuum before

being detected by a membrane (Feucherolles *et al*., 2019). Feucherolles et al. (2019) further reported that the time of flight of molecules depends on their electrical charge and mass, which results in specific spectra for each organism. The spectra obtained from the samples were compared to spectra of reference strains in the database of the MALDI Biotyper 3.0 software, to determine each isolate's identity. For each isolate a logarithmic score was obtained that indicated similarity between a reference strain and tested isolate (Zulu, Z. 2020, Researcher, Faculty of Natural and Agricultural Sciences, University of Pretoria, Pretoria).

According to the manufacturer's guidelines, these logarithmic scores are classified into four value ranges that serve as indications of the reliability of the MALDI-TOF identification (Table 5.3) (Zvezdanova et al., 2020). A logarithmic score lower than 1.700 could for instance indicate a mixed culture or the absence of reference spectra on the database for the tested isolate. Scores above 2.3 indicate identification is reliable up to species level.

Table 5.3 Logarithmic score interpretations of MALDI-TOF results (Zvezdanova et al., 2020)

| Log value range	Logarithmic score interpretation
<1.700	No identification possible
1.700 – 1.999	Only genus identification
>2.000	Probable species identification
>2.300	Species identification with high accuracy

5.4.3.11 Screening for Shiga toxin producers with Pall GeneDisc STEC Top 7 test

The Pall GeneDisc STEC Top 7 system enables the detection of DNA from Shiga-toxin *E. coli* (STEC) belonging to O-serogroups O26, O103, O111, O145, O45, O121 and O157 using real time polymerase chain reaction analysis. The wells of the disc contain specific reagents for PCR analysis including internal inhibition controls and specific primers and probes for the detection of virulence genes and *E. coli* Top 7 serogroups (O26, O103, O111, O145, O45, O121 and O157*).*

In this study, water samples were enriched in BPW after which these non-selective BPW suspensions were stored in glycerol solution (25% v.v⁻¹) at -80°C until further analysis, similar to Strategy 1 of Chapter 3. These suspensions were resuscitated by defrosting to room temperature, followed by transferring 100 µL of glycerol suspension to 5 mL of sterile BPW (Oxoid, South Africa) tubes. The BPW tubes were then incubated at 37°C for 24 hours.

After incubation, 50 µL of BPW enrichment were transferred into lysis tube (Pall Corporation, France). The lysis tubes were then incubated in a heating block at 102°C ± 2°C. Extracted samples were

placed in a fridge at 5°C ± 3°C, until PCR analysis. For storage longer than 6 hours, samples were frozen at -20°C ± 2°C. Figure 5.2 shows a schematic diagram of the post-sampling process.
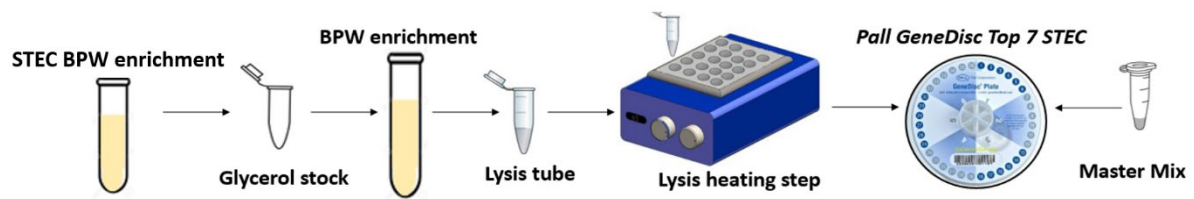
Figure 5.2 Schematic diagram of STEC screening procedure (Chapter 3 optimisation)

5.4.4 Statistical analysis

Statistical analysis was performed by using Statistica software (13.5.0). Significant differences ($p < 0.05$) were determined by utilising a 95% confidence interval. Statistica was used for the calculation of means, standard deviations and Pearson correlation coefficients. Microsoft Excel 2013 were used for table and graph construction in figures.

5.5 Results and Discussion

5.5.1 Objective 1: The effect of a bag filter on the physico-chemical and microbiological characteristics of water from the Plankenburg river.

The main purpose of physical treatments, including filtration techniques, is the reduction of suspended solids, certain biological and chemical compounds (Momba *et al*., 2008). Establishing a pre-treatment step may improve the efficacy of UV disinfection. With regards to previous research done on Western Cape rivers, Sivhute (2019) and Bursey (2020) have stressed the importance of implementing a pre-treatment step, such as bag filtration, prior to UV disinfection on pilot-scale. Sivhute (2019) and Bursey (2020) observed that river profiles vary, as certain rivers have higher solids and turbidity levels when compared to others, suggesting the importance of implementing a pre-treatment before UV irradiation, in order to improve disinfection efficacy. Adhikari et al. (2019) reported that ensuring proper efficiency of any UV system for water disinfection, water quality indicators such as physico-chemical parameters should be improved by pre-treatment filtration. Bag filtration could possibly reduce certain solids present in river water, which may be larger than the specific filter pore sizes. Okpara et al. (2011) noted that the effectiveness of bag filtration as a pre-treatment method may be limited by the pore size of the filter.

During this study, the effect of a bag filtration system was determined by comparing the variations in physico-chemical and microbial characteristics before and after filtration with four different bag filters (5, 20, 50 and 100 µm pore sizes). In this study, the Plankenburg river was sampled on three separate sampling occasions The effects of filtration on the physico-chemical characteristics are presented in Table 5.4. Furthermore, Figures 5.4, 5.5 and 5.6 represent the microbial results obtained for the HPC, *E. coli* and coliforms, respectively, before and after filtration.

Table 5.4 Physico-chemical analyses done on water from the Plankenburg river, before and after bag filtration on each of the three sampling occasions during the months of August 2020 – September 2020

|			Plankenburg river																	
	Before				100 µm				50 µm				20 µm				5 µm			
Characteristics	1	2	3	Avg. / SD	1	2	3	Avg. / SD	1	2	3	Avg. / SD	1	2	3	Avg. / SD	1	2	3	Avg. / SD
UVT%	9.2	52.2	31.6	31.0 / 21.50	9.2	52.2	32.1	31.2 / 21.51	9.2	52.5	32.5	31.4 / 21.67	9.4	53.2	33	31.9 / 21.92	10.9	53.8	33.3	32.7 / 21.45
TDS (mg.L^{-1})	245.0	354.0	311.0	303.3 / 54.90	246.0	361.0	304.0	303.7 / 57.50	247.0	358.0	306.0	303.7 / 55.53	242.0	352.0	298.0	297.3 / 55.00	257.0	351.0	301.0	303.0 / 47.03
TSS (mg.L^{-1})	115.3	7.3	57.3	59.9 / 54.04	104.7	7.3	46.3	52.8 / 49.02	101.8	5	46.7	51.2 / 48.55	101.3	3.7	45.3	50.1 / 48.97	88.7	3.7	40	44.1 / 42.65
COD (mg O$_2$.L^{-1})	55.0	12.0	29.0	32.0 / 21.65	31.0	16.0	25.0	24 / 7.54	42.0	13.0	26.0	27 / 14.52	34.0	24.0	22.0	26.7 / 6.42	44.0	16.0	19.0	26.3 / 15.37
pH	7.3	7.6	7.4		7.3	7.6	7.4		7.4	7.6	7.4		7.3	7.7	7.3		7.4	7.4	7.5	
Turbidity (NTU)	129.0	14.8	33.0	58.9 / 61.35	130.0	13.9	31.6	58.5 / 62.55	129.0	12.1	31.5	57.5 / 62.64	124.0	11.4	30.6	55.3 / 60.23	119.0	11.6	30.1	53.6 / 57.41
EC (mS.m^{-1})	0.35	0.39	0.35	0.36 / 0.02	0.35	0.41	0.35	0.37 / 0.02	0.35	0.41	0.36	0.37 / 0.03	0.35	0.41	0.35	0.37 / 0.03	0.35	0.41	0.35	0.37 / 0.03
Alkalinity (mg CaCO$_3^{-1}$.L)	71.0	116.0	127.0	104.6 / 29.67	61.0	108.0	121.0	96.6 / 31.56	68.0	111.0	128.0	102.3 / 30.92	67.0	101.0	128.0	98.7 / 30.56	68.0	107.0	127.0	100.7 / 30.00
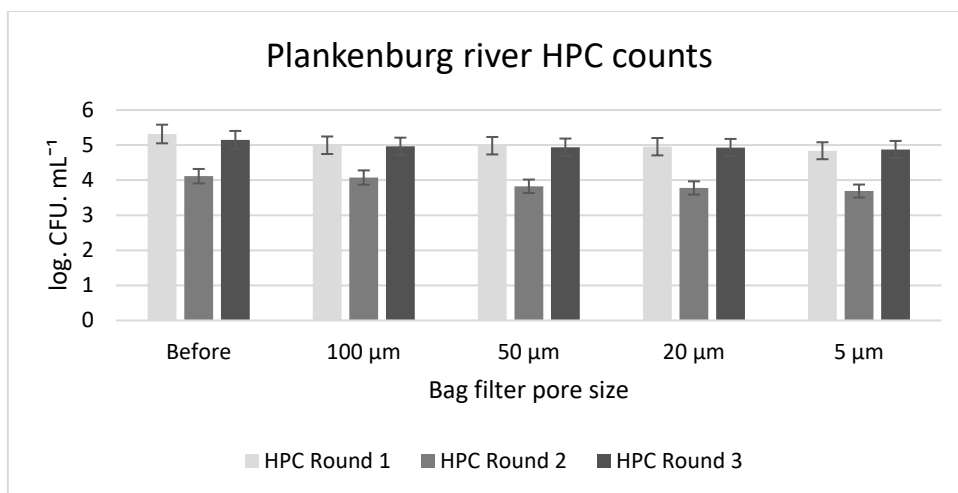
Figure 5.3 HPC counts expressed in log CFU. mL before and after various bag filters, with error bars indicating standard deviation across thee sampling occasions.
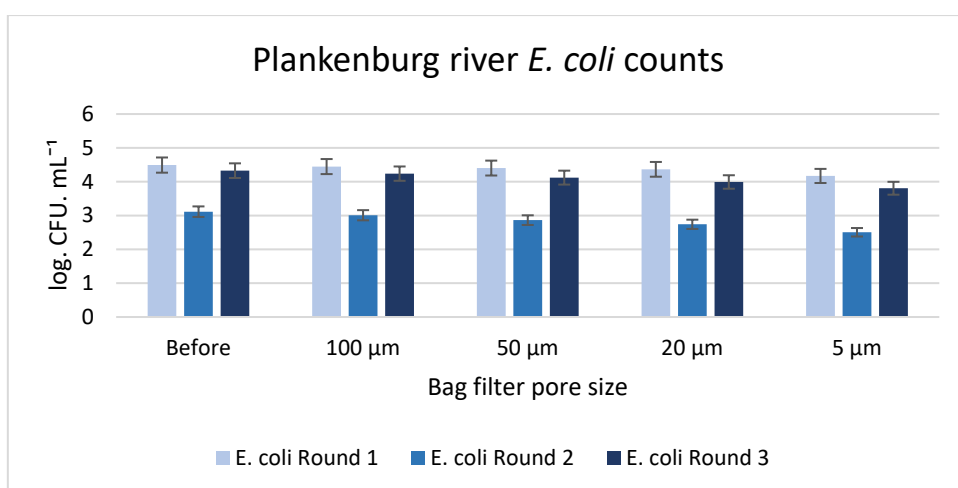
Figure 5.4 *E. coli* counts expressed in log CFU. mL before and after various bag filters, with error bars indicating standard deviation across thee sampling occasions.
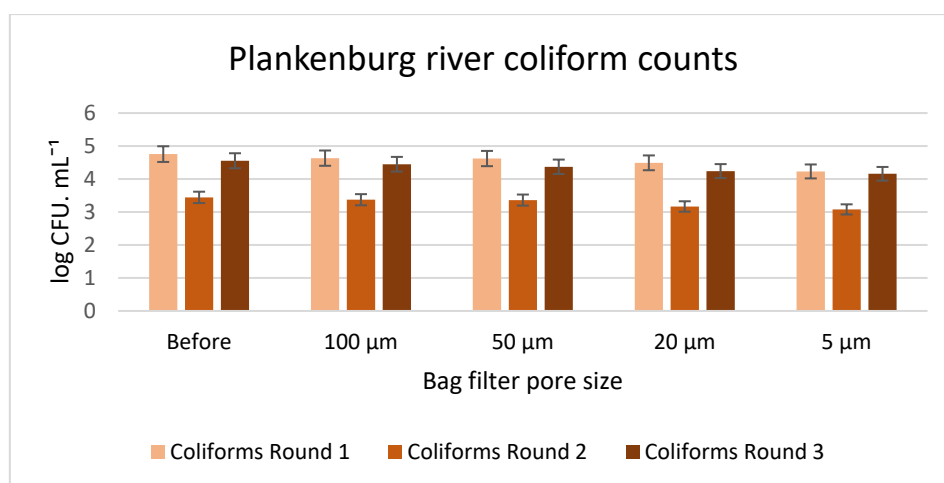
Figure 5.5 Coliform counts expressed in log CFU. mL before and after various bag filters, with error bars indicating standard deviation across thee sampling occasions.

As seen in Table 5.4, various physico-chemical characteristics were considered in this study. However, with regards to the bag filtration, it was observed that not all physico-chemical characteristics were equally affected by bag filtration. It was, for instance, expected that the dissolved solids content will not change significantly. In this study, dissolved solids content influenced the physico-chemical characteristics measured as part of EC, TDS and pH. With regards to the treatment of dissolved solids, specifically TDS, more complex techniques such as ion exchange pre-treatment and reverse osmosis would be needed to reduce TDS levels along certain ions (Dong *et al*., 2020). Gayán et al. (2012) reported that the physico-chemical characteristics of UVT%, COD, TSS and TDS affects the effectiveness of a UV disinfection plant the most.

The UVT %, which is one of the most important parameters of water quality to be considered for UV irradiation treatment, varied significantly ($p<0.05$) between the three sampling rounds (Table 5.4). During the first sampling occasion, water was sampled after the river experienced flooding after heavy rain. This weather pattern possibly caused the stirring of sediment and organic material in the river water, resulting in low a UVT % of 9.2% (Table 5.4), which could be the reason for the high levels of turbidity (129 NTU) observed (Table 5.4). The flooding could also have caused the high TSS and COD values observed here after the first sampling occasion (Table 5.4).

Considering the guideline limits, it should be noted that the Irrigation Water Guidelines (DWAF, 1996a) do not stipulate a limit for UVT %, however, the higher the UVT %, the higher the UV disinfection efficacy would be. Furthermore, the results in Table 5.4 indicate that, overall, bag filtration does not have a significant effect on UVT %, as the average 5 μm bag filter results only indicated improvement by only 1.7 %, when compared to the average 'before' sample results (Table 5.4).

With regards to the TDS content, the first sampling round showed an average 'before' value of 245 mg.L^{-1}, which is below the required guideline limit of 260 mg.L^{-1} stipulated by the Irrigation Water Guidelines (DWAF, 1996a). However, the second and third sampling occasions obtained average 'before' values of 354 and 311 mg.L^{-1} (Table 5.4), both exceeding the recommended guideline limit. Comparing the average 'before' filtration results to the average 5 μm bag filter results, TDS improved by 0.3 mg.L^{-1}, which is not a significant difference ($p>0.05$). As TDS consists of dissolved solids which could pass through the bag filters tested in this study, this result was expected. Fluctuations observed during these rounds could possibly be attributed to dissolved matter trapped in suspended solids that were reduced by smaller pore sizes (Table 5.4). Furthermore, the high TDS values are in line with values measured for the Plankenburg river during the previous two research chapters. As mentioned before, the elevated levels of TDS could possibly be attributed to the water site being downstream of industrial and domestic wastewater effluents as described in Table 5.1.

It was expected that the suspended solids content of the river water would be the most affected by bag filtration. The results in Table 5.4 indicated that this was indeed the case when considering the TSS values. As mentioned before, the first sampling occasion was after river flooding, which may have contributed to the significantly higher TSS value of 115 mg.L^{-1}, as sediment and organic matter was possibly stirred in turbulent river water (Table 5.4). In contrast, during sampling occasion two, the river water level was notably lower than the first occasion, possibly resulting in less stirring with less turbulent river water flow (Table 5.4). When considering the 'before' bag filtration values obtained during sampling round one and three (Table 5.4) both exceeded the Irrigation Water Guidelines (DWAF, 1996a) (Table 5.2), which stipulate levels only below 50 mg.L^{-1} are acceptable for agricultural irrigation water use.

When considering COD, there was a reduction of 8 mg O$_2$.L^{-1} between the 'before' sample and after the 100 µm bag filter (Table 5.4). However, as the pore size were lowered to a size of 5 µm, fluctuations occurred in COD levels with no constant reduction, suggesting that COD levels were not influenced markedly by different bag filtration pore sizes. Furthermore, bag filtration did not significantly ($p<0.05$) reduce the physico-chemical characteristics of pH, EC and alkalinity (Table 5.4). As pH is measured by hydrogen ion concentration along with EC, which is the measurement of total soluble and dissolved salts, it was expected that bag filtration will not have an effect on these parameters.

With regards to the microbial results, it was expected that the bag filtration will have minimal effect on the initial microbial counts, as reported before by Ong et al. (2018) that significantly reducing microbial matter is a limitation of the bag filtration method. Speer et al. (2019) states that the average length and width of an *E. coli* cell is 1.7 µm and 1.2 µm, respectively, suggesting that the smallest bag filter pore size (5 µm) will not stop suspended *E. coli* cells from passing through. This statement could be supported by the results in Fig 5.4, indicating that *E. coli* loads were not significantly reduced ($p>0.05$) by bag filtration. When considering the initial loads of the three rounds, it can be seen that the first round had the highest loads (Fig. 5.3, 5.4 & 5.5). This statement could be supported by Nag et al. (2021), which reported that rainfall events may cause microorganisms to migrate to surface water, ultimately polluting surface water resources such as rivers. Furthermore, the slight decrease observed between the 'before' sample and 5 µm bag filter sample (Fig 5.4) could possibly be attributed to microbial cells being attached to larger suspended particles. In support of this statement, Oliver et al. (2007) and Jeng et al. (2005) reported that *E. coli* regularly attach to soil particles within a size range of 5-30 µm. The results from Figure 5.4 indicated an average 'before' count of 4.31 log. CFU.mL^{-1}, compared to the 5 µm bag filter sample that had an average count of 3.82 log CFU.mL^{-1}. These results confirmed that bag filtration could never be used as a stand-alone treatment for reducing microbial

loads, as a bag filter of 5 µm only reduced the initial loads by 0.49 log CFU. mL⁻¹ (Fig 5.4). After filtration, the water from the Plankenburg river still exceeding the recommended irrigation water guideline for *E. coli* of 2 log CFU. mL (DWAF, 1996 a & c).

Overall, this study (Objective 1) indicated that bag filtration did not reduce physico-chemical and microbial characteristics of this river water source significantly (Table 5.4). However, considering the slight improvements associated with the four bag filter options, the results indicated that, if a bag filter had to be chosen, the smallest pore size of 5 µm was the best option.

Bursey (2021) also studied the same four bag filter options using a different river water source (Mosselbank river) and reported that the difference between physico-chemical and microbial characteristics before and after bag filtration were also not significant (p>0.05). It was however noted in her study that COD, TSS and turbidity levels were reduced slightly by the 5 µm bag filter, which is similar to findings of this study (Table 5.4). During this study UVT % of the Plankenburg river water did not improve significantly after filtration (Table 5.4). A similar observation was made by Bursey (2021), on the Mosselbank river.

As a bag filter is required as part of the pilot plant setup, a choice had to be made based on the results of the four bag filters tested during this study and in previous work (Bursey, 2021). Therefore, the bag filter of 5 µm was chosen as the pre-treatment option for a medium-pressure UV disinfection system during objective 2 of this study.

Ong et al. (2018) stated that physical systems such as bag filtration has limitations, as microbial matter and organic particles might not be reduced efficiently. With regards to the effect of bag filtration during this study, TSS was reduced the most compared to the other physico-chemical characteristics (Table 5.4), possibly due to some suspended solids being larger than 5 µm, which was the smallest bag filter pore size tested. Overall, these findings could suggest that pre-treatment should be explored further in future, as physico-chemical fluctuations in river water quality continue to be observed between sampling occasions in this and previous studies. In addition, these fluctuations could influence the reliability of UV treatment at pilot-scale, which in the future could affect the efficacy of the technology if no other cost-effective pre-treatments could be applied.

With regards to treatment methods of physico-chemical characteristics, very few economical options exist. Dong et al. (2020) previously reported in this chapter that complex techniques such as ion exchange pre-treatment and reverse osmosis would be needed to reduce TDS levels along with other dissolved solids. Treatment options for suspended solids are more available, such as ultrafiltration, however the operation cost is an important draw-back and would not be a viable option for irrigation water. Therefore, it remains a challenge to establish the most effective and economic pre-treatment methods.

5.5.2 Objective 2.1: The effect of a medium-pressure UV system at pilot-scale on the microbiological characteristics (HPC, *E. coli* and coliforms) of three different river profiles

Jones et al. (2014) stressed that a study simulating a water treatment process in a real agricultural setting, with the use of significantly larger water volumes, is of great importance to the test the UV-C efficiency. Therefore, as part of this objective, the disinfection efficacy of a medium-pressure UV system, with a 5 µm bag filter as pre-treatment, was investigated using significantly larger river water volumes (1 000 L) and a pilot-scale UV system. Three rivers were chosen for this objective based on their known river water profiles from previous work (Bursey, 2020, Olivier, 2015). These included: the Plankenburg river (poor microbial characteristics); the Mosselbank river (poor physico-chemical characteristics); and the Franschhoek river (good microbial and physico-chemical characteristics) (Table 5.1). As for UV radiation, a UV dose of 20 mJ.cm^{-2}, both as a single (1 x 20 mJ.cm^{-2}) and double dose (2 x 20 = 40 mJ.cm^{-2}), were applied using the medium-pressure UV system (Berson, The Netherlands) on river water.

Each of the three rivers were only sampled once, with all UV treatments (before UV, after first UV dose and after second UV dose) conducted in triplicate using a single 1 000 L river water batch. Anion and cation analysis (BIOGRIP) for each river were performed from one river water sample, with characteristics and measurements illustrated in Table 5.5. Furthermore, the physico-chemical analyses of triplicate samples, taken after one 1 000 L sampling occasion for each river, are presented in Table 5.6.

Table 5.5 Physico-chemical results of the three rivers tested during one sampling occasion, with average values obtained from triplicate water sample measurements

Characteristics	River location		
	Mosselbank river	Franschhoek river	Plankenburg river
Fluorine (mg.L^{-1})	0.48	0.08	0.14
Chlorine (mg.L^{-1})	194.56	26.40	59.10
Sulphate (mg.L^{-1})	70.41	7.69	9.72
Nitrate (mg.L^{-1})	11.36	2.20	0.62
Sodium (mg.L^{-1})	118.17	0.07	0.64
Ammonium (mg.L^{-1})	0.85	0.07	0.64
Magnesium (mg.L^{-1})	20.18	3.92	10.24
Calcium (mg.L^{-1})	63.30	11.33	20.85

Table 5.6 Physico-chemical characteristics of three selected rivers over one sampling round, performed in triplicate per 1000 L water sample

Characterisitcs	Mosselbank river				Franschhoek river				Plankenburg river			
	1	2	3	Avg. SD	1	2	3	Avg. SD	1	2	3	Avg. SD
UVT%	40.0	40.2	40.0	40.1 0.11	68.0	69.2	69.0	69.0 0.64	65.0	69.1	72.4	65.5 3.70
TDS (mg.L^{-1})	701	701	701	701 0.00	84	94	89	89 5.00	206	209	208	208 1.52
TSS (mg.L^{-1})	14	14	14	14 0.00	6	5	6	6 0.57	4	4	4	4 0.00
COD (mg O$_2$.L^{-1})	43	50	47	47 3.41	10	10	10	10 0.00	37	16	37	37 12.12
pH	7.21	7.21	7.21		6.96	6.96	6.96		7.10	7.09	7.10	
Turbidity (NTU)	2.9	2.3	3.1	2.76 0.41	3.3	3.0	3.0	3.10 0.17	2.8	3.1	2.8	2.9 0.17
EC (mS.m^{-1})	0.76	0.75	0.76	0.76 0.01	0.15	0.15	0.15	0.15 0.00	0.22	0.24	0.23	0.23 0.01
Alkalinity (mg CaCO$_3$.L^{-1})	186	186	186	186 0.00	65	65	65	65 0.00	101	100	101	101 0.05

*UVT – ultraviolet Transmittance *TDS – Total Dissolved Solids *TSS – Total Suspended Solids *COD – Chemical Oxygen Demand *EC – Electrical Conductivity

As expected, the physico-chemical results indicate that the river profiles vary (Table 5.5 & 5.6). As previously reported, the Mosselbank river was considered to be the worst river in terms of physico-chemical characteristics. This statement could be supported by the physico-chemical findings of this study (Table 5.5 & 5.6). The results from the Mosselbank river indicated that this river had the poorest UVT % of the three rivers, along with an uncharacteristically high TDS value, (as indicated in previous chapters of this thesis and by Bursey (2020). The Mosselbank river in the Kraaifontein area is situated downstream of wastewater treatment works (Table 5.1), which may suggest the reason for the significantly higher TDS value (Table 5.6), when compared to the Franschhoek and Plankenburg river. This statement can be supported by Belgiorno et al. (2007), which reported that various chemical compounds such as polycyclic aromatic hydrocarbons, alkylphenols and heavy metals can be found in the effluents of wastewater treatment plants.

When considering the anion and cation results (Table 5.5), all characteristics measured were markedly higher at the Mosselbank river, when compared to the other two river profiles. In support of this finding, Hossain et al. (2018) reported that characteristics such as sodium chloride (NaCl), sodium sulphate (Na_2SO_4) and magnesium (Mg) contribute to high TDS readings. As seen in Table 5.5, both the chlorine (Cl), sodium (Na) and sulphate (SO_4) measurements in water from the Mosselbank river site were higher than that of the Plankenburg and Eerste rivers, supporting the findings of Hossain et al. (2018). The reason for these elevated levels could possibly be related to the location of the Mosselbank river site (Table 5.1), downstream of a wastewater treatment plant. According to Wilson et al. (2014), water treatment plants to not have operations to eradicate high TDS levels, resulting in effluents with elevated TDS levels exiting into surface water sources such as river systems. Belgiorno et al. (2007) stated that advanced oxidation processes are used in wastewater treatment works, which could result in elevated TDS levels of final effluent. The addition of various chemicals during the treatment of wastewater could increase TDS levels as Navamani Kartic et al. (2018) reported that sulphate in the form of sodium sulphate results in elevated TDS during treatment.

The microbial results are presented in Figure 5.6 to 5.11 below. The HPC and *E. coli*/coliform counts are expressed in log CFU.mL^{-1} before UV disinfection, after the first UV dose (1 x 20 mJ.cm^{-2}), and after the second UV dose (2 x 20 mJ.cm^{-2}). Table 5.7 also indicates the presence/absence results of Listeria monocytogenes (LM) and Salmonella spp. (S) for three rivers, where three triplicate water samples were pooled together during one sampling round, before UV radiation and after the first and second UV dose
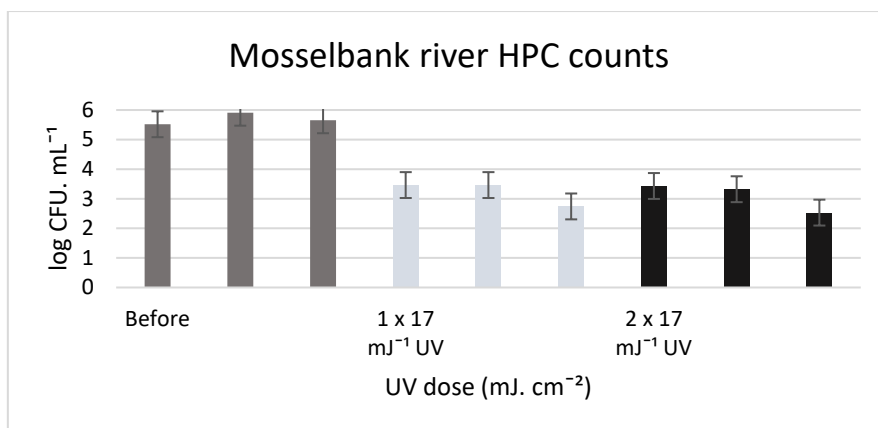
Figure 5.6 HPC counts expressed in log CFU. mL before, after 1 x 17 mJ.cm⁻² UV, after 2 x 17 mJ.cm⁻² UV, with error bars indicating standard deviation across one 1 000 L sampling round.
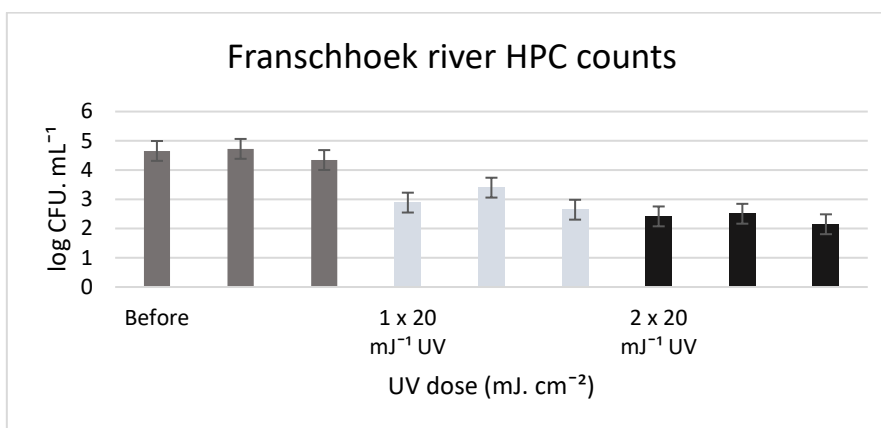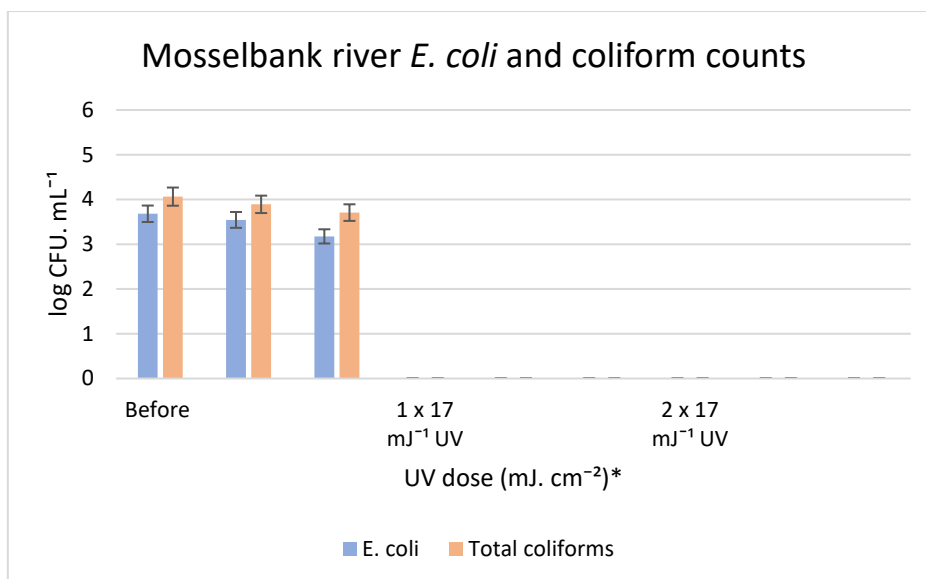

Figure 5.7 HPC counts expressed in log CFU. mL before, after 1 x 20 mJ.cm⁻² UV, after 2 x 20 mJ.cm⁻² UV, with error bars indicating standard deviation across one 1 000 L sampling round.
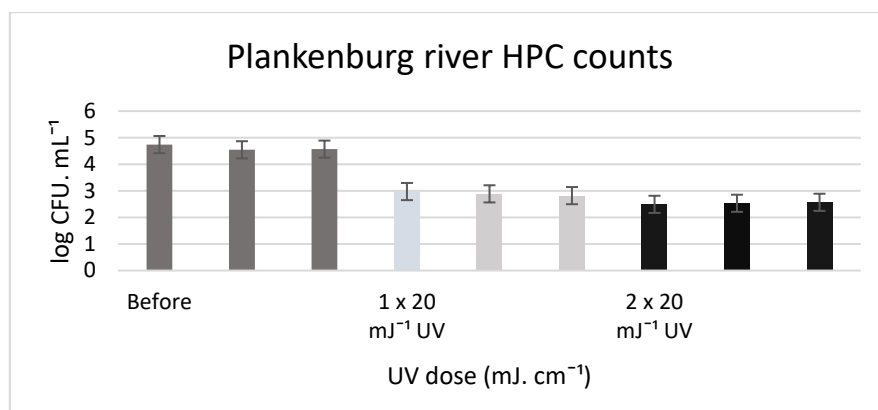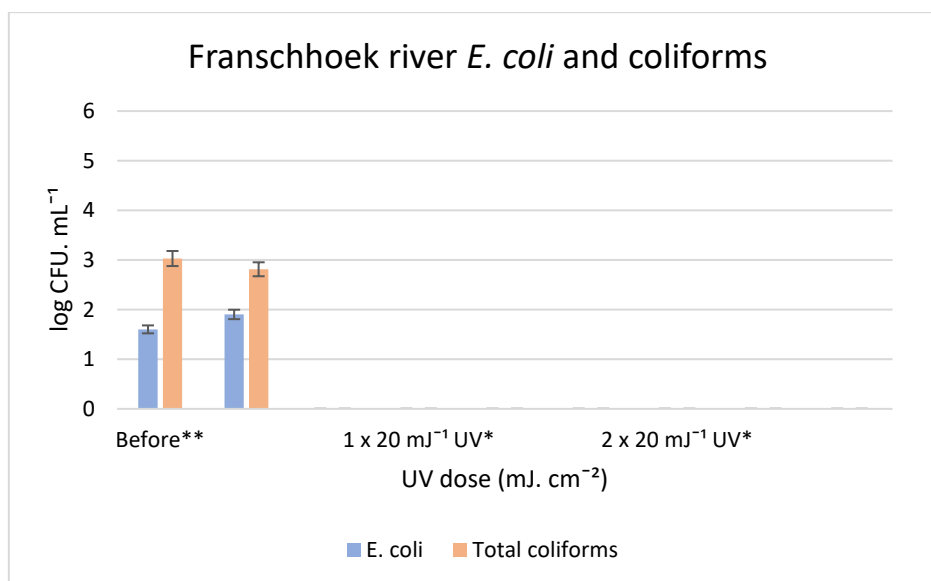

Figure 5.8 HPC counts expressed in log CFU. mL before, after 1 x 20 mJ.cm⁻² UV, after 2 x 20 mJ.cm⁻² UV, with error bars indicating standard deviation across one 1 000 L sampling round.
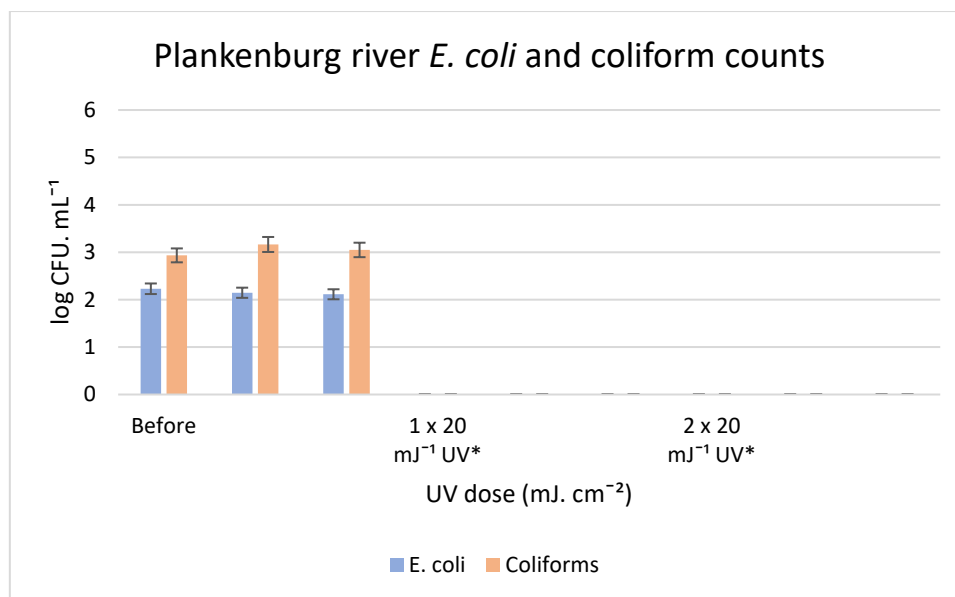
Figure 5.9 *E. coli* and coliform counts from Mosselbank river, expressed in log CFU.mL before UV, after 1 x 20 mJ.cm⁻² UV, after 2 x 20 mJ.cm⁻² UV, with error bars indicating standard deviation across one sampling round. The detection limit for this method is 1.0 log CFU.mL⁻¹, since all colonies below 300 CFU were counted at the lowest dilution. *No growth was observed after any UV doses (mJ.cm⁻²)

Figure 5.10 *E. coli* and coliform counts from Franschhoek river, expressed in log CFU.mL before UV, after 1 x 20 mJ.cm⁻² UV, after 2 x 20 mJ.cm⁻² UV, with error bars indicating standard deviation across one sampling round. The detection limit for this method is 1.0 log CFU.mL⁻¹, since all colonies below 300 CFU were counted at the lowest dilution. *No growth was observed after any UV doses (mJ.cm⁻²) **Third water sample of 'Before' sample showed no growth of *E. coli* and coliforms

Figure 5.11 *E. coli* and coliform counts from Plankenburg river river, expressed in log CFU.mL before UV, after 1 x 20 mJ.cm⁻² UV, after 2 x 20 mJ.cm⁻² UV, with error bars indicating standard deviation across one sampling round. The detection limit for this method is 1.0 log CFU.mL⁻¹, since all colonies below 300 CFU were counted at the lowest dilution. *No growth was observed after any UV doses (mJ. cm⁻²)

Table 5.7 Presence/absence results of *Listeria monocytogenes* (LM) and *Salmonella spp.* (S) for three rivers, where three triplicate water samples were pooled together during one sampling round, before UV radiation and after the first and second UV dose

River	Organism	UV dose (mJ. cm⁻²)		
		0 mJ. cm⁻²(Before)	1 x 20 mJ. cm⁻²	2 x 20 mJ. cm⁻²
Mosselbank river*	LM	+	-	-
	S	-	-	-
Franschhoek river	LM	-	-	-
	S	-	-	-
Plankenburg river	LM	+	-	-
	S	-	-	-

*Mosselbank river. A UV dose of 1 x 17 mJ.cm⁻² and 2 x 17 mJ.cm⁻² could only be achieved at this site.

LM – Listeria monocytogenes

S – Salmonella spp.

It should be noted before discussing microbial results that a limitation of the medium-pressure UV system was encountered when UV radiation was applied on one of the three rivers. Reiterating the UV dosing procedure as described before, a desired UV dose of 20 mJ.cm⁻² and flow rate of 5 m³.h⁻¹ has been set before operation. When the UV system was used to treat water from the Mosselbank river, it was noted that the Berson EC tronic Ώ medium-pressure UV system could not administer a dose of 20 mJ.cm⁻². This was due to the river quality of the Mosselbank river being too poor, as a UVT

% of 40.1 % and TDS value of 701 mg.L^{-1} was noted (Table 5.6). The highest possible UV dose that could be applied (as automatically measured and displayed on the UV system's display screen) for the Mosselbank river water was 1 x 17 mJ.cm^{-2} and 2 x 17 mJ.cm^{-2} at 100% system power (Figure 5.6 & 5.9).

With regard to these findings, it was decided that the system will be set to a desired (theoretical) UV dose of 20 mJ.cm^{-2} and flow rate of 5 m^3.h^{-1} for all water samples tested, and that the actual UV dose that could be applied, and was measured in real-time (e.g., 17 mJ.cm^{-2}), would be recorded. In addition, it was decided that river water with a UVT % was below 40 %, would be considered untreatable given the practical limitations of this UV system, as preliminary optimisation testing have revealed.

Furthermore, physico-chemical results from Plankenburg and Franschhoek river (Table 5.6) were shown to be better, when compared to the Mosselbank river. These findings resulted in a UV dose of 20 mJ.cm^{-2} being given to the Plankenburg and Franschhoek river. Considering the limitations of the Berson EC tronic Ὠ medium-pressure UV system, it was possible to apply two lower UV doses (2 x 20 mJ.cm^{-2}), instead of 1 x 40 mJ.cm^{-2}, since the application of higher doses with poor quality river water, (as seen with the Mosselbank river -Table 5.6) could not be achieved

With regards to the results, the initial microbial results (Fig 5.6 & 5.9) of the Mosselbank river are in line with microbiological results from the previous chapter (Chapter 5.4) and other studies (Bursey, 2020). Results from previous work has shown that the microbiological state of the Mosselbank river wase not within the irrigation water guideline limits (Table 5.2). This observation could be supported by Figures 5.6 & 5.9, indicating the highest initial HPC population and *E. coli*/coliform counts, respectively, between the three river systems.

When considering the effect of the UV doses on the HPC population of the Mosselbank river, a reduction of 2.46 log. CFU. mL^{-1} (Figure 5.6) was observed after the first UV dose (1 x 17 mJ.cm^{-2}). However, when the river water sample was exposed to the second UV dose (2 x 17 mJ.cm^{-2} = 34 mJ.cm^{-2}), a very slight reduction of only 0.13 log. CFU.mL^{-1} (Figure 6) was observed. The first 17 mJ.cm^{-2} UV dose from the medium pressure UV system (Berson, Netherlands) resulted in log reductions comparable to the lab-scale low-pressure UV lamp at 20 mJ.cm^{-1}, as investigated in previous chapters. When considering the UV dose response of HPC populations in previous chapter (Chapter 4), the log reduction from a second UV dose, for example 40 mJ.cm^{-1}, were not notable, thus comparable to the results after 2 x 17 mJ.cm^{-2} (Figure 5.6).

As for the *E. coli* and total coliform counts from the Mosselbank river, high initial loads were observed, with *E. coli* exceeding the recommended guideline limit of 2 log. CFU.mL^{-1} (DWAF, 1996a). The first 17 mJ.cm^{-1} caused all *E. coli* and coliforms loads to drop below the lowest detectable limit

(Figure 5.9). The presence of *Listeria monocytogenes* (*L. monocytogenes*) was also observed in the untreated river water, with no growth after UV radiation (Table 5.7). In addition, during this sampling round the presence of *Salmonella spp*. could not be detected (Table 5.7), however, Bursey (2020) had picked up these organisms before at this site. These findings are in line with results from previous chapters and work (Bursey, 2020), ultimately suggesting that *E. coli,* coliforms and *L. monocytogenes* from these river systems are sensitive to a UV dose between 17 and 20 mJ.cm^{-2}.

As previously mentioned, Zimmer & Slawson (2002) reported that the medium-pressure UV system has a broader wavelength range, which may possibly result in targeting more cellular structures, ultimately resulting in a larger reduction. This would suggest that the medium-pressure UV, when compared to the lab-scale low-pressure UV, may result in a larger microbial reduction, especially after a second UV dose. In terms of the results observed for the Mosselbank river, the statement from Zimmer & Slawson (2002) could not be confirmed during this study.

Comparing these results to the Franschhoek river, which was previously considered as the best river in terms of physico-chemical and microbial characteristics (Bursey, 2020), notable difference can be reported. As opposed to the Mosselbank river, the Franschhoek river is not situated close to the effluents from a wastewater treatment plant (Table 5.1), suggesting that better microbiological and physico-chemical characteristics would be expected at this site. The findings of this study supported this statement as results from the Franschhoek river showed lower microbial (Fig 5.7 & 5.10) and physico-chemical (Table 5.5) loads. With regards to the physico-chemical characteristics, the Franschhoek river results indicated the highest overall UVT % together with the lowest TDS and COD levels (Table 5.6). The anion and cation results from the Franschhoek proved to be the lowest of three river sources tested (Table 5.5). This may suggest that a wastewater treatment plant, such as in the case of the Mosselbank river (Table 5.5), can contribute to an increase in dissolved solids in rivers due to wastewater treatment processes (Belgiorno *et al*., 2007).

As for the microbial results at this river site, initial HPC populations results (Figure 5.7) from the Franschhoek river were lower than the Mosselbank river, however, comparable to the Plankenburg river. This is concerning as the results from previous chapters and other studies (Bursey, 2020) at this Franschhoek river site, indicated that the microbial status, in terms of HPC loads, of Franschhoek river was better than that of the Plankenburg river. These findings support observations made in previous chapters that the microbial profile of rivers do fluctuate frequently. As for the HPC populations after the two doses of 20 mJ.cm^{-1}, results from the Franschhoek river indicate a 1.90 log CFU.mL^{-1}and 0.66 log CFU.mL^{-1} reduction for the first 20 mJ.cm^{-1} and second 20 mJ.cm^{-1} UV dose, respectively.

When considering the initial *E. coli* and coliform counts (Figure 5.10) from the Franschhoek river, it can be observed that microbial loads are lower than the Mosselbank (Figure 5.9) and Plankenburg river (Figure 5.11). In Figure 5.10, during the third analysis done on the 1 000 L river water sample, E. *coli* and coliform loads were not detected (Figure 5.10), suggesting that microbial concentrations in a 1 000 L Franschhoek river water sample also fluctuate. In addition, *L. mono* and *Salmonella* spp. could not be detected from the untreated river water sample (Table 5.7), however, previous research has shown the presence of both the pathogens at these sites (Bursey, 2020). This finding stresses the importance of sampling larger river water volumes continually during a pilot plant procedure, as fluctuations may occur. Furthermore, as for the two UV doses applied, results were similar to the Mosselbank river, as all *E. coli* and coliform counts (Figure 5.10) were below the detection limit after the first 20 mJ.cm^{-1} already.

Lastly, the Plankenburg river was chosen for this study as results from previous chapters in this thesis as well as previous studies (Britz, 2013; Bursey, 2020; Sivhute, 2019) established that the microbial levels present were very concerning. These findings could possibly be attributed to the Plankenburg river being situated downstream from non-point pollution sources, such as effluents from informal settlements and exposure to wastewater from an industrial area (Table 5.1).

With regards to the initial HPC population, results from the Plankenburg river (Figure 5.8) proved to be marginally higher to the HPC population loads observed at the Franschhoek river (Figure 5.7) and slightly lower than the Mosselbank river (Figure 5.6). As for microbial reductions, 1.73 and 0.35 log CFU.mL^{-1} reductions were observed after the first and second 20 mJ.cm^{-1} UV dose, respectively. These findings were consistent in terms of the log reduction effect of the first and second medium-pressure UV dose. It should however be noted that the HPC population results from the Plankenburg river indicated more UV resistant microorganisms (Figure 5.8). This conclusion could be made due to the tempo of log reduction of the Plankenburg river water being much lower when compared to results of the Mosselbank river loads (Figure 5.6), even though the initial HPC population of the Plankenburg river (Figure 5.8) were lower than Mosselbank river (Figure 5.6). These findings were also observed in previous chapters, where these results suggest that irrespective of the initial microbial loads observed, population composition and resistance to a certain UV dose should be considered as well.

Furthermore, *E. coli* and coliform counts exceeded the recommended irrigation water guidelines of 2 log. CFU.mL^{-1} (Figure 5.11). The presence of *L. mono* was also observed before UV, with no growth after treatment (Table 5.7). In addition, Bursey (2020) noted that *Salmonella spp*. were frequently present at this site, however, during this round it was not detected (Table 5.7). As with the

Mosselbank and Franschhoek river, the *E. coli*, coliforms and *L. mono* present in the Plankenburg waters did not survive the first UV dose of 20 mJ.cm^{-1} (Figure 5.11).

In addition to the microbial results above, certain HPC population colonies (n=11) which survived the first and second 20 mJ.cm^{-1} UV doses, were isolated and further characterised. The characterisation and identification procedure involved all isolated colonies being characterised by Gram staining and testing whether the microorganisms are catalase and oxidase positive or negative, according to the methods set out by Tarrand & Gröschel (1982) and Chester (1979), respectively. The isolated colonies were prepared for MALDI-TOF analysis, for species identification of pure strains. The MALDI Biotyper® system involves the use of a MicroFlex LT Matrix-Assisted Laser Desorption/Ionisation Time of Flight (MALDI-TOF) mass spectrometer (Bruker Daltonics, Germany) to obtain genus or species identification. The results for all characterisation and identification tests are summarised in Table 5.8, with the MALDI-TOF spectra of each individual organism detected in Appendix C.

Table 5.8 Summary of characterisation and MALDI-TOF results, indicating the microbial species isolated after specified UV doses from HPC populations

River location	UV dose (mJ.cm^{-2})	Gram staining (+/-)	Catalase test (+/-)	Oxidase test (+/-)	MALDI-TOF Score[a]	Identification	Appendix C spectra
Plankenburg	1 x 20	-	-	-	1.85	*Aeromonas hydrophila*[a]	C5
Plankenburg	1 x 20	+	-	-	1.61	*Enterococcus* species [e]	C6
Mosselbank	1 x 17	-	+	+	2.10	*Brevundimonas vesicularis*[c]	C7
Mosselbank	1 x 17	+	+	-	1.84	*Rhodococcus erythropolis*	C8
Plankenburg	2 x 20	-	-	-	2.10	*Aeromonas caviae*[b]	C9
Plankenburg	2 x 20	+	-	-	1.49	*Enterococcus* species	C10
Mosselbank	2 x 17	-	+	+	2.28	*Brevundimonas vesicularis*	C11

[a]MALDI-TOF Score. A logarithmic score lower than 1.70 would indicate a mixed culture or the absence of reference spectra on the database for the tested isolate, score between 1.70 – 1.99 indicate low-confidence identification score between 2.00 – 2.30 indicate high probability species identification and score between 2.30 – 3.00 indicate high confidence species identification.

[a]*Aeromonas hydrophila.* Species of this genus have very similar patterns, therefore, distinguishing their species is difficult.

[b]*Aeromonas caviae.* Species of this genus have very similar patterns, therefore, distinguishing their species is difficult.

[c]*Brevundimonas vesicularis.* No information available with regards to distinguishing their species.

[e]*Enterococcus hirae*. No information available with regards to distinguishing their species.

During chapter 4 the same procedure of identifying certain HPC population were performed, where results indicated that several different microbial species from various rivers were not sensitive to UV radiation. When considering the results shown in Table 5.8 it is in line with chapter 4, as isolated HPC colonies identified as different microbial species. Kizhakkekalam & Chakraborty (2019) also reported the presence of a large diversity of HPC bacteria in water, as 148 HPC bacterial strains of 7 different species were isolated during their study. Kizhakkekalam & Chakraborty (2019) noted that more species may be present in a HPC population, as they could consist of much more species than reported during this study.

Two *Aeromonas* species, *A. hydrophila* and *A. caviae,* were detected in the Plankenburg river water after UV doses of 1 x 17 mJ.cm^{-2} and 2 x 17 mJ.cm^{-2}, respectively (Table 5.8). The *A. hydrophila* and *A. caviae* obtained MALDI-TOF scores of 1.85 and 2.10, respectively (Table 5.8). The MADLI-TOF score of the A. *hydrophila* indicate a low-confidence identification (1.70-1.99), however, it should be noted that distinguishing between species of the genus is difficult when using the as MALDI-TOF spectra as basis (Table 5.8). The *A. caviae* MALDI-TOF score was between 2.00 – 2.40, indicating high probability species identification (Table 5.8). During chapter 4, *A. hydrophila* was also detected after UV radiation at the Plankenburg river, supporting the findings of these study which could suggest a regularly occurrence of UV resistant *Aeromonas* species in this river. Very limited research is available regarding why *Aeromonas* species are UV resistant, as seen in this study. However, their frequent presence in surface water such as river systems can be supported by Liu et al. (2016), who reported their common occurrence in aquatic environments.

Results from the Plankenburg river indicated the presence of *Enterococcus* species (Table 5.8). *Enterococcus* species is Gram-positive cocci that populate in various plants and animals, and their presence in water is often associated with faecal contamination from farm animals (Zaheer *et al*., 2020). Mbanga et al. (2021) investigated *Enterococcus* species in the effluent from a wastewater treatment plant in South Africa, where it was established that this species was frequently detected downstream of these plants. The results from Mbanga et al. (2020) can possibly support the findings of this study, as *Enterococcus* species were detected at the Plankenburg river site, which is downstream of different wastewater effluents (Table 5.1).

With regards to the UV sensitivity of *Enterococcus* species, Monteiro & Santos (2020) reported that *Enterococcus* species, specifically vancomycin-resistant strains, were previously isolated from UV-disinfected wastewaters and irrigation water sources, which is in line with the findings of this study. In addition, Ozawa et al. (1997) discovered that a plasmid, called pAD1, encodes a UV resistance gene, which protects *Enterococcus* species, specifically *E. faecalis,* from UV light and DNA damage. The *Enterococcus* species obtained MALDI-TOF scores of 1.61 and 1.48, respectively (Table 5.8). These

results suggest that no accurate species identifications could be made, as MALDI-TOF scores was below 1.70 (Table 5.8). The results of these MALDI-TOF isolates, which is included in Appendix C, indicated a mixture of *Enterococcus hirae* and *Staphylococcus epidermidis*.

The next species to be isolated was *Brevundimonas vesicularis* (Table 5.8), which is a Gram-negative, aerobic and both catalase and oxidase positive bacteria (Ryan & Pembroke, 2018). *Brevundimonas vesicularis* was isolated after both UV doses at the Mosselbank river (Table 5.8). With regards to their MALDI-TOF scores, both isolates were between 2.00-2.30 (Table 5.8), indicating high probability species identification. Very limited information is available regarding the UV resistance of *Brevundimonas* species, but it has been documented before that these species are emerging pathogens as they can be resistant to various antibiotics, which included β-lactams and fluoroquinolones (Almuzara *et al.*, 2012).

Lastly, *Rhodococcus erythropolis,* which is described as stress-tolerant bacterial species (Ivshina *et al.*, 2021), was also isolated from the Mosselbank river, however, only after 1 x 20 mJ.cm^{-2} (Table 5.8). Very limited research is available regarding the UV response of these species.

Overall, these results indicate that a variety of microorganisms from HPC populations may survive UV disinfection (Table 5.8). And when considering their UV dose response, as with the results in Chapter 4, it could be noted that species show their own unique response to different UV doses (Table 5.8).

5.5.3 Objective 2.2: The effect of a medium-pressure UV system at pilot-scale on the microbiological characteristics (STEC and ESBL -producing *Enterobacteriaceae*) of three different rivers

River water samples from the three rivers were screened for the presence of STEC, both before and after UV radiation, with the use of the Pall GeneDisc STEC Top 7 test. As discussed in the previous chapters, the non-selective isolation strategy (Chapter 3, Strategy 2) was the detection method of choice in this study, as it was considered the most reliable option for detection of STEC in river water. Table 5.9 below contains a summary of the Pall GeneDisc STEC Top 7 results obtained, indicating the presence and absence of targeted gene sequences (included the O-serogroup markers, followed by whether the water samples contained *stx* genes, along with a specific virulence gene). It should be noted that only data from the samples before UV radiation was included, as no STEC virulence genes or any *E. coli* O-serogroups were observed after the first or second 20 mJ.cm^{-1} UV dose.

Table 5.9 Summary of Pall GeneDisc STEC Top 7 results of three pooled water samples from each of the three rivers before UV radiation, with individual spectra figures presented in Appendix C

Targets	UV dose		
	Before (0 mJ.cm^{-2})		
	Mosselbank (Figure C2)	Franschhoek (Figure C3)	Plankenburg (Figure C4)
O103	Presence	Not detected	Presence
O111	Presence	Not detected	Presence
O145	Presence	Not detected	Presence
O157	Presence	Not detected	Not detected
O26	Presence	Not detected	Not detected
O45-O121	Presence	Presence	Presence
stx1: stx2	Presence	Not detected	Not detected
Vir. O111	Presence	Not detected	Presence
Vir. O145-O157	Not detected	Not detected	Not detected
Vir. O26	Presence	Not detected	Presence
Vir. O45-O103-O121	Presence	Not detected	Presence
STEC presence (Yes/No)	Yes	No	No

Overall, the Pall GeneDisc STEC Top 7 test results indicated that only the Mosselbank showed the presence of *stx* genes along with *E. coli* O-serogroups (Table 5.8). Results from the other two river sites, Franschhoek and Plankenburg river, indicated that certain top 7 O-serogroups were detected, however in the absence of *stx* genes (Table 5.8). The Pall GeneDisc STEC Top 7 findings of this study was similar to STEC findings in previous chapters, such as chapter 4, which indicated that *stx* genes among the top 7 O-serogroups were frequently detected at the Mosselbank river site (Table 5.9).

Bell et al. (2021) reported that polluted surface waters, which include wastewater runoff and untreated domestic water, had been associated with high prevalence of STEC. When considering the location of the Mosselbank river site (Table 5.1), the statement by Bell et al. (2021) could support the reason for STEC detection. Even though no STEC was detected from the Plankenburg river site (Table 9), which is also downstream of wastewater effluents (Table 5.1), these results and literature illustrate that river sites being situated downstream of industrial and domestic effluents, could increase the risk of STEC detection.

Lastly, the presence of ESBL – producing *Enterobacteriaceae* was also investigated as part of this study. Interestingly, the Mosselbank river was the only water site where presumptive ESBL-producing *Enterobacteriaceae* were isolated from. No presumptive ESBL-producing organisms could be picked up from Plankenburg or Franschhoek river.

The presumptive positive ESBL-producing *Enterobacteriaceae* strains isolated from the Mosselbank river was prepared for MALDI-TOF identification. The analysis was aimed at species confirmation, as the strains demonstrated the correct phenotypical morphologies on CHROMagar ESBL (MediaMage, South Africa). Table 5.10 indicate the MALDI-TOF species identification results of isolates before UV, as no ESBLs survived after UV radiation was applied.

Table 5.10 Presumptive positive ESBL-producing *Enterobacteriaceae* isolated from the CHROMagar ESBL plates before UV radiation of the Mosselbank river sampling round, were identified using MALDI-TOF analyses

River location	UV dose (mJ.cm⁻²)	Incubation temperature	MALDI-TOF score[a]	Identification
Mosselbank	0	37°C	2.11	*Raoultella ornithinolytic*
Mosselbank	0	37°C	2.20	*Raoultella ornithinolytic*[b]
Mosselbank	0	37°C	2.13	*Raoultella ornithinolytic*
Mosselbank	0	37°C	2.02	*Klebsiella oxytoca*

[a] MALDI-TOF Score. A logarithmic score lower than 1.70 would indicate a mixed culture or the absence of reference spectra on the database for the tested isolate, score between 1.70 – 1.99 indicate low-confidence identification score between 2.00 – 2.30 indicate high probability species identification and score between 2.30 – 3.00 indicate high confidence species identification.

[b]*Raoutella ornithinolytic. Klebsiella oxytoca* and the *Raoultella* species *ornithinolytic, planticola, terrigena* have very similar patterns. Therefore, distinguishing their species is difficult.

[c]*Klebsiella oxytoca. Klebsiella oxytoca* and the *Raoultella* species *ornithinolytic, planticola, terrigena* of have very similar patterns. Therefore, distinguishing their species is difficult.

As all isolated strains were confirmed to be part of the *Enterobacteriaceae* family*,* each of the strains were prepared for the ESBL testing procedure. As previously discussed in Chapter 3 and 4, the disc diffusion-based test involved the microorganism being exposed to β-lactamases inhibitors such as clavulanic acid (EUCAST, 2021), after which inhibition zones were measured to determine whether an isolate was an ESBL producer.

Table 5.11 shows the results of four river water isolates (pre-UV treatment), after the ESBL testing procedure (EUCAST, 2021). EUCAST (2021) indicates that if the inhibition zone diameter is ≥ 5 mm larger with discs containing the clavulanic acid (CV) alongside the disc without the clavulanic acid, strains are considered ESBL producers. A positive control, *Klebsiella pneumoniae ATCC 700603* and a negative control, *E. coli ATCC 25922*, were also included for each testing round to ensure accuracy of the test. Two of the four Mosselbank river isolates were identified as ESBL producers (Table 5.11).

Table 5.11 Results obtained with the ESBL testing procedures (EUCAST, 2021) of all *E. coli* isolates pre-UV treatment

| River location | Organism | Zone diameter (mm) | | | | | | | | | | | | ESBL producer |
| | | CPM | | CPM/CV | | CTX | | CTX/ CV | | CAZ | | CAZ/ CV | | |
		1	2	1	2	1	2	1	2	1	2	1	2	(Yes/No)
Mosselbank	*Raoultella ornithinolytic*	23	23	26	26	14	15	17	20	21	20	24	24	No
Mosselbank	*Raoultella ornithinolytic*	22	20	27	25	15	14	21	20	22	22	28	27	Yes
Mosselbank	*Raoultella ornithinolytic*	20	20	25	25	16	15	21	20	23	21	28	29	Yes
Mosselbank	*Klebsiella oxytoca*	19	20	21	22	23	21	24	22	15	16	17	16	No
	Klebsiella pneumoniae ATCC 700603	22	21	27	26	20	21	26	26	17	17	24	23	Yes
	Escherichia coli ATCC 25922	32	33	32	33	31	32	32	32	30	31	30	30	No

*CPM – Cefepime, CTX – Cefotaxime, CAZ – Ceftazidime, CV – Clavulanic Acid

During the previous chapters, various ESBL producing *E. coli* were isolated. However, during this study two new ESBL producing *Enterobacteriaceae*, *Raoultella ornithinolytic* and *Klebsiella oxytoca,* have been identified on CHROMagar ESBL at the Mosselbank river site (Tables 5.10 & 5.11). *Klebsiella* and *Raoutella* are considered opportunistic pathogens from the *Enterobacteriaceae* family (Sękowska, 2017). *Klebsiella pneumoniae* is a very well-known ESBL producing organism, however, Sękowska (2017) reported that recently attention has also been given to other ESBL producers, *Raoultella ornithinolytic* and *Klebsiella oxytoca,* which could also cause severe illness. The fact that these two bacterial species were isolated during this study, raises concerns with regards to possible contamination of irrigation water and fresh produce, which could contribute to public health issues. These two organisms can be found in various environments, from soil, plants, animals, and wastewaters (Brisse *et al*., 2006). Their findings support the results of this study, as the Mosselbank river setting (Table 5.1) is situated after wastewater effluents and the river water site is exposed to soil and plants.

According to the ESBL testing results (EUCAST, 2021) (Table 5.11), the *Klebsiella oxytoca* strain was not identified as an ESBL producer, however, two of the three *Raoultella ornithinolytic* were (EUCAST, 2021) (Table 5.10). Even though only two of the four strains isolated during this study were identified as ESBL producers, the presence of antibiotic resistant bacteria in river water systems is of great concern, raising concerns regarding public health and food safety. In addition, Muzslay et al. (2017) reported that *Raoultella ornithinolytic* and *Klebsiella oxytoca* strains isolated during their study were identified as ESBL producers. Although only two of the four strains in this study tested as ESBL producers, the statement Muzslay et al. (2017) suggest this is known characteristics of these species. Furthermore, the fact that ESBL producers could only be detected before UV radiation, shows that the medium-pressure UV disinfection step successfully eliminated ESBL producers of this study.

5.6 Conclusion

During this study, the transition from lab-scale volumes to pilot-scale volumes was investigated with the aim to optimise a medium-pressure UV disinfection system at pilot-scale using water from three different rivers. During Objective 1, it was established that bag filtration does not have a noteworthy effect on physico-chemical characteristics, specifically dissolved solids (Table 5.4). In addition, with regards to the effect of bag filtration on microbial loads, minimal reductions occurred (Figures 5.3 – 5.5). It was however noted during this study that the smallest bag filter of 5 µm showed the best reduction of the four bag filters tested. As the pilot plant system require a bag filter as pre-treatment, the 5 µm was chosen to be used for the rest of the study.

During Objective 2, the Berson EC tronic Ὡ medium-pressure UV system was used as UV disinfection option for the pilot plant study. Water from three rivers, the Mosselbank, Plankenburg and Franschhoek river, was tested. The results of the 'before UV' samples of these three rivers indicated the presence of specific microorganisms, with Plankenburg and Mosselbank river being above the recommended limits (DWAF, 1996). In addition, important pathogens such as STEC and ESBL-producing organisms were also detected in the 'before' samples of the Mosselbank river, which raises concerns regarding the water quality of untreated river water, which is often used as irrigation water. These findings indicate the increased risk of microbial transfer from polluted river water to fresh produce.

Furthermore, a medium-pressure UV system at pilot-scale was investigated to treat the three chosen rivers. Early in the study it was noted that the UV dose is highly dependent on the water quality of the river, especially the physico-chemical profile of the river water sample. During objective 2 the aim was to treat water with a single 20 mJ.cm^{-1} UV dose, followed by another 20 mJ.cm^{-1} UV dose, as the UV system was set to a theoretical UV dose of 20 mJ.cm^{-1} for each of the three rivers. However, in practice, the Berson EC tronic Ὡ medium-pressure UV system had certain, system limitations. The UV system could only achieve a UV dose of 17 mJ.cm^{-2} for the Mosselbank river, probably as a result of the poor physico-chemical profile (Table 5.5 & 5.6). The water profiles of the Plankenburg and Franschhoek river did not interfere with the UV irradiation process as a 20 mJ.cm^{-2} UV dose could be successfully applied (Figures 5.7, 5.8, 5.10 & 5.11).

With regards to the microbial effect from the medium-pressure UV system, reduction in HPC populations was noted after the first and second UV doses. These findings followed the same trend for all three rivers. The *E. coli*, coliforms and *L. monocytogenes* detected during this study did not survive the medium-pressure UV radiation applied during this study. Furthermore, STEC and ESBL-producing *Enterobacteriaceae* were also sensitive to UV radiation, with no growth observed after any UV doses of 20, 40 and 60 mJ.cm^{-2}. These findings suggest that UV radiation is effective in reducing *E.*

coli, coliforms, *L. monocytogenes*, STEC and ESBL-producing organisms to below detectable limits in water from various rivers.

Despite the promising effect of UV radiation on these specific microorganisms, limitations were observed with the HPC population counts. As mentioned before, HPC populations also occurred after UV radiation, with the most reduction after the first UV dose applied. These results indicated that HPC populations show resistance to UV doses applied by the medium-pressure UV system of this study. In addition, Gram-positive & Gram-negative microorganisms such as *B. vesicularis, A. hydrophila* and *R. erythropolis* were identified from the HPC populations post UV radiation (Table 5.8).

These findings suggest that the microbiological profile of river water is of public health concern, if not treated appropriately. If the HPC strains that show UV resistance are identified as pathogenic microorganisms, along with microorganisms which can carry microbial resistant genes, it would increase the risk of resistant genes being transferred to pathogens and making them resistant. These findings suggest that a follow-up study should be performed to further optimise a medium-pressure UV system at pilot-scale. The inclusion of more river profiles will aid in optimising the Berson EC tronic Ώ medium-pressure UV system by investigating the UV effect on microbial counts, which include HPC population identifications, from different river waters.

5.7 References

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Appendix C

Rank (Quality)	Matched Pattern	Score Value	NCBI Identifier
1 (-)	Enterococcus hirae 139 RLT	1.61	1354
2 (-)	Enterococcus hirae LMG 12286 LMG	1.49	1354
3 (-)	Enterococcus hirae LMG 20868 LMG	1.46	1354
4 (-)	Enterococcus hirae DSM 20160T DSM	1.44	1354
5 (-)	Enterococcus hirae DSM 3320 DSM	1.39	1354
6 (-)	Enterococcus hirae LMG 17176 LMG	1.38	1354
7 (-)	Enterococcus hirae IBS_MS_18 IBS	1.35	1354
8 (-)	Staphylococcus epidermidis ATCC 12228 CHB	1.32	1282
9 (-)	Staphylococcus simiae DSM 17639 DSM	1.31	308354
10 (-)	Enterococcus hirae LMG 11492 LMG	1.30	1354

Figure C1 Additional MALDI-TOF results of the mixed *Enterococcus* isolates presented in Table 7 of Objective 2
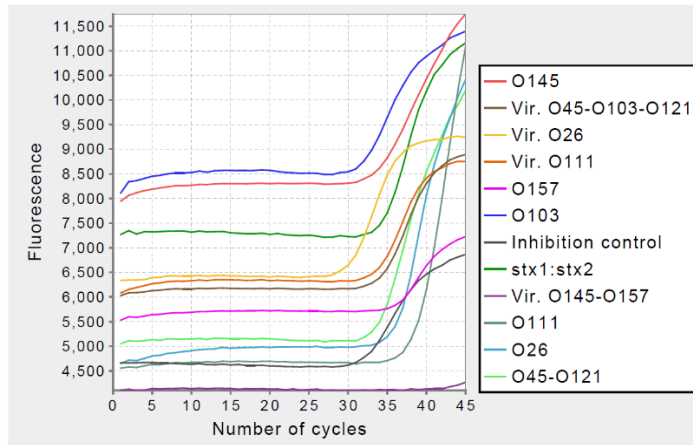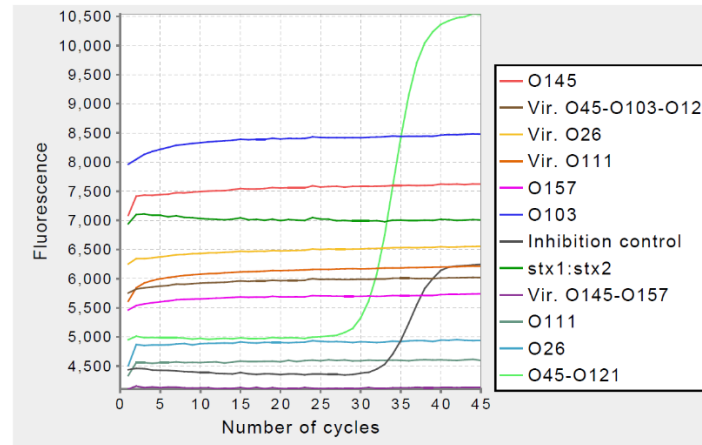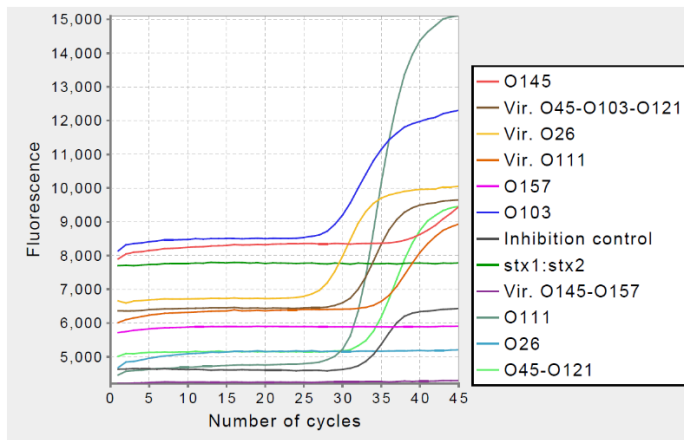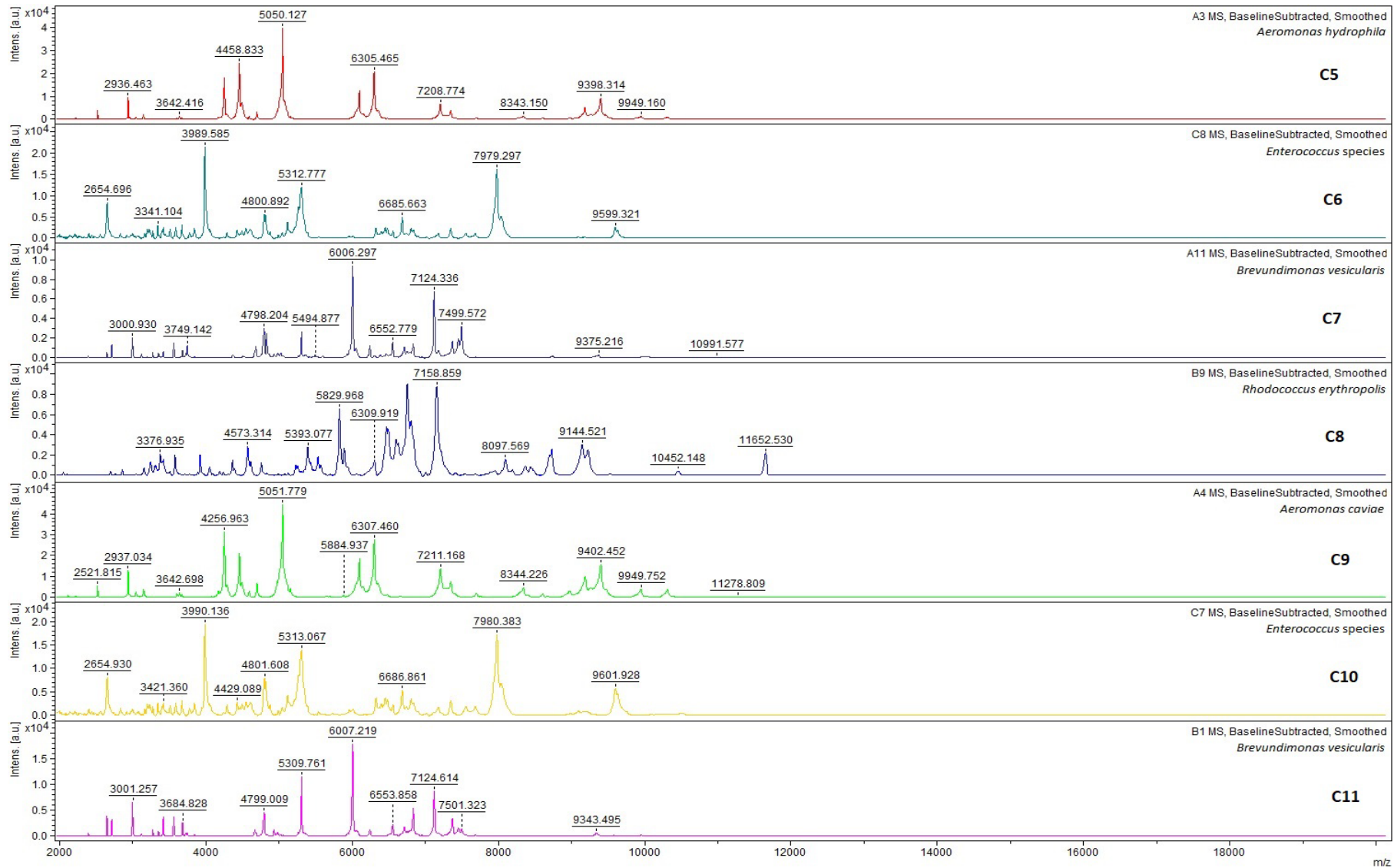
Figure C2

Figure C3

Figure C4

Figures C2-C4 Representation of the spectra obtained from the Pall GeneDisc STEC Top 7 test results (objective 2)

Figures C5-C11 MALDI-TOF spectra of individual organisms detected from Objective 2.1

CHAPTER 6
GENERAL CONCLUSIONS AND RECOMMENDATIONS

6.1 General conclusions

It has been established that access to clean, safe water is very important for various aspects of life (Ao *et al*., 2019). The exponential growth of the global population coupled with the increased use of water, has led to diminishing water resources, which are also regularly polluted with concerning levels of harmful microorganisms and chemicals (Hanjra & Qureshi, 2010). Literature has shown that various water sources such as river water are frequently used for irrigation purposes (Jongman *et al*., 2017). Furthermore, Ziervogel & Ericksen (2010) reported that climate change has also caused a decrease in available water sources. Considering this fact and the increase demand for water, the findings suggest that the water quality of available sources is also of great concern with regards to microbial content (Ao *et al*., 2019).

Microbiological pathogens in water are for the most part of faecal origin (Olivier *et al*., 2015), and are an important source of pathogenic contamination, particularly in the area of irrigation water (Uyttendaele et al., 2015). These pathogenic microorganisms may transfer from polluted river water to fresh produce during irrigation, possibly increasing the risk of foodborne outbreaks.

The consumption of fresh fruits and vegetables has significantly increased over the past decade (Alegbeleye *et al*., 2018). As fresh fruits and vegetables undergo minimal processing and are regularly consumed raw (FDA, 2011), the risk for microbial infection is increased when fresh produce is exposed to polluted river water from irrigation systems. This statement can be supported by literature, which has shown that fresh fruits and vegetables are considered a high-risk food group for foodborne infection outbreaks, as most outbreaks are associated with fresh produce (Painter *et al*., 2013).

In South Africa, river water is the favoured water supply for irrigation on farms (Chami & Moujabber, 2016). As South Africa is a semi-arid country, concerns have been raised regarding the availability of water in the country (Schulze, 2008). However, apart from fears regarding water availability, concerns related to the quality of water have increased considerably (Britz *et al*., 2013). Several researchers have indicated that microbial contamination in some Western Cape rivers exceeded the irrigation water guidelines on a frequent basis (Barnes & Taylor, 2004; Paulse *et al*., 2009; Lamprecht *et al*., 2014; Olivier, 2015, Sivhute, 2019). During this study it was also reported that some Western Cape rivers exceeded the irrigation water guidelines (DWAF), suggesting that the microbial status of these rivers has not improved over a longer period. This statement is supported by previous studies performed on the Mosselbank, Eerste, Plankenburg and Franschhoek rivers (Britz *et al*., 2013; Bursey, 2020, Sivhute, 2019, Olivier, 2015).

Various food pathogens such as *E. coli*, *Salmonella* spp., and *L. monocytogenes,* are important in irrigation water scenarios. These pathogens have shown the ability to survive in soil, water, and edible parts of crops in a fresh produce environment (McEgan *et al*., 2013). In addition, screening methods for STEC and ESBL - producing *Enterobacteriaceae* are of great importance. Consumers are at risk, as STEC could cause severe illness such as haemolytic uraemic syndrome (Byrne *et al*., 2015), and ESBL-producing *Enterobacteriaceae* make treating illnesses more difficult due to being resistant to beta-lactam antibiotics (Kar *et al*., 2015).

In order to reduce or prevent microbial transfer from polluted irrigation water to fresh produce, water treatment methods can be implemented to address this problem. Treatments can be divided into three main categories, namely physical, chemical, and photochemical treatment options. Physical treatment includes various filtration methods; however, the effectiveness of filtration methods has proven to be limited to certain surface water sources, due to their variability and complexity (Adhikari *et al*., 2019). Chemical treatment includes various chemical derivates such as chlorine and hydrogen peroxide. Chlorine, however, causes the formation of by-products, which could negatively affect the environment (Hassenberg *et al*., 2017). Hydrogen peroxide, on the other hand, is sensitive to various factors, such as temperature and concentration, which could alter the treatment efficacy (Raffelini *et al*., 2011).

Ultraviolet (UV) radiation falls within photochemical treatment, which was the chosen option of this study. The disinfection mechanism of UV works on the principle of microorganisms absorbing the UV light, resulting in the damage and destruction of multiple cellular components with emphasis on the alteration and damage of genetic material, resulting in cell mortality (Premi *et al*., 2015). In addition, as the damage and destruction of cellular compounds occur, no detrimental by-products are generated during the process (Gayan *et al*., 2014). With regards to the limitations of UV treatment, the water quality, which includes the physico-chemical profile, affect the UV treatment efficacy (Farrell *et al*., 2018). One of the most important physico-chemical characteristics is ultraviolet transmission percentage (UVT %), as it specifies the percentage of UV light not being absorbed by different constituents in water. Therefore, a high UVT% indicates a higher UV dose is achievable, which would increase the efficacy of the UV radiation. As the UVT % should be considered during UV treatment of water, the physico-chemical profiles of river water were constantly investigated during this study.

This study comprised of three research chapters. Chapter 3, the first research chapter, investigated the physico-chemical and microbial profile of the Plankenburg river system at five sites. It was observed that there was variation in microbial and physico-chemical characteristics of water from these five different water sites of the Plankenburg river system. It was concluded that several

point and non-point pollution sources along the Plankenburg river system could have contributed to the variations in water quality at the five locations, as some sites had poorer microbial and physico-chemical characteristics compared to others. In addition to the microbial loads observed, STEC and ESBL-producing *E. coli* were frequently detected in the Plankenburg river system, specifically at water sites with exposure to industrial wastewaters and other non-point faecal contamination (Location 2,4 & 5). Furthermore, Location 1 & 2 had the poorest profiles, which Location 3 being the best.

Results from the first research chapter indicated that river water raises concerns regarding microbial contamination, as pathogenic microorganisms may pollute river water from various sources. Untreated river water, which is intended to be use as irrigation water, may result in microbial transfer to fresh produce. Therefore, a water disinfection system, such as UV-C radiation, was implemented to reduce microbial loads of untreated river water to acceptable limits. As results from this study also indicated, water quality variations within one river system in terms of microbial and physico-chemical characteristics, suggest that a site-specific approach should be followed when implementing on-farm UV irrigation plants. A site-specific approach and custom UV system design could be more economically viable, by appropriately sizing and optimising the UV system according to the physico-chemical profile of the water at a specific site.

During the second research chapter (Chapter 4), the effect of UV-C irradiation on heterotrophic plate counts (HPC) and total psychrotrophic aerobic bacteria count (TPAC) indicators, along with STEC and ESBL present in the Plankenburg, Eerste, Franschhoek and Mosselbank river were investigated. This chapter served as a follow-up study to work done by Bursey (2020). It was established during Bursey's study (2020) that certain HPC colonies survived various low-pressure UV doses from a collimated beam system. During this follow-up study a year later, HPC were again observed after UV radiation, with the addition of another microbial group called known as TPAC. As for the TPAC populations, UV resistant colonies were also reported. It was assumed that HPC and TPAC populations would differ from each other based on their microbial characteristics and enumeration condition, but no significant difference in counts was observed from the initial populations and colonies surviving UV. Furthermore, TPAC and HPC colonies were successfully isolated, characterised and identified after three low-pressure UV doses (20, 40 and 60 mJ.cm^{-2}).

The HPC and TPAC populations which survived UV radiation, included various microbial species, such as *Bacillus cereus*, *Aeromonas hydrophilia*, *Bacillus megaterium* and the *Exiguobacterium* genus, which were identified using MALDI-TOF. In addition, results indicated that each species showed their own unique response to UV-C exposure. In addition, STEC and ESBL-producing *Enterobacteriaceae* were detected in water from the rivers tested in this study, especially rivers downstream of wastewater effluents, posing a health risk to consumers and food safety concern.

However, results showed that STEC and ESBL-producing *Enterobacteriaceae* were sensitive to UV radiation, with all strains being eliminated by the lowest UV dose of 20 mJ.cm⁻² in treated water. As for physico-chemical characteristics, it was noted that a strong negative correlation occurs between UVT% and TDS, which may indicate that TDS is the main contributor of the low UVT%, as seen in the Mosselbank river system.

In Chapter 3 it was established that the physico-chemical profile varies at different water sites of one river system. In Chapter 4 it was illustrated that the physico-chemical profile varies between different river systems. These findings imply that water quality variations are common within and between river systems, which could have consequences for UV treatment efficacy. However, with regards to the lab-scale collimated beam, certain pathogens were effectively inactivated, regardless of the water characteristics. These findings should be considered when upscaling a UV system. When transitioning from a lab-scale to pilot-scale UV system, it is advised to implement a pre-treatment with correct UV dose contact times for best UV efficacy, when working with large water volumes on farm.

The third research chapter (Chapter 5) studied the optimisation of a medium-pressure UV disinfection system at pilot-scale, to treat water from three different rivers. During this chapter, the use of a bag filtration method as pre-treatment to UV was studied as the first objective. Results from the bag filtration study (Study One) indicated that the bag filters tested do not have a notable effect on physico-chemical characteristics, specifically dissolved solids, and microbiological content of river water. However, the smallest pore size bag filter of 5 µm showed the biggest reduction of the four bag filters tested and was the chosen bag filtration pore size for the second study.

For the UV pilot-scale testing of large river water volumes (objective two), the results from the 'before UV' samples showed that the Mosselbank and Plankenburg river water are polluted. These loads could cause microbial transfer to irrigated fresh produce, ultimately increasing the risk for consumer safety. As for the UV system, it was noticed that the Berson EC tronic Ω medium-pressure UV system had some operational limitations, as the efficacy of the UV system was influenced by the physico-chemical profile of the water. The theoretical dose that was set during the study could not be achieved in practice, as river water with poor physico-chemical characteristics limited the UV dose achieved. As for the UV disinfection effect, HPC were reduced to lower microbial loads after each UV dose. And *E. coli*, coliforms, STEC, ESBL-producing *Enterobacteriaceae, L. monocytogenes* and *Salmonella* spp. were sensitive to the UV, where no growth was recorded after any UV doses. As for the HPC that survived, various Gram-positive and Gram-negative species were identified by MALDI-TOF as *B. vesicularis*, *A. hydrophila* and *Enterococcus* species.

6.2 Recommendations for future work

During Chapter 3 (1st research chapter), the Plankenburg river system was characterised, in terms of physico-chemical and microbial characteristics, at five different river sites to establish whether water quality variations occur over a longer distance. As variations were observed it would suggest that more river systems, specifically the four chosen rivers of this study, should be characterised over a longer distance as well. In addition, investigating the different pollution sources, such as wastewater treatment effluents, domestic wastewater and run-off from agricultural activities on the water quality profile. Furthermore, during this study the presence of STEC and ESBL-producing *Enterobacteriaceae* were observed in the Plankenburg river system. However, with regards to STEC screening, it was noted that limitations might occur regarding the isolation method used. Melli et al. (2020) reported that the inclusion of STEC immunocapture is a valuable and sensitive method, as it uses serogroup-specific antigens for the most common STEC serogroups. De Boer & Heuvelink (2000) suggest that immunocapture should be implemented as a confirmation step after selective STEC media, resulting in the most accurate results.

Chapter 4 (2nd research chapter) investigated the UV-C irradiation treatment effect on heterotrophic plate counts (HPC) and total psychrotrophic aerobic bacteria count (TPAC) indicators, along with pathogens such as STEC and ESBL in four selected rivers in the Western Cape. *Bacillus cereus* (*B. cereus*), which is considered a food pathogen as it can produce toxins in foods (Begyn *et al*., 2020), was one of the microbial isolates identified from the HPC populations after UV radiation. Gdoura-Ben Amor et al. (2018) reported that selective media, such as Mannitol-egg yolk-phenol red-polymyxin-agar (MYP), are available for the detection and isolation of *B. cereus,* which would be ideal for screening a large number of samples during such a study.

Chapter 5 (3rd research chapter) studied the optimisation of a medium-pressure UV disinfection system, with bag filtration as pre-treatment, at pilot-scale on three rivers. As the bag filter resulted in only slight improvement of the physico-chemical characteristics, it would be suggested to rather explore the economic viability of other pre-treatment methods prior to UV disinfection. However, literature has indicated that very few economical options exist for the treatment of physico-chemical characteristics, which remains a challenge. Furthermore, as some HPC and TPAC populations survived the medium-pressure UV, it would be important to further identify these colonies by MALDI-TOF. The medium-pressure UV doses should also be increased, for example repeating the 20 mJ.cm^{-2} in triplicate for one water sample, resulting in a 60 mJ.cm^{-2} (3 x 20 mJ.cm^{-2}) UV dose applied.

Furthermore, the working of on-farm UV treatment plants should also be considered. As for common farm activities, river water might be stored in reservoirs or storage tanks after exposure to

an on-farm UV treatment plant. During these periods photoreactivation might occur. As briefly mentioned in the literature study (Chapter 2), photoreactivation is the phenomenon in which bacteria, which were inactivated by UV light, show the ability to be reactivated and repaired by a DNA photolyase enzyme in the presence of visible light (Van der Spek *et al.*, 1996). Studying the effect of low-pressure and medium-pressure UV on the photoreactivation phenomenon would be important to determine if microbial counts can increase again after UV disinfection.

Finally, this study has shown that some Western Cape rivers are not fit for agricultural irrigation use. Microbial loads above the recommended irrigation water limits may cause microbial transfer to fresh produce, ultimately putting the consumers and fresh produce industry at risk. The use of UV treatment for untreated irrigation water showed the potential to reduce microbiological levels to acceptable limits and inactivate specific food pathogens. During the study it was observed that the physico-chemical profile of a river could influence the efficacy of the UV treatment. However, the implementation of a pre-treatment step to enhance the UV efficacy, along with a site-specific approach to UV optimisation, could overcome these limitations.

6.3 References

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