ULTRA-VIOLET (UV) TREATMENT OF IRRIGATION WATER AT FARM LEVEL TO REDUCE MICROBIAL CONTAMINATION FOR IMPROVED FOOD SAFETY

Volume I: Laboratory-scale collimated beam and Pilot-scale UV treatment dose responses of selected indicators and specific food pathogens present in various irrigation water sources and screening of environmental isolates for antimicrobial resistance.

Report to the
Water Research Commission

by

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This is the first in a set of two reports. The other report is *Ultra-violet (UV) treatment of irrigation water at farm level to reduce microbial contamination for improved food safety, Volume II: Guidelines and recommendations for the cost feasibility estimation of UV treatment of irrigation water* (WRC Report No. 2965/2/23).

EXECUTIVE SUMMARY

BACKGROUND

The poor microbial quality of many South African rivers is an undeniable threat to consumer health. Previous and current research (as discussed in Chapters 1 and 2) has highlighted the continuing deterioration of the microbial quality of South African surface waters. Various sources of pollution, both of point source and non-point source origin, have been reported, and include, among others, the poor state of municipal wastewater treatment facilities across South Africa. The recent Green Drop Report (DWS, 2022) furthermore emphasises the fact that a very limited number of Wastewater Treatment Plants (WWTPs) function properly, which implies that improperly treated wastewater is released into the environment on a daily basis in the South African context. This is concerning from both a food security and food safety perspective, as most of South Africa's irrigation water is sourced from surface waters. The potential health implications this could have for the consumers of fresh produce urgently warrants some form of water treatment prior to crop irrigation, to prevent pathogens from entering the food distribution chain. Disinfection of surface water prior to agricultural irrigation has thus become a necessity rather than a choice, given the current South African context.

A variety of water treatment methods have been used in the past, of which the most commonly used ones are of a chemical nature. As concerns rise regarding the environmental impact, and detrimental health effects of disinfection byproducts, the advantages of residue-free UV-based disinfection become apparent. It is, however, not without its challenges and it is against this backdrop that the previous scoping study (Sigge et al., 2016), as well as the current project have been undertaken. The technology has its limitations, and certain knowledge gaps were revealed which included the following:

- The previous scoping study (WRC Report No. 2174/1/16) focussed only on the effect of UV irradiation on the levels of *E. coli* (< 1000 cfu.100 mL^{-1}) (WHO, 1989; DWAF, 1996) in river water. Other important food pathogens linked to fresh produce outbreaks, such as *Salmonella*, *Listeria monocytogenes* and Shiga toxin-producing *E.coli* (STEC), might, however, also be present in river water. Further research into the efficacy of UV disinfection on these pathogens is warranted.
- One of the limitations of the previous scoping study was that the effect of water quality on the UV treatment of river water was previously investigated using water from one site only. Fluctuations in water quality and composition (measured with quality parameters such as UVT%) could impact UV treatment efficacy, and this critical knowledge area should be expanded by including different river water sources.

- The effect of water quality on photo reactivation and dark repair of microbial populations after UV treatment, and how recovery can be minimised by increased UV dosages should be considered.
- Make recommendations as to expanding current guidelines pertaining to the microbiological quality of irrigation water for fresh produce, over and above the faecal coliform guideline levels;
- To provide practical guidelines around implementation costs (capital and operational) that should be considered for full-scale UV treatment of river water prior to irrigation.

AIMS AND OBJECTIVES OF THIS STUDY:

The purpose of the current project was thus to address the issues listed above, and expand the existing body of knowledge by formulating the following separate and specific aims:

Chapter 4.1 The aim of this study was to determine how specific microbial populations (including Heterotrophic plate count (HPC), Total Psychrotrophic Aerobic counts (TPACs), *Enterobacteriaceae* populations, as well as *E. coli* naturally present in river water (from four different sources) responded to three different doses of UV radiation (20, 40 & 60 $mJ.cm^{-2}$). The presence of specific pathogens (STEC, *Salmonella* and *Listeria monocytogenes*) was also determined before and after UV irradiation treatment. This was done in order to establish the dose response information of the selected microbial populations. In addition, some of the bacterial strains present after UV radiation were isolated and identified to ascertain the potential risk to the consumer. The antimicrobial resistance of the environmental isolates to a limited number of antibiotics were also determined as part of this study.

Chapter 4.2 The aim of this study was to determine the recovery potential of specific food pathogens and microbial populations in water after UV-C treatment. This was done to establish which UV dose would decrease the recovery ability of the selected microbial populations most.

Specific objectives included:

- Comparison of UV susceptibility of six environmental isolates and reference strains to three UV doses in a collimated beam device.
- Comparison of microbial recovery, under light and dark conditions, of selected strains after three different UV doses in three different sterile water matrices in a collimated beam device.
- Comparison of microbial recovery, under light and dark conditions, of certain microbial populations (naturally present in river water) after UV treatment in a collimated beam device.

Chapter 4.3 The aim of this study was to determine the disinfection efficiency of a pilot-scale, medium pressure UV-C system treating larger volumes of river water (from different sources), with a single UV radiation dose (1 x 20 mJ.cm^{-2}), a double (2 x 20 mJ.cm^{-2}) or triple (3 x 20 mJ.cm^{-2}) UV radiation dose.

- The first objective was to establish the efficacy of the UV system by comparing microbial loads present before and directly after each of the UV treatments.
- The second objective was to determine the recovery potential of microbial populations naturally present in river water by comparing microbial loads directly after UV treatment with samples that had time to recover for three hours after different UV treatments. Both objectives were tested as part of two studies (Study 1 and Study 2) conducted in 2021 and 2022.
- The third objective was to isolate and identify surviving colonies after UV treatment AND recovery and comment on the health risk these isolates may have for consumer health.

Chapter 4.4 Antimicrobial resistant microbes in general, and extended spectrum beta lactamase (ESBL) producers in particular, pose a threat to human health globally. In this study it is of particular interest as ESBL positive isolates have been identified in the rivers included in this project (Mosselbank, Eerste, Plankenburg and Franschhoek rivers) (Oosthuizen, 2022; Chapter 4.1, Tables 4.1.16 and A1). Thus, while the presence of ESBL-producing microorganisms in the above-mentioned rivers has been investigated and reported on in previous chapters, more in-depth antibiotic susceptibility profiles have not yet been established. This study therefore aimed to expand on the work performed by Oosthuizen (2022) and Jankowitz (In press) included in previous chapters and determine the extent of the antimicrobial resistance observed. This was done by extensively testing antibiotic resistance of river isolates to 19 antibiotics: ampicillin, amoxicillin/clavulanic acid, cefalexin, cefalotin, cefpodoxime, cefovecin, ceftiofur, imipenem, amikacin, gentamicin, neomycin, enrofloxacin, marbofloxacin, pradofloxacin, doxycycline, tetracycline, nitrofurantoin, chloramphenicol, trimethoprim/sulfamethoxazole, using the Vitek® 2 compact system.

GENERAL CONCLUSIONS

In order to fill the knowledge gaps previously identified, various iinvestigations (summarised in Chapters 4.1, 4.2 and 4.3) included testing for the presence of *Listeria monocytogenes,* STEC and *Salmonella* spp. before and after UV treatment. UV resistance profiles and recovery potential of isolates obtained from the rivers were also tested (Chapters 4.2 and 4.3), and included antimicrobial resistance testing (Chapters 4.1, 4.2, 4.3 and 4.4). Lastly, by moving from an LP laboratory-scale UV system (Chapters 4.1 and 4.2) to a pilot-scale MP UV system (Chapter 4.3) this study intended to fill

knowledge gaps and contribute towards the successful future application of UV radiation in irrigation water treatment at farm-scale.

Findings related to the physico-chemical and microbial profiles of rivers

As the previous scoping study (Sigge et al., 2016) evaluated aspects of UV disinfection while focusing on water from only one site, this project aimed to evaluate the efficacy of UV radiation – both at laboratory-scale and pilot-scale – on a variety of river water sources of varying water qualities. Based on irrigation water guidelines (summarised in Section 3.1, Chapter 3), the previous scoping study and other research (Sigge et al., 2016; Banach et al., 2021) have focused mainly on *E. coli* as indicator organism for UV disinfection efficiency. This is in spite of the fact that a number of other pathogens can be associated with contaminated fresh produce and cause disease (as discussed in Chapter 2). The effect of UV on important food pathogens other than *E. coli* was thus an important aim of this project. Including this research aim in the project was well justified considering the findings that related to the presence of specific pathogens in river water samples during the course of this study. Evidence of the presence of *Salmonella*, *Listeria monocytogenes*, ESBL positive strains and *E. coli* (>3 log CFU.mL^{-1}) and STEC was found (as summarised in Table 5.1).

The motivation to include other river water sources in this study was also based on the findings of the previous study which reported fluctuations in the physico-chemical nature of river water quality over time at the same site. This observation was also confirmed by the results of the current project. UVT% is an important parameter to consider in UV-irradiation applications, and if the results of this project over time are considered (as summarised in Figure 5.1), it is apparent that substantial variations occurred in UVT% over time at the different sites included in this project (Figure 5.1).

As highlighted in Figure 5.1 (and in Chapters 4.1-4.3), the Mosselbank river consistently had the poorest UVT%, compared to the Franschhoek river, which had the best UVT% profile. The causes for the poor quality observed at the Mosselbank site – both in terms of physico-chemical profiles and microbial risks (Table 5.1) have been discussed in detail (Chapters 4.1-4.3, Bursey, 2021; Oosthuizen, 2022) and could be directly related to the WWTP situated upstream of the sampling site, which is not unique in the South African context (Green Drop Report, 2022). What should be noted, though, are the variations in UVT% observed at the three "better" sites over time. If these variations are compared UVT% values reported in literature for water in other countries (Table 5.2), and how UVT% values should be classified (Table 5.3), it can be concluded that at some sampling occasions during the course of this project, water from all four sites could have been classified as similar to a standard of secondary wastewater effluent (UVT% equal to 60% and lower). This brings with it certain design requirements

for wastewater (USEPA, 1999) that should be considered in large-scale UV installations treating river water in the South African setting.

Findings related to UV treatment efficacy

Considering the poor UVT% values observed in this study, the addition of pre-treatments is also a possibility, but it does inevitably add to the total cost of treatment and might also have additional environmental impacts. The only pre-treatment included as part of the pilot plant UV treatment done in this study was 5 µm bag filtration (Chapter 4.3, Appendix D). It has however, been demonstrated in other research (Cantwell & Hoffmann, 2008) that UV disinfection of unfiltered surface waters, although partially inhibited, still lead to significant reductions in coliform levels.

In agreement with the findings of Catwell & Hoffmann (2008) significant reductions in microbial indicator levels were observed throughout this project, for UV doses up to 60 mJ.cm^2 in both the LP UV and the MP UV-based studies in spite of the varying UVT% levels observed. In addition, UV treatments could also successfully inactivate *Listeria monocytogenes* and *Salmonella* at the levels that they were present in the river water samples. Molecular detection of STEC also did not show any presence after UV treatment. Laboratory studies on the UV susceptibility of pure *Salmonella* (Chapter 4.2) did reveal that it might be more prone to recovery post-UV than *L. monocytogenes*.

It has also been demonstrated that certain bacteria can survive and recover post-UV after the doses applied (20-60 mJ.cm^2) (Chapters 4.1-4.3). Identification and characterisation of the strains has revealed the presence of opportunistic pathogens and strains that carry a wide range of antimicrobial resistance (AMR) determinants, even to critically important antibiotics (Chapter 4.1-4.4). The latter is a great concern, as this study provides further proof of the rapid spread of AMR within the South African aquatic environment.

Treatment cost considerations and implications

It is clear from the results presented throughout this project that UV-C treatment of irrigation water is an efficient treatment technology to reduce microbial contamination in the water. It has, however, also become clear (and this is stated in the relevant literature also) that there are several factors which influence the efficiency of the UV disinfection process. Many of the parameters are interlinked to varying degrees, but measurement of only a few parameters is often not enough to make an informed decision.

The parameters which affect the UV efficiency are as follows:

1) UV Transmittance (UVT%)
2) Total Suspended Solids (TSS)
3) Total Dissolved Solids (TDS) and/or Electrical Conductivity (EC)
4) Turbidity
5) Alkalinity & pH
6) Anions and Cations
7) Chemical Oxygen Demand (COD)
8) Microbial population
9) Flow rate and flow type
10) Water Guidelines or Targets
11) Geography on site
12) Manufacturer and reactor design

Recommendations

From the discussion in the WRC Report: *Ultra-violet (UV) treatment of irrigation water at farm level to reduce microbial contamination for improved food safety, Volume II – Guidelines and recommendations for the cost feasibility estimation of UV treatment of irrigation water*, the following information, without which an accurate estimation of cost cannot be made, should be gathered and supplied to reputable UV installers to be able to quote on a UV disinfection system for irrigation water treatment:

- Full description of the current (or required) irrigation system, including:
    - Pumps sizes and power ratings
    - Filtration equipment specifications
    - Pipe lengths and diameters
    - Head pressures
    - Flowrates required/used during irrigation
    - Hours of operation
- Full physico-chemical analysis of the irrigation water (covering seasonal variations) including:
    - UV Transmittance (UVT%)
    - Total Suspended Solids (TSS)
    - Total Dissolved Solids (TDS) and/or Electrical Conductivity (EC)
    - Turbidity
    - Alkalinity & pH

    - Anions and Cations
    - Chemical Oxygen Demand (COD)
- Full microbiological analysis (covering seasonal variations) including:
    - Coliforms
    - Faecal coliforms or *E. coli* (indicator organisms)
    - Pathogens of interest (*E.coli*, *Salmonella*, *Listeria* or others)
- Target reduction required (a decision is needed as to which organism/s are selected as the target reduction organism, and what reduction is required (e.g. 3 log reduction)

The full discussion and explanation of the above recommendations can be found in the WRC Report: *Ultra-violet (UV) treatment of irrigation water at farm level to reduce microbial contamination for improved food safety, Volume II – Guidelines and recommendations for the cost feasibility estimation of UV treatment of irrigation water*

PROPOSALS FOR FUTURE RESEARCH

The potential threat that UV surviving strains entering the fresh produce food chain holds for the consumers of fresh products does, however, depend on a variety of factors. These factors do, for instance, include the microbes' ability to attach and form biofilms in irrigation water distribution systems and on plant surfaces. This is an area that warrants urgent further research within the South African agricultural production chain.

Considering the impact that the Mosselbank river's poor quality had on results, it is recommended that the impact of municipal wastewater treatment plants (WWTPs) on both the South African environment and human health be investigated further. These WWTPs can facilitate the spread of AMR phenotypes in the environment by two means: 1. Through the discharge of diluted antimicrobials not fully metabolised by the human body; 2. Through the discharge of resistant bacteria not removed during the water treatment processes. Diluted antimicrobials in the environment can further select for resistant environmental strains as it is not present at lethal concentrations. UV disinfection leaves no residue and would be the treatment method of choice for disinfection before discharging WWTP effluent into the environment. A very important consideration would however be humic substances, which are naturally present at high concentrations in faecal matter, and in the resultant effluent. These substances have very high UV absorption characteristics and can interfere with UV treatment efficacy. The true impact it may have on South African rivers in general, and on the UV technology implementation in particular, need to be determined.

Lastly, as mentioned in the literature, LED-based UV treatment is a new field of research which aims to address some of the most important practical issues associated with UV lamps by: 1. lowering energy requirements by using LED-based lamps; and 2. replacing the mercury-based UV lamps with more environmentally friendly alternatives. Given the current energy crisis, further research in the application of UV-LEDs in the disinfection of river water in the South African context is justified.

CAPACITY BUILDING AND PRODUCTS

This WRC Project has culminated with the completion of four MSc projects and two WRC Reports.

Capacity Building:

1. Caroline Rose Bursey (MSc in Food Science – graduated March/April 2021)

Thesis title: CHARACTERISING THE MICROBIAL PROFILES OF VARIOUS RIVER SOURCES AND INVESTIGATING THE EFFICACY OF UV RADIATION TO REDUCE MICROBIAL LOADS FOR IMPROVED CROP SAFETY

2. Marco Oosthuizen (MSc in Food Science – graduated March/April 2022)

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

3. Corani Jankowitz (MSc in Food Science – In process)

Thesis title: SURVIVAL POTENTIAL OF FOOD PATHOGENS IN RIVER WATER AFTER UV-C IRRADIATION TREATMENT

In process – Estimated graduation date: December 2023

4. Margot Küster (MSc in Food Science – In process)

Preliminary thesis title (still to be finalised): THE IMPACT OF UV ON ANTIBIOTIC RESISTANT BACTERIA FROM RIVER WATER

In process – Estimated graduation date: December 2023

Products (WRC Reports):

1. Ultra-violet (UV) treatment of irrigation water at farm level to reduce microbial contamination for improved food safety, Volume I: Laboratory-scale collimated beam and Pilot-scale UV treatment dose responses of selected indicators and specific food pathogens present in various

irrigation water sources and screening of environmental isolates for antimicrobial resistance. (WRC Report No. 2965/1/23 – this report).

2. Ultra-violet (UV) treatment of irrigation water at farm level to reduce microbial contamination for improved food safety, Volume II: Guidelines and recommendations for the cost feasibility estimation of UV treatment of irrigation water (WRC Report No. 2965/2/23).

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LIST OF ABBREVIATIONS

AMR	Antimicrobial resistant
ARB	Antibiotic resistant bacteria
ARG	Antibiotic resistance genes
AST	Antibiotic susceptibility testing
BHI	Brain Heart Infusion agar
BPW	Buffered peptone water
CFU	Colony forming units
COD	Chemical Oxygen Demand
CPDs	Cyclobutane pyrimidine dimers
DBPs	Disinfection byproducts
DoH	Department of Health (South Africa)
DWAF	Department of Water Affairs and Forestry
DWS	Department of Water and Sanitation
EC	Electrical Conductivity
ESBL	Extended Spectrum Beta Lactamase
EUCAST	European Committee on Antimicrobial Susceptibility Testing
HPC	Heterotrophic plate count
L-EMB	Levine's Eosin Methylene-Blue Lactose Sucrose agar
LP	Low-Pressure
MALDI-TOF	Matrix-assisted Laser Desorption Ionisation – Time of Flight
MDR	Multi-drug resistant
MIC	Minimum Inhibitory Concentration
MP	Medium-Pressure
NTU	Nephelometric Turbidity Units
PCA	Plate Count Agar
PCR	Polymerase Chain Reaction
RV	Rappaport Vassiliadis broth
SANS	South African National Standards
Spp.	Species
STEC	Shiga toxin producing *E coli*
TDS	Total Dissolved Solids
TPAC	Total Psychrotrophic Aerobic count
TSB	Tryptic Soy Broth
TSS	Total Suspended Solids
USEPA	United States Environmental Protection Agency
UV	Ultraviolet
UVT%	Ultraviolet Transmission percentage
VRBG	Violet Red Bile Glucose agar
WHO	World Health Organisation
WRC	Water Research Commission
WWTP	Wastewater treatment plant
XLD	Xylose Lysine Deoxycholate agar

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1. INTRODUCTION

Please note: This chapter contain extracts from the MSc theses of Bursey (2021) and Oosthuizen (2022)

Water is a natural resource that is indispensable for the production of food. Approximately 63% of the available fresh water is used for agricultural purposes in South Africa (Donnenfeld *et al.,* 2018). However, population and economic growth continue to place immense pressure on the freshwater availability, limiting the quantity available for the agricultural irrigation of fresh produce (Liu *et al.,* 2017).

In South Africa, surface water, which includes rivers, dams and lakes, is the preferred source for agricultural irrigation due to the low cost and ease of usage (Singh, 2013). According to Zhou *et al.* (2012), rivers and other surface waters are, however, frequent recipients of contaminants from the surroundings. Based on the type and extent of the contaminant, the resulting water may have a negative impact on the functions that it is required for. According to Donnenfeld *et al.* (2018), over 60% of rivers in South Africa are currently overexploited.

Apart from the concerns regarding water availability, concerns regarding water safety and quality have increased dramatically in South Africa (Britz *et al.,* 2013). This could have serious consequences for food safety, as microorganism carry-over from irrigation water to crops can result in food-borne disease outbreaks (Iwu & Okoh, 2019). Irrigation water is a major pre-harvest contributor to the contamination of fresh produce with clinically significant species such as pathogenic *E coli* (including Shiga-toxin producing strains), *Salmonella, Listeria monocytogenes, Enterobacter*, *Citrobacter, Klebsiella,* viruses and parasites, as well as carriers of antimicrobial resistance determinants (Jung *et al.,* 2014; Iwu & Okoh, 2019). Other environmental sources of contamination include faecal contamination, pesticides and other chemicals and contaminated soil (Olaimat & Holley, 2012).

Painter *et al.* (2013) has furthermore reported that 46% of all foodborne outbreaks in the United States from 1998 to 2008 were traced back to produce-associated illnesses. Fresh produce-related outbreaks have increased globally in the last two decades, (Machado-Moreira *et al*., 2019; Aiyedun *et al*., 2020). Numbers related to fresh produce-linked foodborne disease in sub-Saharan Africa are, however, lacking. This is probably due to the fact that the majority of foodborne illnesses in sub-Saharan Africa, like in other developing countries, are not reported due to a lack of governmental monitoring and surveillance systems (Akhtar et al., 2014).

The Department of Water and Sanitation previously stipulated the limits for microbial loads in irrigation water, as well as other physical characteristics (DWAF, 1996a). This has since been updated by Du Plessis *et al.* (2017) with the Water Research Commission in the form of the Decision Support

System for risk-based and site-specific guidelines for irrigation water. According to the DWAF guidelines, the limit for faecal coliforms is 1 000 colony forming units (CFU) per 100 mL in water intended for the irrigation of fresh produce. Studies performed by multiple researchers in the Western Cape (Barnes & Taylor, 2004, Paulse *et al.,* 2009, Lamprecht *et al.,* 2014, Olivier, 2015, Sivhute, 2019) have indicated that microbial contamination in water from specific rivers continuously exceeded the guideline limits.

Water samples from certain sites in the Plankenburg, Eerste, Mosselbank and Krom Rivers have been analysed in a number of studies over the last decade (Lötter, 2010, Huisamen, 2012, Olivier, 2015, Sivhute, 2019). Lötter (2010) reported faecal coliform levels of 160 000 and 460 000 CFU.100 mL^{-1} in the Plankenburg and Mosselbank Rivers, respectively. Huisamen (2012) reported findings of up to 7 x 10^6 *E. coli* CFU.100 mL^{-1} in the Plankenburg and Eerste Rivers. In 2016, Alegbeleye *et al.* investigated the microbial loads in the Plankenburg and Eerste Rivers. It was reported that average bacterial counts in the Plankenburg River ranged between 3.1 x 10^5 to 6.9 x 10^8 CFU.mL^{-1}. More recently, Sivhute (2019) noted *E. coli* levels of over 3.1 x 10^6 CFU.100 mL^{-1} in the Plankenburg River. *E. coli* is frequently used as an indicator organism of faecal pollution in water (Britz *et al.,* 2012). The presence of such high levels of *E. coli* indicates that the presence of pathogens is highly likely.

There are also no guidelines in South Africa, nor many other countries around the world, that specifically address the presence of common food pathogens such as *Salmonella* species (spp.), pathogenic *E. coli* or *Listeria monocytogenes* in irrigation water. This may result in underreporting, as there is no legislative pressure to test for these organisms. Furthermore, there are currently no guidelines that address the presence of bacteria that carries antimicrobial resistance (AMR) in fresh produce irrigation water. This is in spite of the fact that contaminated fresh produce can be instrumental in AMR transmission (Samreen et al., 2021).

Both ESBL-producers and antimicrobial resistant bacteria have frequently been identified within surface waters around the world (Blaak *et al.,* 2015, Vital *et al.,* 2018). Limited research has been recorded with regard to the persistent presence of antimicrobial resistant genes in South Africa. However, the isolation of antimicrobial resistant bacteria from river water has been reported (Romanis, 2013, Lamprecht *et al.,* 2014, Sivhute, 2019). These results provide an indication of the consistent contamination of the rivers and emphasise the need for an effective method of disinfection.

Sigge *et al.* (2016) suggested that the ultimate solution for the contaminated river water problem is treating the pollution at the source, or better yet, prevention of the pollution itself. Based on water quality studies of local rivers, Britz *et al.* (2012) has suggested that treatment strategies that result in a target microbial reduction of 3-4 log units should be sufficient to result in water with *E. coli* loads that fall within the guideline limits.

Water disinfection includes physical, chemical and photochemical methods (NHMRC, 2004). Olivier (2015) states that the efficacy of these methods of water treatment is dependent on the water quality, which is highly variable in surface waters. The oldest method of water disinfection is the use of filtration techniques, where particulates are physically removed from the water (Kesari *et al.,* 2011). Chemical treatment methods such as chlorine, peracetic acid and hydrogen peroxide are of the most commonly used chemicals for water disinfection (Jyoti & Pandit, 2004). These chemicals are associated with the development of carcinogenic disinfection by-products (DBPs), particularly in the case of chlorine. This has resulted in a push towards environmentally friendly methods of water disinfection (Galvéz & Rodrígues, 2010).

Ultraviolet (UV) radiation has gained momentum as a method of disinfection due to the reduced environmental impact, no residual chemicals, and efficacy of water treatment (Liu*,* 2005, Guo *et al.,* 2009). Bolton and Cotton (2008) state that UV radiation in the germicidal UV-C range is effective at disinfecting pathogens such as *Cryptosporidium* and *Giardia* spp. which are organisms that are known to be resistant to chlorine disinfection. The nucleic acids of the microorganisms absorb the UV radiation, predominantly in the region of 253.7 nm, which results in the formation of either cyclobutane pyrimidine dimers (CPDs) or pyrimidine 6-4 pyrimidones (6-4PPs) (Dai *et al.,* 2012, Cutler & Zimmerman, 2011). This process results in the prevention of transcription, resulting in mutagenesis, and ultimately leads to cell death (Cutler & Zimmerman, 2011, Gayán *et al.,* 2012).

UV-C application technology is constantly improving, and UV-C radiation can be applied using a variety of different lamps, including low-pressure (LP) or medium-pressure (MP) mercury vapour lamps, as well as the more recent UV-LED applications (Bolton & Cotton, 2008; Umar *et al.,* 2019). Developments in the UV-LED application field are driven primarily by a need for a more environmentally friendly UV technology, as the mercury content of the original lamps, and some of the operational difficulties associated with their use and disposal, can be problematic (Umar *et al.,* 2019).

UV-C dosage requirements are dependent on a number of factors, which include both intrinsic and extrinsic characteristics (Gayán *et al*., 2014). Intrinsic characteristics include factors such as cell size, presence of UV absorbing proteins, cell wall thickness and presence of repair mechanisms, amongst others (Koutchma, 2009). Extrinsic characteristics include the physical and chemical properties of the water, such as the UV transmission (UVT%), turbidity and suspended solids, amongst others (Olivier, 2015, Farrell *et al*., 2018). Inevitably, a great deal of variation exists with regard to microbial resistance or sensitivity towards UV disinfection and needs to be taken into account when determining dosage requirements (Liu, 2005).

Overall, this method of water treatment has shown to be effective for producing a safe supply of water for the irrigation of fresh produce, as noted in previous studies (Hassen *et al.,* 2000, Jones *et al.,* 2014, Sivhute, 2019). Several factors need to be taken into account for ensuring a consistent water disinfection, which includes the variabilities in river water quality, microbial loads present and type of UV radiation equipment employed as well as recovery potential of microbial populations after treatment.

Findings from previous research (Olivier, 2015; Sivhute, 2019) indicated that the physico-chemical characteristics of the water sample may impact the UV-C disinfection efficacy, as well as increasing the exposure time required for disinfection. These studies were limited to water sampled from rivers with relatively similar physical profiles. It was, thus, recommended that the impact of the physico-chemical profile on UV treatment is studied across a broader range of river water sources.

Previous studies have investigated the effect of UV-C radiation on *E. coli strains* (Olivier, 2015, Sivhute, 2019). No research has, however, been performed in South Africa, with regard to the application of UV radiation on environmental strains of other food pathogens such as *Salmonella* spp. and *Listeria monocytogenes*, resulting in a literature gap in this area. It was, thus, recommended that the effects of UV treatment on pathogens such as *Salmonella* spp. or *Listeria monocytogenes,* are also investigated (Olivier, 2015; Sivhute, 2019).

The greater aim of the current research study was to evaluate the efficacy of UV radiation – both at laboratory-scale and pilot-scale – on a variety of river water sources of varying water qualities, and on different microbial populations in and from these rivers. This study specifically investigated the effect of UV on food pathogens, including *Listeria monocytogenes* and *Salmonella* spp. UV resistance profiles and recovery potential of isolates obtained from the rivers were tested, including antimicrobial resistance testing. Lastly, by comparing disinfection results obtained in an LP laboratory-scale UV system and a pilot-scale MP UV system. This study intends to fill knowledge gaps and contribute towards the successful future application of UV radiation in irrigation water treatment at farm-scale.

2. LITERATURE REVIEW

Please note: This chapter contain extracts from the MSc theses of Bursey (2021) and Oosthuizen (2022)

2.1 Background

The use of sunlight to inactivate microorganisms was first reported in 1877 by Downes and Blunt who noted that the germicidal effect was dependent on dosage, wavelength and microorganism sensitivity (Reed, 2010). Whitby & Scheible (2004) reported that the application of UV irradiation technology in the water treatment industry was first introduced in 1910 and has since continued to gain momentum due to its efficacy. More recently, societal trends toward more environmentally sustainable living have contributed to scientific research into alternative disinfection technologies in order to lower dependence on thermal processing and the use of chemicals, which may contribute to the formation of harmful disinfection by-products (DBP's) (Koutchma, 2009).

UV radiation occurs within the light wavelengths of 100-400 nm in the electromagnetic spectrum and UV light is divided into four spectral areas: Vacuum UV (100-200 nm); UV-A (315-400 nm); UV-B (280-315 nm); and UV-C (200-280 nm) (Dai *et al.,* 2012). Cutler & Zimmerman (2011) describe vacuum UV as being of the highest energy-containing spectral area of UV. Dohan & Masschelein (1987) reported that this type of UV radiation can be used to produce ozone for use in water treatment via the production of free radicals. USEPA (1999) has, however, stated that this type of technology is impractical due to the rapid dissipation of this energy over very short distances.

According to Gabros & Zito (2019), the ozone layer around the Earth is able to absorb all of the UV-C radiation from the sun, 90% of UV-B and minimal amounts of UV-A radiation. The reduction in the ozone layer, due to carbofluoro-carbons and other chemicals, can result in the penetration of more harmful UV rays, causing damage to human, animals and vegetation on Earth (Gabros & Zito, 2019). UV-A and UV-B radiation are notably associated with skin damage and cell injury from sun exposure. This is confirmed by experiments on fruit flies and other experimental animals that were treated with light within the UV-A and UV-B range, resulting in the formation of tumours, with the UV-A rays being the most carcinogenic (Hockberger, 2002). The damaging effects of UV-A and UV-B radiation include that of photo-aging and photo-carcinogenesis. UV-A radiation in particular is responsible for the harmful health effects as it can penetrate deep within the skin to produce free-radical oxygen species, resulting in damaged cell DNA. UV-B, on the other hand, is responsible for causing sunburn which could lead to non-melanoma skin cancers. UV-B also plays a role in the synthesis of vitamin D within the body, whereas UV-A is responsible for the tanning of human skin (Cutler & Zimmerman, 2011, Gabros & Zito, 2019). Broad spectrum sunscreens can protect the skin from irradiation by absorbing both UV-B and UV-A radiation (Gabros & Zito, 2019).

The UV-C portion of the light spectrum is strongly absorbed by the nucleic acids of the microorganisms, and therefore, has application potential in the inactivation of pathogenic and indicator organisms in both food and water samples. Within the germicidal UV-C spectrum a germicidal peak has been reported at 253.7 nm (Dai *et al.,* 2012). Most UV-C irradiation studies have been performed *in vitro* and *ex vivo* and the technology has shown great success in germicidal activity over the past 100 years (Dai *et al.,* 2012).

According to Gayán *et al.* (2012), UV-C irradiation is lethal for Gram-positive bacteria, yeasts, spores, viruses, moulds. Gram-negative bacteria show even less resistance than Gram-positive bacteria to this treatment method, due to structural cell wall differences. The resistances and lethal dosages vary widely amongst different microorganisms, as well as strain-to-strain within the same species (Gayán *et al.,* 2014). There are three considerations to take into account for quality assurance of this treatment method, the first includes that extensive knowledge must be obtained regarding the resistance of the microorganisms under investigation to the UV light, which will aid in the decision-making process for dosage requirements. Secondly, surveillance and inspection must be conducted throughout the application of UV treatment to ensure sample meets specifications, which includes the use of a calibrated UV sensor. Lastly, a broad knowledge of the available commercial UV equipment should be ensured to ascertain which is best suited for the application (Sommer *et al.,* 2008).

Developments in the field of UV-C lamp and installation technology are dynamic and constantly evolving. Traditionally however, according to Sommer *et al.* (2008), two types of UV-C irradiation technologies have been employed for water disinfection purposes. The first involves using different types of low-pressure mercury lamps that produce monochromatic UV-C emissions at a wavelength of 253.7 nm, where damage to microbial DNA is at its highest. The second type of UV-C irradiation technology require the use of mercury lamps of medium-pressure, which emit polychromatic light across a broader wavelength range. Due to the broader wavelength range, it is difficult to ascertain the exact cause of microbial inactivation, due to the fact that structural changes might not only be limited to DNA during UV-C irradiation (Sommer *et al.,* 2008).

2.2 Basic principles and equipment required for UV radiation

Traditionally, three types of commercial UV equipment design used for disinfection purposes dominate the industry, namely; open-channel systems, closed channel systems or closed-pipe systems. The most commonly used design for wastewater treatment are the open channel reactors, whereas closed-pipe systems are employed for the treatment of drinking water (Bolton & Cotton, 2008, Libhaber & Jaramillo, 2012). In both closed-pipe and channel systems, UV lamps are placed

inside of covered UV-transmitting quartz that is submerged in the flow of the water (Libhaber & Jaramillo, 2012). The function of this quartz sleeve is to protect the lamps from damage as well as maintaining a constant temperature for effective lamp functioning (IWG, 2002). The use of medium-pressure (MP), low-pressure (LP), or low-pressure high output (LPHO) mercury lamps are most regularly employed for disinfection purposes in water and food industries, the main difference being the vapour pressure at which they operate, as well as wavelength bands emitted. Mercury is the metal of choice for these applications due to the low vapour pressure and the ability to activate with ease as compared to other metals (Koutchuma, 2009). Low-pressure lamps emit single or narrow wavelength bands, targeted at the wavelength of maximum DNA absorbance, located at 253.7 nm on the electromagnetic spectrum (Kowalski, 2009). In open-channel systems, these lamps are placed perpendicularly or in parallel to the flow (Bolton & Cotton, 2008). Medium-pressure lamps emit a wider polychromatic spectrum of wavelengths between 200 and 600 nm, with only between 15-23% of emissions at the maximal DNA absorbance wavelength of 253.7 nm (Kowalski, 2009). According to Zimmer & Slawson (2002), the increased pressure and intensity of this radiation from a MP lamp reduces the number of lamps that are required. The lamps are always placed in a manner to provide the highest intensity.

2.3 Mechanism of inactivation by UV radiation

As the UV light spreads through a water sample, it interacts with particles within the water resulting in it being reflected, absorbed, refracted or scattered. This interaction with intrinsic matter determines the efficacy of microorganism inactivation (Koutchuma, 2009). The mechanism of microbial inactivation by UV radiation is as a result of lethal DNA damage (Reed, 2010). The nucleotides and proteins of a microbial cell are the only components that are able to absorb UV radiation in significant amounts in this specific wavelength range (Bolton & Cotton, 2008). Proteins are responsible for most of the absorption of UV radiation below 230 nm, whereas nucleotides dominate absorbance above 230 nm. Due to the fact that water is able to absorb light below 230 nm, higher dosages of UV radiation will be required for disinfection at lower wavelengths. Therefore, maximum disinfection efficacy can be obtained by utilising wavelengths higher than 230 nm, as water absorbance at these wavelengths is decreased, and the DNA of an organism can be effectively targeted. This results in the requirement for higher UV doses to disrupt protein activity within microorganisms as compared to doses required for DNA and RNA disruption (Bolton & Cotton, 2008). DNA contains phosphate groups, sugar moieties and either a pyrimidine or purine molecule. Due to the fact that the phosphate and sugar molecules absorb UV radiation below 210 nm, the purine and pyrimidine molecules absorb UV-C at higher wavelengths, and are thus targeted (Gayán *et al.,* 2014).

A small increase in UV-C absorption observed at 280 nm for proteins can be attributed to the aromatic amino acids such as tryptophan, tyrosine and phenylalanine, with tryptophan absorbing the strongest at 280 nm. These aromatic compounds, along with pyridines, pyrimidines, and flavins, contain conjugated double bonds that hold two electron pairs. The two molecular bonds occurring within these structures are Sigma (σ) and Pi (π) orbitals. Within conjugated ring structures of these compounds; large, non-localized π-orbitals dominate, which are stable and exhibit a longer wave function than σ-orbitals (Cutler & Zimmerman, 2011). The absorption of the photon energy by these bonds, results in the promotion of the electrons to high energy levels, and leads to the conversion to vibrational energy. This unstable state must return to ground state either via the dissipation of this gained energy or through bond rotations (Cutler & Zimmerman, 2011).

Within RNA, uracil and cytosine are the targets of UV inactivation whereas thymine and cytosine are targeted in DNA. Damage to the DNA occurs following the absorption of photons of energy and the formation of one of six possible photoproduct formations. These include cyclobutane pyrimidine dimers (CPDs) and to a lesser extent, pyrimidine 6-4 pyrimidones (6-4PPs) as described by Cutler & Zimmerman (2011) and Dai *et al.* (2012). It is interesting to note that pyrimidines are ten times more sensitive to UV energy than purines (Gayán *et al.,* 2013). Gayán *et al.* (2014) further report that CPDs are formed when a pyrimidine molecule absorbs a photon of energy, causing it to covalently bond via the carbon-5 and -6 of an adjacent pyrimidine molecule forming a ring structure. Unstable products such as oxetane and azetidine are produced in the formation of 6-4PP molecules, when the carbon-5 and -6 of a pyrimidine molecule reacts with the fourth carbon on a carbonyl or amino group for the 3' neighbour. Photoproducts of UV irradiation include thymine-thymine, uracil-uracil, cytosine-cytosine, cytosine-thymine, uracil-thymine and uracil-cytosine dimers, with the first two dimers requiring the least energy to form. Thymine dimers are produced with the highest yield, and form after the hydrogen bond linkage between bases is lost, resulting in carbon-5 and -6 becoming cross-linked. This ultimately results in the prevention of further transcription and replication, resulting in mutagenesis and ultimately leading to cell death (Reed, 2010, Cutler & Zimmerman, 2011, Gayán *et al.,* 2013). Bolton & Cotton (2008) noted that once a certain number of dimers is formed within microbial cells, DNA replication will be inhibited and this, then, is the primary UV disinfection mechanism.

2.3.1 Ultraviolet dosage requirements

The UV dose can be defined as the measurement of the energy per unit area that falls upon a surface and is most commonly expressed in $mJ.cm^{-2}$ (Johnson *et al.,* 2010). According to Gayán *et al.* (2014) UV dosage requirements are dependent on microbial sensitivities and may be attributed to intrinsic

and extrinsic factors. Cell wall structure and thickness, the presence of UV absorbing proteins, cell and genome size and most importantly, the ability of the microorganism to repair the genetic damage are amongst the many intrinsic factors that determine microbial susceptibility to UV light (Koutchuma, 2009). Differences in UV sensitivity between different microorganisms result in difficulty in determining UV dosage requirements to achieve a predetermined log reduction in mixed microbial populations (Bolton & Cotton, 2008). To overcome this limitation, UV dose-response curves have been developed, using collimated beam tests, to effectively treat samples to achieve certain log reduction, taking resistant microorganisms into consideration. As a general rule of thumb, Gram-negative microorganisms are more sensitive to UV radiation than Gram-positive organisms, yeasts, spores, moulds and viruses (Gayán *et al.,* 2011). Therefore, it is of great importance to determine the microbial type and load present within the water source in order to apply an effective dose to achieve the required result.

The USEPA (1999) suggests that a dose of between 21-36 $mJ.cm^{-2}$ should be sufficient in the inactivation of bacterial and viral pathogens depending on water quality parameters such as turbidity and COD. Table 2.1 provides a summary of the estimated dosages required to achieve a 1-log reduction (D_{10}) in specified microorganisms within certain groups.

Table 2.1 Dosage requirements for different microorganisms groups as well as specific organisms treated with UV radiation at 253.7 nm to achieve a 1-log reduction (Amoah *et al.,* 2005, Koutchuma, 2009, Gayán *et al.,* 2014, USEPA, 1999)

Microorganism group	Average UV dose required ($mJ.cm^{-2}$)
Enterobacteriaceae	2.0-8.0
E. coli O157: H7	3.5
Salmonella typhi	1.9
Others from Bacteria domain	
Listeria monocytogenes	2.6
Pseudomonas aeruginosa (ATCC 10145)	4.6
Staphylococcus aureus (ATCC 6538)	5.6
Cocci and micrococci	1.5-20.0
Enterococcus faecalis	4.2
Enterococcus faecium	3.4
Spore formers	4.0-30.0

Microorganism group	Average UV dose required ($mJ.cm^{-2}$)
Clostridium perfringens spores	16.7
Bacillus subtilis spores	16.9
Protozoan pathogens	5-120.0
Cryptosporidium parvum	<5
Giardia lamblia	<10
Yeast	2.3-8.0
Fungi	30.0-300
Algae	300.0-600.0

According to Gayán *et al.* (2011), it is difficult to accurately measure the UV dose that is absorbed by microorganisms since it is highly dependent on the exposure time and radiation. The magnitude of this dependence is determined by the flow of the water through the equipment. It has also been reported that a turbulent flow will increase the efficiency of treatment as compared to laminar flow (Gayán *et al.,* 2011). Gayán *et al.* (2014) state that larger sized cells have a greater resistance to UV radiation due to an increased probability of the energy-containing photons being absorbed by other components within the cell, and therefore, not causing damage to the DNA. This can be used to explain the much higher dosage requirements from larger cells such as moulds and yeasts as compared to bacteria. Olivier (2015) states that complex samples such as irrigation water that contains a broad microbial population with various pathogenic strains may reduce the efficacy of this treatment method, and needs to be taken into account when evaluating the disinfection efficacy.

2.3.2 Microbial resistance and photo-repair mechanisms

A combination of intrinsic and extrinsic parameters is responsible for the ability of microorganisms to resist inactivation due to UV radiation. Dai *et al.* (2012) state that fast multiplication rates allow microorganisms to rapidly adapt to environmental stresses, which result in favourable competitive advantages that will eventually spread throughout the entire population. Gayán *et al.* (2014) described that the main parameters that determine microbial resistances to UV treatment are wavelength and UV dosages. A higher lethal efficiency is achieved at wavelengths that are close to the absorption peaks of DNA. Intrinsic factors include the species and strain of the microorganism. It has been noted that *Enterococci* spp. are some of the most tolerant bacteria to UV treatment whereas *Staphylococcus* spp. show a much greater sensitivity to this treatment method, with dosage requirements of 12 $mJ.cm^{-2}$ and 2 $mJ.cm^{-2}$, respectively (Gayán *et al.,* 2014). Increased resistances in bacterial spores

can be attributed to their relatively dehydrated state and the thick spore protein-coating preventing the formation of pyrimidine dimers (Riesenman & Nicholson, 2000). A study performed by Sommer *et al*. (2000) noted that widely-diverse doses are required for enteropathogenic *E. coli* inactivation, which exhibited 13 different D_{UV} values for the same log reduction. Gayán *et al.* (2014) state that, in general, pathogenic bacterial strains are more resistant to UV radiation than non-pathogenic strains.

A study performed by Gayán *et al.* (2012) on the effect of UV radiation on different *Salmonella* species with regard to their individual resistances, noted that *Salmonella typhimurium* STCC 878 was the most resistant strain, requiring 18.03 $mJ.cm^{-1}$ to achieve a 4-log reduction. The most resistant microorganism to date is that of *Deinococcus radiodurans,* an extremophilic bacterium, which requires a dose of between 19.7 and 145 $mJ.cm^{-2}$ for inactivation (Koutchuma, 2009). According to a study performed by Dai *et al.* (2008), excessive repetition of UV radiation may result in resistances developed by microorganisms.

Kalisvaart (2004) explains there are two mechanisms of DNA repair, the first is that which requires light and therefore, termed photoreactivation. The other is that which does not, namely, dark-repair. The use of either mechanism is dependent on the biological organisation level as well as the kind of damage inflicted. The ability of microorganisms to repair their DNA after replication errors or endogenous and exogenous DNA-damaging agents is due to various enzymatic repair pathways. Mismatch repair (MMR), base excision repair (BER) as well as nucleotide excision repair (NER) are the repair mechanisms that have been adopted to repair damage to DNA without the dependence of light. These are all based on the principle of the splicing out of the damaged region and the insertion of new bases and ligation of the damaged pieces (Friedberg, 2003, Rastogi *et al.,* 2010, Gayán *et al.,* 2013). Nucleotide excision repair is one of the most versatile and flexible repair mechanisms found in organisms and is highly conserved in eukaryotes (Rastogi *et al.,* 2010). Two modes of NER have been established, and include repair of damage over an entire genome and repair of transcription-blocking lesions that are present in transcribed DNA strands (De Laat *et al.,* 1999). Nucleotide excision repair involves the direct removal of the nucleotides containing lesions, and can remove a broad spectrum of lesions through a variety of cascade reactions carried out by the U_{VR}ABC exonuclease enzyme. This excision method removes large lesions such as CPDs, 6-4PPs as well as some forms of oxidative damage (Rastogi *et al.,* 2010).

Base excision repair (BER) arises from hydrolytic deamination and involves the removal of the damaged base, resulting in an apurinic or apyrimidinic site that is subsequently removed (De Laat *et al.,* 1999). This repair mechanism is dependent on glycosylase enzymes for the recognition of specific lesions within the nucleotide bases, which are then removed. The efficiency and specificity of this repair process is dependent on the different forms of this enzyme which are responsible for the

removal of differently affected bases (Rastogi *et al.,* 2010). Mismatch repair (MMR) mechanisms are used to repair mistakes made in the replication processes, performed by DNA polymerase enzymes. This is achieved by a group of proteins that detect and correct base errors which occurs when bases are incorrectly inserted, deleted and incorporated within the DNA strand.

Water is treated within enclosed treatment systems, but is often pumped into holding tanks or drained into surface waters before use in irrigation systems. This exposure to sunlight may affect the quality of the water post-treatment, where microorganisms that have been previously deactivated are able to repair themselves and cause an increase in pathogenicity (Quek & Hu, 2013). Photoreactivation is a light-mediated enzymatic repair mechanism that involves the action of the photolyase enzyme, which binds to the CPD lesions for removal. The photolyase enzyme contains FAD as a cofactor and a chromophore as a light harvesting antenna (Thoma, 1999). Together with the chromophore, which is responsible for the conversion of light energy to chemical energy, the enzyme directly reverts the damaged DNA to its undamaged form (Clancy, 2008). In the case of CPDs, this enzyme functions by binding to the DNA, flipping the pyrimidine dimer out of the DNA strand and into a hole that contains the FAD molecule. Light-initiated electron transfer reactions cause the cyclobutane ring to be split. Other enzymes required for photoreactivation include endonuclease, polymerase and ligase (Kalisvaart, 2004). This type of repair is not limited to bacteria, but extends to viruses, algae, protozoa, vertebrates and mammals (Das, 2001).

Kalisvaart (2004) states that for the initiation of the photoreactivation process, exposure to light between 310 and 480 nm is required for a few minutes, depending on the organism. Organisms have developed such diverse repair mechanisms to environmental stresses that below the lethal UV dose, the potentially lethal effects of this treatment method can be avoided. In water treatment systems, indicator organisms are often able to reactivate easier than the pathogens, which results in an overestimation of the pathogen numbers (Zimmer & Slawson, 2002). Zimmer (2003) noted that pathogenic *E. coli* O157:H7 was able to undergo dark repair as well as photoreactivation when treated with LP lamps but showed no detectable repair when treated with MP lamps. Quek & Hu (2013) state the possible reasons for this includes increased dimer formation, damage to critical replication enzymes or amino acids and damage to the photolyase enzyme occur in the presence of polychromatic MP UV radiation, which does not occur under monochromatic light emitted during LP UV radiation.

The damage to the photolyase was also reported in a study by Quek & Hu (2008) where it was determined that MP UV radiation caused the oxidation of the FAD molecule within the enzyme. A study performed by Oguma *et al.* (2005) investigated the effect of various wavelengths using MP UV radiation to determine dimer repair ability *in vivo*. It was noted that dimer repair was not affected by single wavelengths and therefore, the action of simultaneous exposure to multiple wavelengths

facilitated photoreactivation suppression (Oguma *et al.,* 2005). Quek & Hu (2013) performed a study on photolyase to determine the effect of radiation, prior to the UV treatment, on the enzyme activity at different wavelengths. This study utilised one photolyase enzyme which was exposed to UV radiation and one that was not. A decreased dimer repair rate was noted (between 8 and 25%) in the exposed photolyase experiment as compared to the unexposed enzyme experiment.

For applications such as irrigation systems, it is important to take the contribution of natural sunlight into account when determining photoreactivation ability. Guo *et al.* (2009) performed a study in which a lamp emitting a spectrum representing that of natural sunlight was utilised to determine the ability of sunlight to initiate photoreactivation post-treatment. It was reported that for the *E. coli* strain *CGMCC 1.3373* showed 50% and 20% photoreactivation after initial UV treatment using 5 $mJ.cm^{-2}$ using LP and MP UV systems, respectively. This provides an indication that, at low UV doses and more specifically the use of LP UV systems, sunlight may play a significant role in microbial repair (Guo *et al.,* 2009). Areas where sunlight is plentiful and temperatures between 23-37 °C are a cause for concern for photoreactivation (Quek & Hu, 2008).

Furthermore, it was established that higher wavelengths result in increased ability for photolyase repair as compared to lower wavelengths. This could be attributed to photolyase absorbing strongly below 300 nm, with maximum absorbance peaks noted at 280 m and emission peaks at 266 nm and 365 nm, and therefore, damage to the enzyme could alter its repair mechanisms. In the study performed by Quek & Hu (2013), wavelengths of 254 nm exhibited the lowest dimer repair rate, as compared to wavelengths of 365 nm which showed to have increased dimer repair rates. This may be due to the fact that the enzyme utilises light energy between 300 and 500 nm to perform its repair and therefore, wavelengths of 365 nm are likely to aid in the process of photoreactivation (Quek & Hu, 2013). The study concluded by noting that shorter wavelengths have a greater ability at preventing dimer repair, however, filtered MP UV radiation at 254 nm has a greater limiting effect than LP UV radiation at 254 nm due to the filtered MP UV including peaks at 266 nm (Quek & Hu, 2013).

2.3.3 Advantages and Limitations of this treatment method

Several advantages of the use of UV technology for disinfection purposes have been proposed by Bolton & Cotton (2008). These include that this treatment method is effective at not only inactivating bacteria, but extremely effective for the disinfection of *Cryptosporidium* and *Giardia* spp. The equipment is relatively simple to use and can be adjusted according to site-specific water quality and target organisms. This is a rapid treatment method, requiring only a few seconds as compared to upwards of 30 minutes of contact time for certain chemicals. Possibly the biggest advantage of this

treatment method is that there are no DBPs formed and therefore, can be considered to exert a small footprint on the environment as well as no residuals remaining in the water (Bolton & Cotton, 2008; USEPA, 1999).

Disinfection residuals are maintained in chlorine-treatment systems via the addition of chlorine or chloramines after initial treatment, this however, is not possible for UV treatment systems and therefore, can be seen as a limitation (Bolton & Cotton, 2008). Difficulties in the monitoring of the UV dosages result in reliance on sensor readings or water flow rates and thereby, providing some level of inaccuracy. Mercury lamps provide some level of hazard due to the possibility of breakages. Highly turbid water samples may result in decreased water treatment efficiency and therefore, the requirement for pre-treatment methods such as filtration becomes essential, which could inevitably increase treatment costs (IWG, 2002). Lastly, limited water disinfection can occur when the lamp is warming up or if there are power interruptions and therefore, would affect water treatment capacity (Bolton & Cotton, 2008). These limitations could be overcome by using a solar power installations or the utilisation of a battery back-ups (Bolton & Cotton, 2008).

There are also several factors related to water quality that can influence the efficacy of UV as a treatment method. Absorption of light is defined by Koutchma (2009) as the transformation of photon energy to other energy forms following the interaction with a substance. The higher the level of absorbance by substances, other than the target organism within the sample, the lower the dose delivery for the intended microbial inactivation. Hassen *et al.* (2000) and Gayán *et al.* (2011) describe the factors that affect the efficacy of this treatment method in disinfection applications as lamp ageing, turbidity, Total Suspended Solids (TSS), Chemical Oxygen Demand (COD), and the percentage UV transmittance (UVT%). Liu (2005) describes that temperature and pH have no direct effect on disinfection efficacy. However, this statement may come as a contradiction to a study performed by Gayán *et al.* (2012) on the effect of UV radiation on *Salmonella* spp. in combination with mild heat treatments (UV-H). This study showed that temperatures of 50-60°C increased the lethality of the radiation by between 1.18 ± 0.06 and 6.62 $\log_{10}$ cycles, respectively. The inclusion of heat proved successful in comparison to the application of UV irradiation at room temperature, which hardly achieved a reduction of 0.64 ± 0.08 $\log_{10}$ cycles for the same *Salmonella* strains. The mechanism of increased inactivation with this combination method is as a result of bacterial cell envelope damage due to the applied heat.

The pH of the sample may play a role in negatively impacting treatment, as an increased pH may result in dissolved metals precipitating out of solution, leading to an increase in turbidity, and a subsequent decreased inactivation efficacy (Farrell *et al.,* 2018). The COD provides an indication of the level of organic pollution within a water sample. Total organic carbon and phenols contribute to the

absorption coefficient of water. Since both organic and inorganic compounds are able to absorb UV light in water, it is noted that both COD and Total Dissolved Solids (TDS) are inversely proportional to the UVT% (Olivier, 2015).

Suspended solids, according to Abdul-Halim & Davey (2016), reduce the efficacy of this treatment method either by shielding the microorganisms from the UV radiation or by absorbing the UV radiation, thereby, reducing the energy available for microbial inactivation. When UV light is absorbed by suspended solids, it is no longer available for the inactivation of microorganisms and should be taken into account when determining the dosage requirements (Liu, 2005).

High turbidity levels in water impacts disinfection efficiency by lowering the UVT%, whereas lowering turbidity improves the disinfection capacity by increasing the UVT%. This is due to the fact that, much alike suspended solids, microorganism aggregates can be enclosed within the particulates in the turbid samples, which increases the microorganism's resistance to UV penetration dramatically (Farrell *et al.*, 2018). Liu (2005) reported that an increase in turbidity from 1 to 10 NTU would reduce the average dosage by between 5% and 33%.

Olivier (2015) states that the age of the lamp used in the UV system may affect the disinfection efficiency as a result of microorganism's potential to repair themselves following treatment with older lamps. High levels of dissolved substances such as iron, calcium and hydrogen sulphide negatively impact this treatment method by the formation of a thin coat around the unit, which decreases the UV intensity and therefore reduces output power (USEPA, 1999, IWG, 2002).

The UV dose applied for disinfection can be expressed as being the product of intensity and exposure time ($J.m^{-2}$ or $mJ.cm^{-2}$ or $mW.s.cm^{-2}$). Intensity is affected by both water quality and the output of the lamps within the system. Intensity decreases with an increase in turbidity and suspended solids as the UV light becomes obstructed or absorbed by other particulates in the water. Optimal disinfection results may require a pre-filtration step to remove these particulates within the water (IWG, 2002).

Photoreactivation is of concern in surface waters that are exposed to sunlight as it provides the radiation required for organisms to repair and increase the pathogenicity. This can be overcome by utilising MP UV lamps as well as dosages that would completely inactivate the photolyase enzyme.

2.4 Concluding remarks

The promotion of healthy eating has resulted in an increased consumption of fresh produce around the world. Due to the fact that fresh produce is mostly eaten raw or after minimal processing, there is a potential health risk for the consumption of pathogens (Alegbeleye *et al.,* 2018). Contamination of fresh produce has frequently been associated with irrigation water of poor microbial quality

(Pachepsky *et al.,* 2011). Microorganism carry-over from irrigation water to crops is a major concern in the case of food safety, since it can result in foodborne outbreaks (Huisamen, 2012). Surface waters are the preferred source of water supply for irrigation purposes in South Africa due to the fact that it is more economically feasible to obtain than ground waters, however, the risk of contamination of surface waters as compared to ground waters is substantially higher (Singh, 2013). The microbial and physico-chemical characteristics of surface waters vary widely. This can be attributed to a number of factors including upstream commercial and recreational activities, resulting in the contamination of water (Sousa *et al.,* 2007). These factors need to be taken into account when determining the appropriate treatment to ensure a consistently safe water supply. Continued investigations into the efficacy of different water treatment methods are imperative to make accurate recommendations for

Ultraviolet radiation is now a well-established method of disinfection and is routinely used in multiple sectors. As with all treatment methods, there are both advantages and limitations of this treatment method. Short-contact time, ease of operating and limited microbial resistance are possibly the main advantages of UV radiation. Ultraviolet radiation is effective for the treatment of organisms such as *Cryptosporidium* and *Giardia* spp. which have shown resistances to chemical treatments such as chlorine (Bolton & Cotton, 2008). This method of treatment does not result in the formation of DBPs, however, as there are no residuals that remain in the water (as there is with chemical treatments), the chance of recontamination downstream is a possibility. Photoreactivation of microorganisms is another limitation of UV radiation, however, the use of MP lamps and higher dosages are effective measures to combat this possibility.

The efficacy of UV radiation has routinely been tested at laboratory-scale, using LP and/or MP lamps and most often using laboratory-cultured, reference strains of *E. coli* only. The need for investigations regarding efficacy of this treatment with the goal of upscaling to farm-level treatment is imperative to understand how effective this treatment would be against naturally occurring microbial populations.

Knowledge gaps also exist in South Africa regarding the efficacy of UV radiation on the disinfection of irrigation water containing food pathogens such as *Listeria monocytogenes* and *Salmonella* spp. This could be attributed to the fact that limits related to these organisms are not specified in the irrigation water guidelines (DWAF, 1996a). These pathogens are frequently present in surface waters, and therefore, might pose a significant risk to irrigation water safety. They do, however, often go undetected as there is no legislative pressure to test for them.

One way in which this potential threat to irrigation water safety can be addressed is by investigating how these pathogens respond to UV light in water with different physico-chemical

profiles. Optimising UV radiation processes under varying conditions is essential to improve disinfection efficacy and address any potential threat to irrigation water safety.

Discrepancies in literature exist with regard to specific UV dose requirements. These, can be attributed to variations in physico-chemical characteristics of the surface waters investigated, as well as a lack of insight into environmental pressures that might cause genetic mutations and increased resistances of specific strains and the variety of microbial populations naturally present in the water that varies over time (USEPA, 1999, Campbell *et al.,* 2002, Amoah *et al.,* 2005, Hijnen *et al*., 2006, Gayán *et al.,* 2011, Gayán *et al.,* 2014). Therefore, the current study was performed in order to address some of these knowledge gaps, and provide recommendations to individuals treating irrigation water with UV irradiation technologies.

It can, however, be concluded from literature that the use of UV technology is an attractive and effective treatment method for water. The prevention of DBPs, the reduction of requirements on harmful chemicals further increase the attractiveness of this method. This treatment method has proven to be successful as a treatment method for water, even being able to produce water of a high enough standard for drinking.

3. GENERAL METHODS

The aim of this section is to describe a collection of general methods that were used throughout this study in all the respective MSc projects involved (See 'Chapter 8. Capacity building and Products' for more information on the individual MSc projects that were conducted as part of the overall study). By including this section, the authors intend to prevent repetition and promote readability of 'Section 4. Research Chapters'. All experimental design descriptions, as well as any methodologies that were only used once as part of certain individual sections, are described in the related sections under 'Section 4. Research Chapters'.

3.1 Guideline limits for irrigation water

Throughout the study results were compared to the existing Water Quality Guidelines for Irrigation Water (DWAF, 1996a) that specifies guideline limits for various physico-chemical and microbial characteristics of water intended for agricultural irrigation. These limits are presented in Tables 3.1 and 3.2. Some of the physico-chemical parameters that were recorded in this study are not stipulated in the irrigation water guidelines (DWAF, 1996a) and results were compared to other standards instead (as indicated in Table 1c).

It is acknowledged that the DWAF guidelines have been augmented by more recent publications (Du Plessis *et al.,* 2017) including the Water Research Commission which propose a site, and purpose-specific approach to determine water quality acceptability, rather than absolute standards. The latter does, however, not provide absolute guideline targets which can be used as "targets" for UV irradiation optimisation purposes.

Table 3.1 Suggested limits for Faecal Coliforms (*E. coli*) in irrigation water (DWAF, 1996a)

Faecal coliforms (*E. coli*) levels in irrigation water	**Effects on crops**
1-1 000 CFU.100 mL^{-1} (or <10 CFU.mL^{-1})	• Direct irrigation of crops eaten raw might result in pathogen transfer to consumers. • Direct grazing of cows on irrigated pastures that is still wet, might result in contaminated milk. • Crops not eaten raw and animal pastures could be irrigated directly but must be allowed to dry before harvest/grazing. • Fruit trees and grape vines can be irrigated only if fruits are not wetted.
>1 000 CFU.100 mL^{-1} (or >10 CFU.mL^{-1})	• Irrigation of fodder, tree plantations, nurseries and parks are allowed, but human contact with water is not advisable.

Table 3.2 Guideline limits used in this study for monitoring and evaluation of physico-chemical characteristics of river water (based on suggested limits for 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
Conductivity	40 $mS.\ m^{-1}$

Table 3.3 Additional guideline limits used in this study for monitoring and evaluation of physico-chemical characteristics NOT specified as part of the suggested limits for agricultural irrigation water (DWAF, 1996a)

Water quality characteristics	Chosen limit
Turbidity [1]	10 NTU
Alkalinity [2]	< 120 mg $CaCO_3.L^{-1}$
COD [3]	< 75 mg $O_2.L^{-1}$.
UVT%[4]	Not specified

[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 UVT% values were interpreted according to the literature summarised Section 4.1

As listed in Table 3.1, the Water Quality Guidelines for Irrigation Water (DWAF, 1996a) only provide microbial guideline limits for Faecal coliforms (*E. coli*) and protozoan cysts that need to be monitored to ensure water and ultimately crop safety. Table 3.4 compares these guidelines for irrigation water (DWAF, 1996a), to guidelines given for water intended for domestic use (DWAF, 1996c) and guidelines for fresh produce safety (DoH, 2002).

Table 3.4 Microbiological standards comparison (for selected food and water-borne pathogens) between irrigation water guidelines (DWAF, 1996a and 1996c) and fresh produce (DoH, 2002) in South Africa, specifically for important food pathogens

Microorganism	Microbial load		
	Irrigation water	**Domestic use**	**Fresh Produce**
E. coli (CFU.100 mL^{-1})	< 1 000	< 10	0
L. monocytogenes (CFU.g^{-1})	NS	NS	absent in 25 g
Salmonella spp. (CFU.g^{-1})	NS	NS	absent in 25 g
Protozoan pathogens	< 1 cyst	< 1 cyst	absent

3.2 River water sampling procedure

The South African National Standards (SANS) 5667-6 method for sampling(SANS, 2006) was followed to obtain water samples from the rivers using a sterile 1 L Schott bottle. Where necessary, samples were collected using a sampling pole to which the 1 L sterile bottle was attached. Water samples were then transported to the laboratory on ice. Microbiological tests were performed within six hours, and all physico-chemical analyses were performed within 24 hours.

3.3 Physico-chemical analysis of river water samples

In this section the standard methods used throughout this study to determine physico-chemical parameters of river water samples, are described.

3.3.1 Total Dissolved Solids

The total dissolved solids (TDS) content of each water sample was determined using a (TDS)-3 meter (HM Digital). This handheld meter was used to determine the total amount of mobile charged ions, which is directly proportional to the electrical conductivity of the sample. The meter expresses the reading in parts per million (ppm) which equates to $mg.L^{-1}$. Each sample was analysed in duplicate, after which an average value was obtained.

3.3.2 Total Suspended Solids

The Standards Methods (APHA, 2005) were consulted to obtain instructions for the measurements of total suspended solids (TSS). The TSS was determined by filtering a river water sample through a Munktell glass microfiber filter, which was then placed in a crucible at 105°C for 2 hours. The weights of the crucible were recorded before and after heating. Cooling was allowed in a desiccator after

heating, and the respective calculation was performed to determine the values for TSS, which were expressed in units of $mg.L^{-1}$. All tests were performed in duplicate.

3.3.3 Turbidity

The turbidity of river water samples was measured using a portable Orion AQ3010 Turbidity Meter (Thermo Scientific, USA). Values were expressed in Nephelometric Turbidity Units (NTU). Prior to use, the instrument was calibrated with samples of known turbidity, starting with the standard of 800 NTU. All samples were then analysed in duplicate.

3.3.4 Chemical Oxygen Demand (COD)

This is a measure of the quantity of oxygen that is available for consumption by oxidative reactions in a solution. The COD was measured photometrically using the Spectroquant Nova 60 COD Cell Test (Merck Millipore, South Africa) and expressed in units of mg $O_2.L^{-1}$. River water samples were tested in the range of 10-150 mg $O_2.L^{-1}$. Following the standard testing procedure – three millilitres of each sample were added to a standardised test vial that contained the required reagents, and vortexed. Samples then were allowed to digest at 148°C for 2 hours in a thermal reactor (Hach, USA). The samples were allowed to cool following digestion before COD values were measured using the Spectroquant NOVA 60 Spectrophotometer (Merck Millipore, South Africa) in duplicate.

3.3.5 Ultraviolet Transmission percentage (UVT %)

A Sense T254 UV Transmission (%) Photometer was utilised to determine the UVT% (Berson, Netherlands). The procedure provided by the manufacturer was followed, and the photometer was calibrated using distilled water which represented a UVT % of 100%. Analyses was performed in duplicate.

3.3.6 pH

Using a portable pH meter (WTW, Germany) according to the instructions of the manufacturer, duplicate readings for each river sample were obtained. The instrument was calibrated before each use using standard pH solutions (pH 7, pH 4 and pH 10).

3.3.7 Alkalinity

The alkalinity value of each water sample was obtained by performing a titration according to Standard Methods (APHA, 1999). A solution on 0.1 N H_2SO_4 was prepared and titrated into a beaker

containing 50 mL of sample. The titration was performed until a pH of 4.3 was recorded. The volume of H_2SO_4 required was then used in a calculation to determine the alkalinity in units of mg $CaCO_3.L^{-1}$. *Alkalinity values were not specified in the Irrigation Water Guidelines (DWAF, 1996a) and therefore, the Industrial Water Guidelines were consulted (DWAF, 1996b). A value of < 120 mg $CaCO_3.L^{-1}$ was selected as these guidelines state little to minor impairment of product quality.

3.3.8 Electrical Conductivity (EC)

The quantity of the dissolved salts in the river samples was determined by using a portable HI 8733 conductivity meter (Hanna Instruments, USA). The instructions for calibration provided by the manufacturer were followed. Samples were then measured in duplicate with the readings recorded (in mS. m^{-1}) and an average value obtained.

3.4 Microbiological analysis of river water samples and microbial inoculums

Throughout this project, river water samples were analysed for the presence of a variety of indicators and pathogens, both before and after UV irradiation. The choice of indicator organisms varied between studies and will be discussed in more detail as part of separate studies under under 'Section 4. Research Chapters'. In this section the most commonly used methodologies are summarised.

3.4.1 Dilution series preparation

Standard dilution series were prepared (10^{o}-10^{-6}) in 9 mL units of autoclaved Buffered Peptone Water according to the SANS 6887-1 method (SANS, 1999). These dilution series were used for the enumeration of *Enterobacteriaceae*, *E. coli* and Heterotrophic plate count (HPC) populations in river water, as well as for certain pure strain inoculums. Dilutions were transferred to the relevant agars using standard pour plate methods (in duplicate). Colony numbers between 10 and 300 per plate were recorded for all indicator organisms.

3.4.2 Heterotrophic Plate Count (HPC) identification and enumeration

Heterotrophic plate count was determined using Plate Count Agar (PCA) (Oxoid, South Africa) according to the SANS method 5221 (SANS, 2011a). Straw-coloured colonies (between 10 and 300) were counted following inverse incubation at 30°C for 72 hours.

3.4.3 Escherichia coli identification and enumeration

The presence and number of *E. coli* was determined using Brilliance Coliform/*E. coli* Selective Agar (Oxoid, South Africa) following instructions provided by the SANS method 5221 (SANS, 2011a). Purple

colonies were regarded as indicative of the presence of *E. coli,* while both purple and pink colonies represented the Coliform count, after inverse incubation at 37°C for 24 hours.

3.4.4 Enterobacteriaceae identification and enumeration

Enterobacteriaceae counts were determined using Violet Red Bile Glucose (VRBG) Agar (Oxoid, South Africa), and the standard pour plate technique according to the SANS method 4832 (SANS, 2007a). Following inverse incubation at 35°C for 24 hours, presumptive positive results were identified as purple/ pink coloured colonies.

3.4.5 Salmonella species identification

Salmonella spp. were identified using the presence/absence method, which required a two-step enrichment strategy (non-selective, and a selective enrichment), followed by streaking on two selective agars, Xylose Lysine Deoxycholate Agar (XLD) and Hektoen Enteric Agar (Oxoid, South Africa), according to SANS method 19250 (SANS, 2011b). For the enrichment steps, a 25 mL sample of river water was first incubated in autoclaved Buffered Peptone Water for 24 hours at 35°C, after which 0.1 mL was transferred to 10 mL of autoclaved Rappaport Vassiliadis (RV) broth (Oxoid, South Africa), and incubated at 42°C for a further 24 hours. Using a sterile loop, each sample was then streaked onto XLD and Hektoen Enteric Agar plates, in duplicate. These plates were incubated at 35-37°C for 24 hours. Red colonies with black centres were considered as indicative of *Salmonella* spp. on XLD Agar and blue-green coloured colonies with black centres indicated *Salmonella typhimurium* on Hektoen Enteric Agar.

3.4.6 Listeria monocytogenes identification

In order to determine the presence of *Listeria monocytogenes* in water samples, SANS Method 11290-1 (SANS, 2007b) was followed. This method applies to both foodstuffs and environmental samples, including water samples. A 25 mL sample of river water was inoculated into 225 mL of autoclaved Half-Fraser Broth that contained a *Listeria* Selective Supplement (BioRad, South Africa) and incubated for 24 hours at 30°C. A sterile loop was then used to streak this solution onto duplicate plates of Rapid' *L. mono* Agar Plates (BioRad, South Africa), and incubated at 35°C for 24 hours. A presumptive positive result for this test showed blue/black coloured colonies on the red agar plates after 24 hours of incubation at 35°C.

3.4.7 *Identification of bacterial isolates using MALDI-TOF*

Environmental isolates were purified and prepared for species identification with a MicroFlex LT

Matrix-Assisted Laser Desorption/ Ionisation Time of Flight (MALDI-TOF) mass spectrometer (Bruker Daltonics, Germany). The MALDI Biotyper 3.0 software was utilised to determine the identity of each isolate by comparing spectra with reference strains in the database. The similarity of a reference strain to the tested isolate was correlated using a logarithmic score, which was interpreted according to the manufacturer's guidelines (Zulu, Z. 2020, Researcher, Faculty of Natural and Agricultural Sciences, University of Pretoria, Pretoria). A log value of ≥ 2.300 indicates the species identification with a high level of confidence. An intermediate log value score of ≥ 2.000 indicates a probable species identifcation. A log value of between 1.700 and 1.999 indicates only the identification of a genus. Any scores below 1.700 does not allow for any identification to be made. (Zvezdanova et al., 2020)

3.5 UV treatment of microbial innoculums and river water samples

3.5.1 Collimated Beam LP UV system – laboratory scale treatments

A collimated beam low-pressure (LP) UV system was used for treatment of water samples at laboratory scale. A simplified design can be seen in Fig. 1 below. This instrument utilises an Amalgam LP mercury vapour lamp (UV-Technik, Germany) that has an output power of 40 W and an arc length of 25 cm. The single beam of light emitted by the instrument is predominantly at the germicidal wavelength of 253.7 nm.

For each sample the UVT% of the river water was first established, while the UV lamp was allowed to heat up for 10 minutes, before treatment commenced. Before each treatment was applied, the intensity of the UV light was determined at the surface of the liquid sample, using an ILT1400 radiometer (International Light Technologies, USA) coupled with a XRL140T254 detector and recorded intensity in units of $mW.cm^{-2}$. The UV light intensity measured, and the UVT% determined, were used to calculate the exposure time required for each UV dose.

Equation 1 below represents the method of calculating the average intensity of the UV light (Hallmich & Gehr, 2010). This value is required to further calculate (in Equation 2 below) the required time of exposure to UV radiation to achieve a desired dose (in $mJ.cm^{-2}$).

$$I_{\text{avg},\lambda\,(mW.cm^{-2})} = I_0\lambda \left[\frac{1-e^{d\ln(\text{UVT}(\lambda))}}{-d\ln(\text{UVT}(\lambda))}\right] \quad \text{.... [1]}$$

In this equation (Equation 1), $I_{(\text{avg},\lambda\,.)}$ refers to the average UV light intensity over sample depth (*d);* UVT (λ) is the UV transmission of the sample measured at a wavelength of λ (254 nm), using an optical path length of 1 cm, and $I_0(\lambda)$ refers to the UV light intensity measured at the sample surface. The

value obtained is then inserted into Equation 2 below, which provides the exposure time required to achieve a specific UV dose (Hallmich & Gehr, 2010).

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

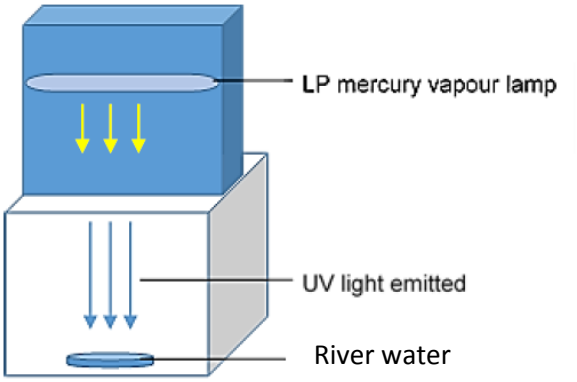
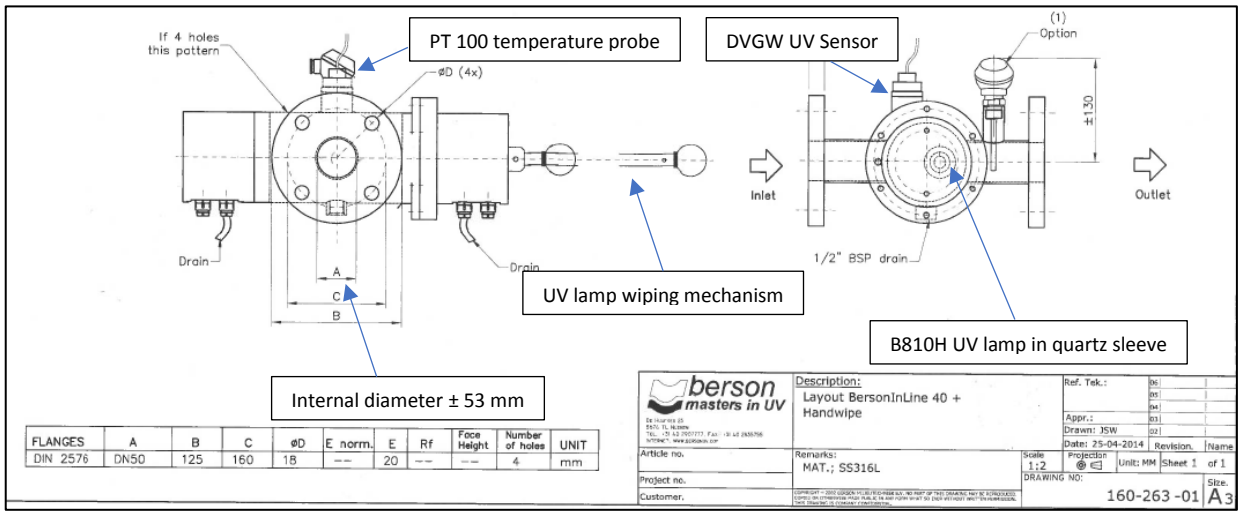


Figure 3.1 Diagram to indicate the design of the bench-scale collimator device used to perform the laboratory-scale UV experiments (Berson, The Netherlands) (Olivier, 2015).

3.5.2 Pilot-scale MP UV system

The medium-pressure (MP) UV system employed in the pilot-scale studies is similar to the one used in previous work (Sigge et al., 2016). UV irradiation was performed using a Berson InLine 40+ UV (Fig. 3.2) disinfection system (Berson, The Netherlands). This utilised a B810H MP UV lamp installed perpendicular to the flow of water in the piping network. Light was emitted in the range of 220 to 580 nm. The flow rate required to deliver a desired UV dose was calculated with reference to the UV transmission percentage (UVT%) of the river water at the time. The computerised UV system allowed the operator to adjust the flow rate, in units of $m^3.h^{-1}$, and quantified the delivered UV dose in units of $mJ.cm^{-2}$. The rate was adjusted on the digital interface of the UV system to establish a value that corresponded to the desired UV dose. The UV system has its own inline DVGW UV sensor (Berson, The Netherlands) measuring the UV dose applied to the water, which measures the UV dose ($mJ.cm^{-2}$) 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). Water flow was then regulated manually by manipulating a valve installed in the piping system before the UV lamp and flow rate was measured by means of an in-line rotameter.



FLANGES	A	B	C	ØD	E norm.	E	Rf	Face Height	Number of holes	UNIT
DIN 2576	DN50	125	160	18	--	20	--	--	4	mm

Figure 3.2 Schematic diagram of Berson InLine 40+ UV reactor disinfection system

Water was collected in 1 000 L batches and pumped to a holding tank through a bag filter (5 micron), before UV treatment commenced, as demonstrated in Fig. 3.3. Higher doses were applied cumulatively by pumping UV treated water into another clean holding tank and recirculating it through the MP UV system again. Specific doses applied are specified in the research chapters under section 4.

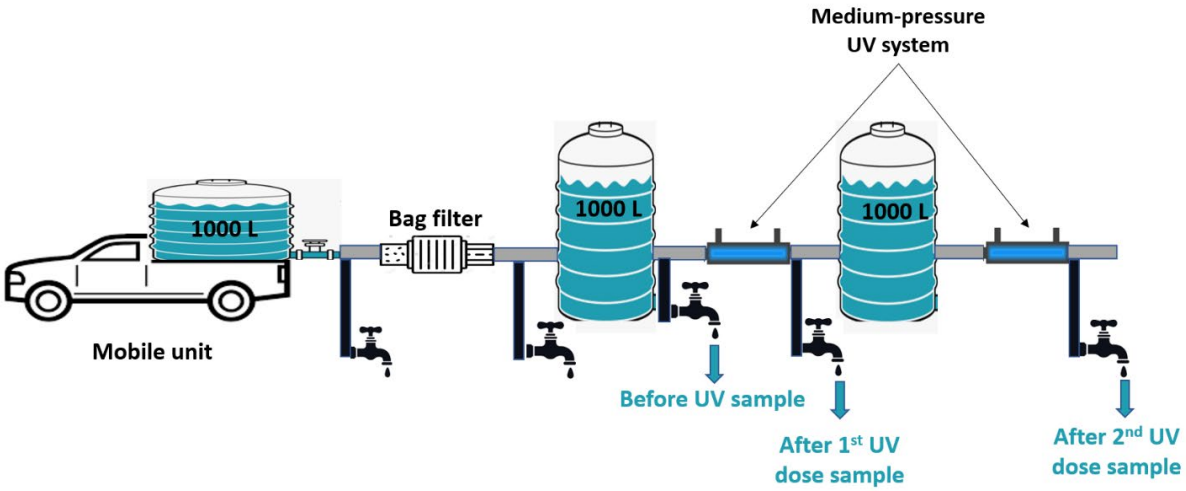


Figure 3.3 Visual illustration of the pilot plant system that includes bag filtration and UV disinfection. (Sampling points, where water samples were withdrawn for further analysis, are indicated) (Oosthuizen, 2021)

3.5.3 Pilot plant cleaning procedure

After use, 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 doesn't leave a residue (Stericlear, 2021. [Internet Document] URL. https://stericlear.co.za/sp/oxyfect-starter-kit/ Accessed 25/1/2023)

4. RESEARCH CHAPTERS

4.1 Laboratory-scale collimated beam UV treatment dose responses of selected indicators and specific food pathogens present in various irrigation water sources

Please note: This chapter contain extracts from the MSc theses of Bursey (2021) and Oosthuizen (2022)

4.1.1 Background & Aim

Important food pathogens, such as Shiga toxin producing *E coli* (STEC), *Salmonella* and *Listeria monocytogenes*, are associated with both contaminated fresh produce and contaminated water sources. The effect these organisms can have on human health are summarised in Table 4.1.1. The Guideline limits for irrigation water do, however, not include any limits for these three organisms, although food safety regulations indicate that they should be absent in fresh produce (as indicated in Table 3.4, 'Section 3. General methods'). As discussed, foodborne disease outbreaks linked to fresh produce have been traced to contaminated irrigation water (Jung *et al.,* 2014; Iwu & Okoh, 2019). In the interest of irrigation water safety, it is important to know the extent of the presence of clinically significant populations, and specific pathogens such as STEC, *Salmonella* and *Listeria monocytogenes* in river water sources, as well as the effects of UV irradiation on them.

The aim of this study was to determine how specific microbial populations (including Heterotrophic plate count (HPC), Total Psychrotrophic Aerobic counts (TPACs), *Enterobacteriaceae* populations, as well as *E. coli*) naturally present in river water (from four different sources) responded to three different doses of UV radiation (20, 40 & 60 $mJ.cm^{-2}$). The presence of specific pathogens (STEC, *Salmonella* and *Listeria monocytogenes*) was also determined before and after UV irradiation treatment. This was done in order to establish the dose response information of the selected microbial populations. In addition, some of the bacterial strains present after UV radiation were isolated and identified to ascertain the potential risk to the consumer. The antimicrobial resistance of the environmental isolates to a limited number of antibiotics were also determined as part of this study.

Table 4.1.1 Three food pathogens, linked to both water and Food safety, and their effect on human health as well as sources of contamination (Bursey (2021, p25))

Microorganism	Infectious dose	Incubation period	Symptoms	Source of contamination	Reference
E. coli (O157:H7)	10-100 CFU	3-4 days	Watery diarrhoea, low grade fever, stomach cramps	Raw meat (beef), dairy products, fresh produce, faecal matter and water sources	Baylis *et al.* (2011), Rahal *et al.,* (2012)
L. monocytogenes (listeriosis)	10-10 million CFU in healthy individuals 0.1-10 million CFU in high-risk individuals	1-4 weeks Febrile gastroenteritis from *L. monocytogenes* *18-20 hours*	Fever, vomiting, diarrhoea, weakness, confusion	RTE foods, dairy products, poultry, vegetables and water sources	Schweon, (2015)
Salmonella spp. (Enteric fever).	20-10^6 CFU depending on health of individual	12-36 hours	Headache, abdominal pain, diarrhoea or constipation, fever	Eggs, poultry, pork, beef, dairy, fruits and vegetables and faecal matter, contaminated water sources	Forsythe (2010), Eng *et al.,* (2015), Andino & Hanning (2015)

4.1.2 Materials & Methods

4.1.2.1. Experimental design

Selected microbial populations and specific pathogens from four different rivers, and their responses to UV treatment, were investigated as part of two studies during two summer irrigation periods: Study 1 (October 2019-January 2020) (Bursey, 2021); and Study 2 (January 2021-March 2021)(Oosthuizen, 2022). The physico-chemical profiles of all water samples were analysed before UV treatment. Microbial analyses (which were different for the two studies) were conducted before and after three different UV doses: 20, 40 & 60 $mJ.cm^{-2}$.

4.1.2.2 Site selection for both studies

The same four river sites (representing four different rivers close to the Cape Winelands District, Western Cape, South Africa) (summarised in Table 4.1.2) were used in both Studies 1 and 2. These 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, acted as a study control site as previous studies from various authors have indicated very high microbial loads at this site (Lamprecht et al., 2014; Sivhute, 2019). 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, 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 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 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.

Table 4.1.2 Four river sampling location names and descriptions of this study (Oosthuizen, 2022)

River site 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

4.1.2.3 General methods

The methods used are presented below in two different parts: Study 1 and Study 2. Similar methodologies are indicated where applicable.

Study 1 (October 2019-January 2020) (Bursey, 2021)

Sampling frequency

The four selected river sites were sampled five times each over the summer irrigation period, from October 2019-January 2020. The river water sampling procedure described in Section 3.2 was followed.

Physico-chemical analysis of river water samples

All analyses were conducted as described in 'Section 3. General methods'. These included Ultraviolet Transmission percentage (UVT %), Total Dissolved Solids (TDS), Total Suspended Solids (TSS), Chemical Oxygen Demand (COD) Turbidity, pH and Electrical Conductivity (EC).

Ultra-violet treatment using collimated beam LP UV system

A bench-top collimated beam UV system was used for treatment of water samples, as described in Section. 3.5.1. Sample size and preparations prior and during UV were done as described by Bursey (2021). Three UV doses (20, 40 and 60 $mJ.cm^{-2}$) were applied in sequential manner on the same sample, and the required volume for microbial testing was removed after each dose. As the sample depth in the beaker decreased, following each dose and test volume removal, the exposure time

required for each dose had to be recalculated for each sample depth according to the dose calculations described in Section 3.5.1.

Microbial analysis of river water samples

Microbial counts of water samples before and after UV irradiation were conducted as described in 'Section 3. General methods' and included the Heterotrophic plate count (HPC) and *Enterobacteriaceae* populations, as well as *E. coli.*

Isolation and identification of environmental strains during Study 1

During the course of Study 1, various microbial strains were isolated from river water samples pre- and post-UV treatment, and stored at -80°C in the presence of 25% ($v.v^{-1}$) glycerol for further identification and characterisation according to methods described in detail by Bursey (Bursey, 2021). A brief summary of the findings are included in this chapter.

Identification of isolates using MALDI-TOF

Environmental isolates were identified using a MicroFlex LT Matrix-Assisted Laser Desorption/ Ionisation Time of Flight (MALDI-TOF) mass spectrometer (Bruker Daltonics, Germany) according to the method described in Section 3.4.7

Molecular confirmation and lineage typing of Listeria monocytogenes strains

The identification of presumptive *L. monocytogenes* strains isolated from river water samples as part of the study of Bursey (2021) were confirmed using PCR analysis according to the method of Rip & Gouws (2020). Strains were then further analysed for lineage type according to the method of Rip & Gouws (2020).

Antimicrobial resistance of environmental strains

A selection of environmental strains was also tested against a limited selection of antibiotics to determine if any antimicrobial resistant phenotypes were present. Breakpoints and testing methods are described in detail in Bursey (2021). A short summary of the results is included in this study.

Study 2 (January 2021-March 2021) (Oosthuizen, 2022)

Sampling frequency

The same four selected river sites (used for Study 1) were sampled on three separate rounds in duplicate over the 2021 summer irrigation period (January 2021-March 2021). The river water

sampling procedure described in Section 3.2 was followed. Each river was sampled on three separate rounds in duplicate.

Physico-chemical analysis of river water samples

Similar to Study 1, all analyses were conducted as described in 'Section 3. General methods'.

Ultra-violet treatment using collimated beam LP UV system

A bench-top collimated beam UV system was used for treatment of water samples, as described in Section 3.5.1. Sample size and preparations prior and during UV were done as described by Oosthuizen (2022). Three UV doses (20, 40, and 60 $mJ.cm^{-2}$) were applied in sequential manner on the same sample, and the required volume for microbial testing was removed after each dose. As the sample depth in the beaker decreased, following each dose and test volume removal, the exposure time required for each dose had to be recalculated for each sample depth according to the dose calculations described in Section 3.5.1.

Microbiological analysis of river water samples

In this study, the HPC population was monitored using Plate Count Agar (PCA) according to the SANS method 5221 (SANS, 2011) (Oxoid, South Africa), as described in '*S*ection 3. General methods'. As described in Study 1 all dilution and UV doses were prepared in duplicate using the pour plate technique where the straw-coloured colonies were counted following the incubation at 30°C for 72 hours.

The enumeration of Total Psychrotrophic Aerobic counts (TPACs) present in River water samples both before and after treatment, were determined using Plate Count Agar (PCA) according to the SANS method 5221 (SANS, 2011) (Oxoid, South Africa), with the exception that the plates were incubated at 22°C for 72 hours, similar to the method described by Holvoet *et al.* (2014).

Isolation of presumptive positive ESBL colonies

A 100 mL volume of each river water sample was filtered through sterile cellulose nitrate membrane filter with pore size of 0.45 µm and diameter of 47 mm (Millipore, South Africa). Membrane filters were then transferred with sterile forceps to 20 mL of buffered peptone water (BPW) and incubated at 37°C for 2 hours. After incubation, 1 mL of BPW was transferred to 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 re-streaked on CHROMagar ESBL plates (MediaMage, South Africa) to ensure purity of colonies. Pink coloured colonies were picked and streaked onto VRBGA agar and incubated at 37°C for 24 hours. Purple/ pink colonies (*Enterobacteriaceae* indication) were then 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 Tryptic Soy Broth, and incubated at 37°C for 24 hours glycerol stock cultures were prepared and stored at -80°C.

Isolation and identification of environmental strains during Study 2 (including MALDI-TOF)

During the course of Study 2, various microbial strains were isolated from river water samples pre- and post-UV treatment, and stored at -80°C in the presence of 25% ($v.v^{-1}$) glycerol for further identification and characterisation according to methods described in detail by Oosthuizen (Oosthuizen, 2022). A brief summary of the findings are included in this chapter. Characterisation also included MALDI-TOF analysis for strain identification according to the method described in Section 3.4.7.

Isolation and confirmation of ESBL-producing Enterobacteriaceae

A 100 mL volume of each river water sample was filtered through sterile cellulose nitrate membrane filter with pore size of 0.45 µm and diameter of 47 mm (Millipore, South Africa). Membrane filters were then transferred with sterile forceps to 20 mL of buffered peptone water (BPW) and incubated at 37°C for 2 hours. After incubation, 1 mL of BPW was transferred to 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 re-streaked on CHROMagar ESBL plates (MediaMage, South Africa) to ensure purity of colonies. Pink coloured colonies were picked and streaked onto VRBGA agar and incubated at 37°C for 24 hours. Purple/ pink colonies (*Enterobacteriaceae* indication) were then 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 Tryptic Soy Broth, and incubated at 37°C for 24 hours glycerol stock cultures were prepared and stored at -80°C until further analysis.

Confirmation of ESBL-production by *Enterobacteriaceae* isolates was done according to the EUCAST (2020) disc diffusion testing procedure. According to the latter, Mueller-Hinton agar (Oxoid, South Africa) was inoculated with an isolate, after which the discs of ceftazidime [30 µg], cefotaxime

[30 µg] and cefepime [30 µg], each individually and in combination with clavulanic acid [10 µg] (Davies Diagnostics, South Africa), were applied. 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.

Molecular detection of the presence of STEC

The presence of STEC in river water samples was determined using the PALL Gene Disc system according to the methods described by Oosthuizen (2022).

4.1.3 RESULTS

4.1.3.1 Study 1 (October 2019-January 2020) (Bursey, 2021)

The four rivers investigated in this study were sampled five times each over the summer irrigation period (October 2019-January 2020). The physico-chemical analyses of each river were determined in duplicate for each sample and an average was calculated, which can be seen in Table 4.1.3. Where possible, results were compared to the guideline limits provided in the Irrigation Water Guidelines (DWAF, 1996a) (see Section 3.1 in chapter 3).

The UV doses applied were kept constant across all the river water samples treated, but exposure times had to be adapted to compensate for variations in the UVT% values between the different river water samples. Table 4.1.4 provides an example of exposure times for the three UV doses across the four river samples with varying UVT% readings. As observed, longer exposure times in the collimated beam deivce was the result of lower UVT% values, with the Mosselbank sample requiring the longest UV exposure, and the Franchoek river the shortest UV treatment.

Table 4.1.4 Exposure times (min) calculated from the UVT (%) of each river water sample, UV lamp intensity (78.8 mW.cm^{-2}) and sample depth for the three specified UV dosages, based on data obtained in the first sampling occasion

Rivers	Plankenburg	Eerste	Mosselbank	Franschhoek
UVT %	60.3	62.2	22.2	80
UV doses (mJ.cm^{-2})	Exposure time (min: sec)			
20	9:02	8:41	22:24	6:06
40	16:29	15:55	38:42	11:36
60	22:31	21:50	49:22	16:35

Table 4.1.3 Physico-chemical characteristics of the four selected rivers used for irrigation over five sampling occasions during the summer irrigation period (October 2019-January 2020). SD-Standard Deviation

	Plankenburg						Eerste						Mosselbank						Franschhoek					
Parameter	**1**	**2**	**3**	**4**	**5**	**Avg.** **SD**	**1**	**2**	**3**	**4**	**5**	**Avg.** **SD**	**1**	**2**	**3**	**4**	**5**	**Avg.** **SD**	**1**	**2**	**3**	**4**	**5**	**Avg.** **SD**
UVT%	60.3	60.0	64.5	61.2	31.2	55.4 *13.7*	62.2	62.0	63.9	56.1	52.1	59.3 *5.0*	22.2	30.0	28.3	25.0	30.2	27.1 *3.5*	80.0	80.0	79.2	80.2	49.1	73.7 *13.7*
TDS (mg. L^{-1})	180	160	143	165	485	226 *145*	245	316	285	297	272	283 *27*	831	850	802	903	540	786 *142*	96	95	96	83	244	123 *68*
EC ($mS.m^{-1}$)	0.30	0.19	0.17	0.22	0.55	0.28 *0.16*	0.34	0.43	0.32	0.37	0.34	0.36 *0.04*	1.0	1.16	0.91	0.99	0.63	0.93 *0.20*	0.13	0.11	0.14	0.13	0.26	0.15 *0.06*
COD (mg O_2. L^{-1})	29	22	19	52	202	63 *79*	25	12	18	34	16	21 *9*	59	50	57	47	47	52 *6*	12	10	10	13	24	14 *6*
Turbidity (NTU)	18.5	15.5	11.1	16.0	27	17.6 *5.9*	3.4	3.4	3.5	2.6	2.3	3.0 *0.6*	9.9	7.7	7.1	17.8	9.6	10.4 *4.3*	2.0	2.6	2.3	2.2	8.9	3.6 *3.0*
TSS (mg.L^{-1})	19	19	6	5	18	14 *7*	9	11	8	4	14	9 *3*	9	24	8	13	22	15 *7*	6	5	1	3	7	4 *2*
pH	7.21	6.91	6.88	6.72	6.48		7.59	7.41	7.28	7.38	7.35		7.39	7.49	7.31	7.43	7.19		6.76	6.78	6.75	6.79	7.14	
Alkalinity (mg.L $CaCO_3^{-1}$)	75	90	63	70	168	93 *43*	106	106	93	100	95	100 *6*	290	205	220	270	165	230 *50*	50	45	55	45	135	66 *39*

Microbial loads present in untreated river water

Microbial levels for specific indicator populations (HPC, *Enterobacteriaceae* and *E. coli*) present in river water, were determined before UV treatment by recording colony counts between 10-300 CFU.mL^{-1}. Specific pathogens tested included *Salmonella* spp. and *L. monocytogenes,* which were tested on a presence/absence basis only, according to standard methods. Results are presented in Figures 4.1.1 and 4.1.2, as well as in Table 4.1.5.

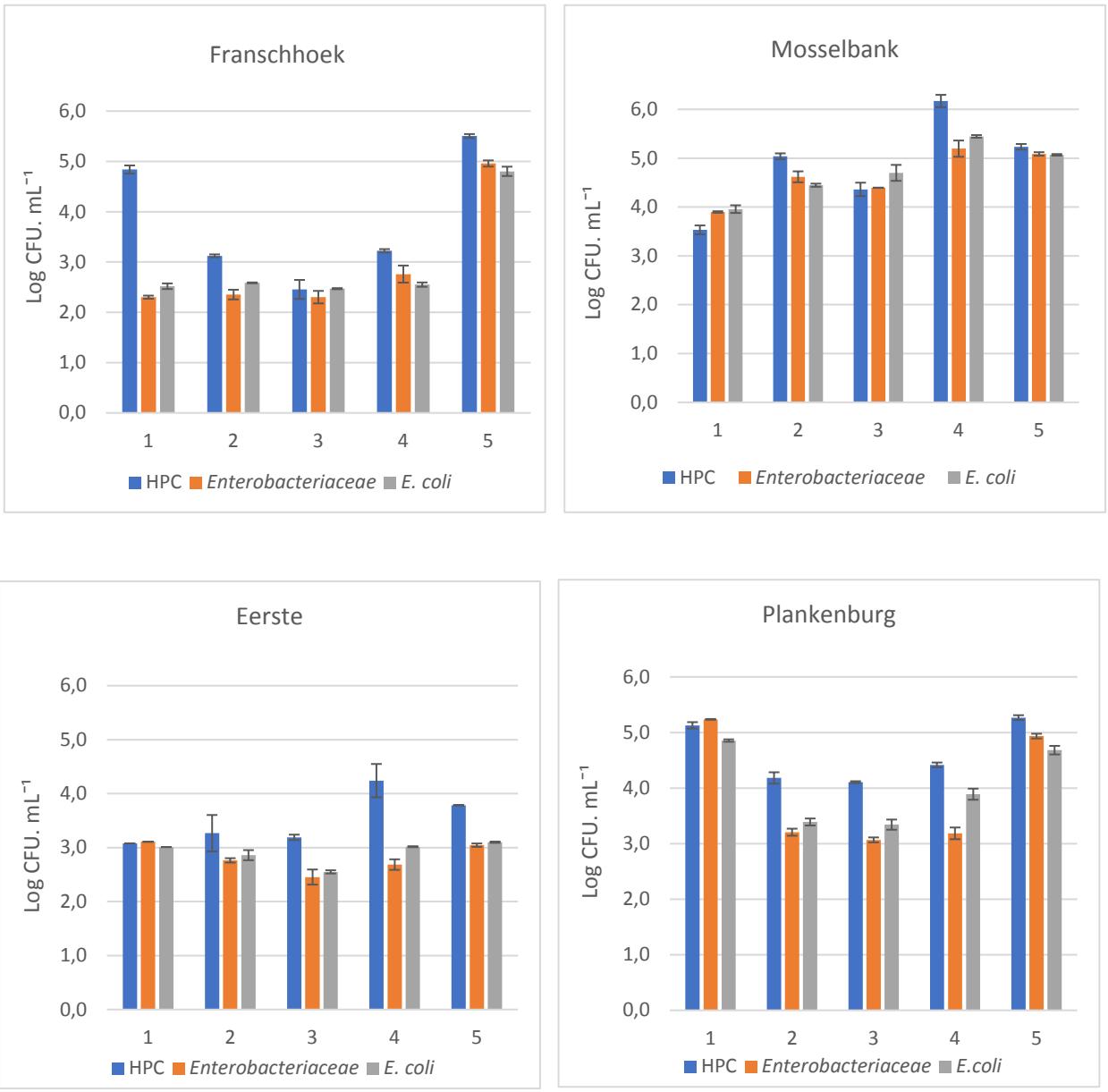


Figure 4.1.1 Results of indicator organism colony counts **before UV** treatment was applied, expressed as log CFU.mL^{-1}, with standard deviation error bars included.

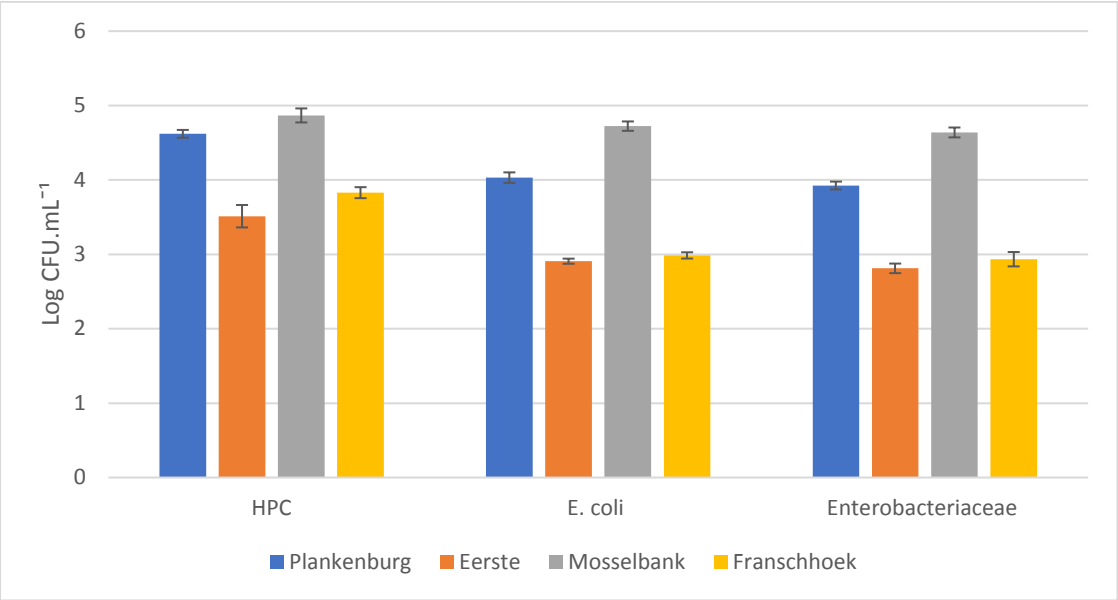


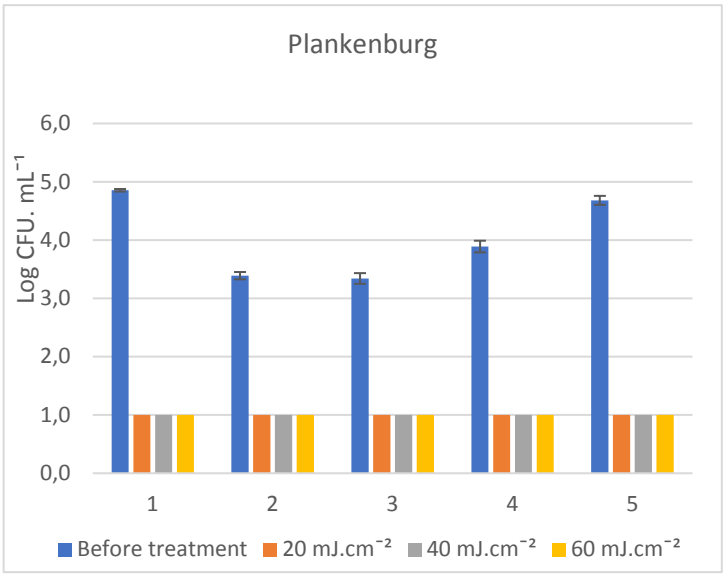
Figure 4.1.2 Average colony counts for all indicator organisms tested across five sampling occasions, indicated as Log CFU.mL^{-1} with standard deviation bars included for error across sampling occasions.

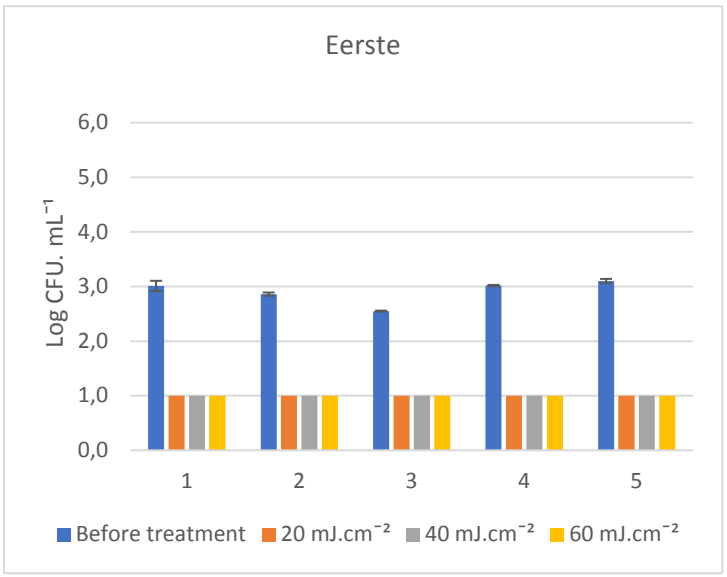
Table 4.1.5 Presence and absence testing results from the testing of *Salmonella* spp. and *L. monocytogenes* in river water samples, prior to the application of UV radiation. Shaded blocks with a "+" sign represent a positive test for the specific organism over the five sampling occasions

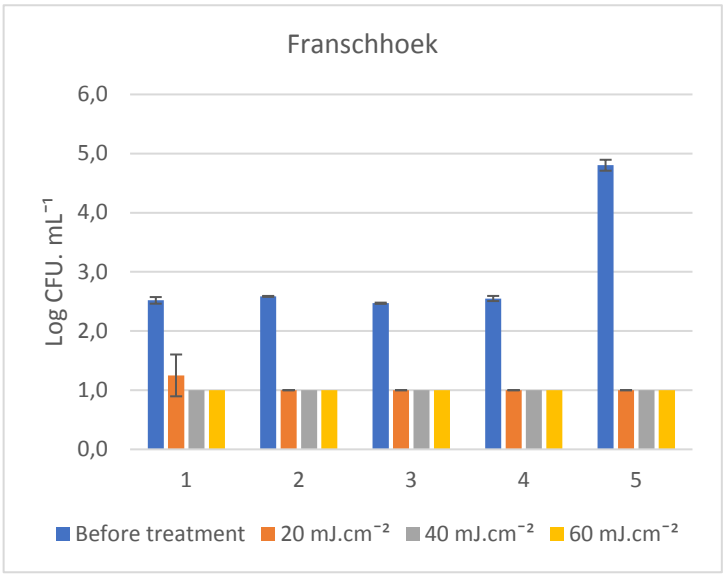
River	Pathogen tested	1	2	3	4	5
Plankenburg	*Salmonella* spp.	+	+	-	+	+
	L. monocytogenes	+	+	+	+	+
Eerste	*Salmonella* spp.	-	-	-	-	+
	L. monocytogenes	+	+	+	+	-
Mosselbank	*Salmonella* spp.	-	+	-	+	+
	L. monocytogenes	+	+	+	+	+
Franschhoek	*Salmonella* spp.	-	-	-	-	+
	L. monocytogenes	-	+	+	-	+

Effect of UV irradiation treatment on microbial loads present in river water

The effect of three doses of UV irradiation (20, 40 and 60 $mJ.cm^{-2}$) on microbial levels of three populations (*E. coli*, HPC, and *Enterobacteriaceae*) are presented in Figures 4.1.3-4.1.5. the effects of treatment on the presence of *L monocytogenes* and *Salmonella* are presented in Table 4.1.6. The results indicated that UV treatment was very effective at lowering microbial numbers across all populations and all four rivers. The HPC population had the highest number of survivors in all the rivers tested, although levels decreased as the UV dose increased. No surviving *L monocytogenes* and *Salmonella* could be detected in any of the rivers after UV doses >40 $mJ.cm^{-2}$.







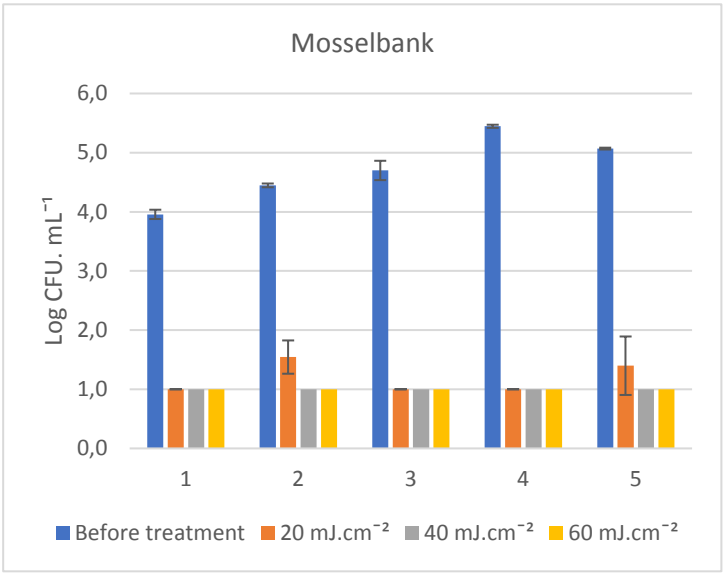
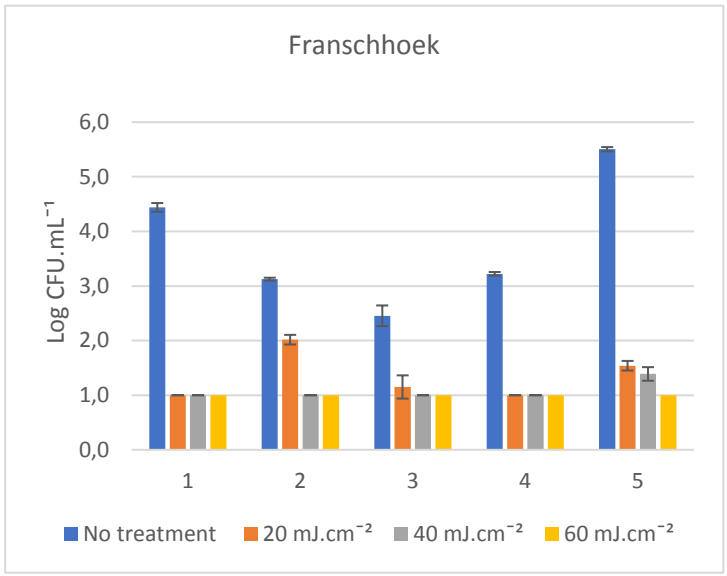
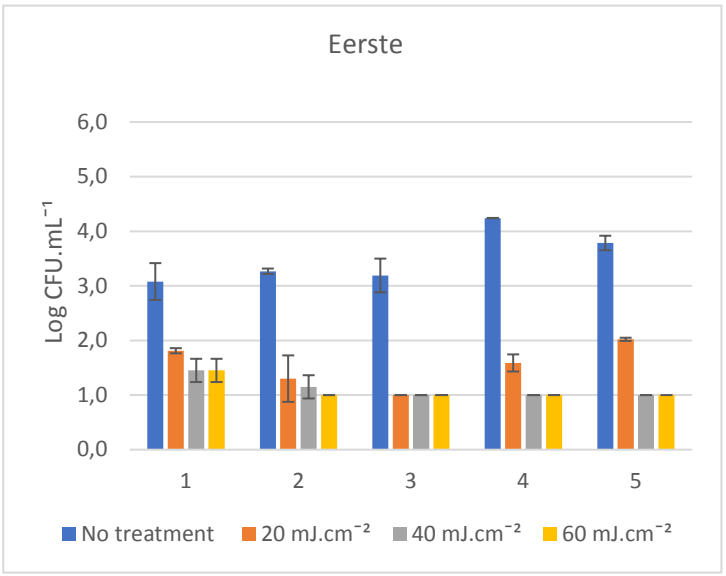
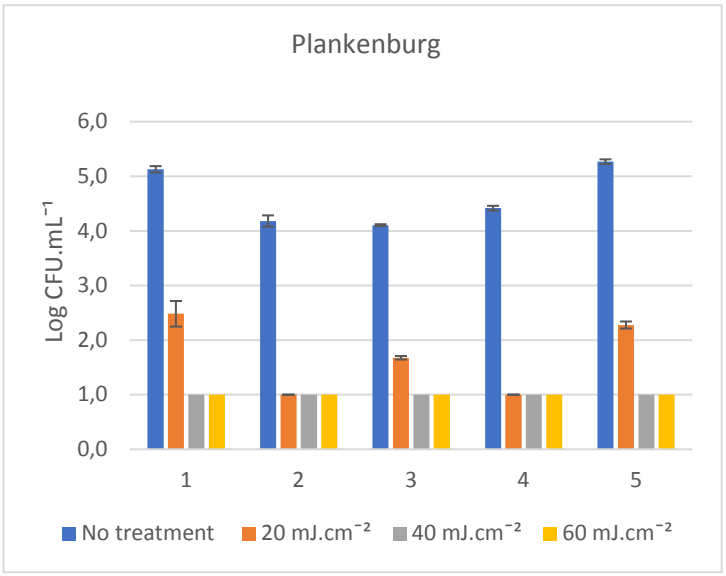


Figure 4.1.3 *E. coli* counts before and after three UV radiation doses for the four selected rivers over five sampling occasions. Please note: Values between 0-1.0 Log CFU.mL^{-1} are theoretical representations as the detection limit for this method was 1.0 Log CFU.mL^{-1}.







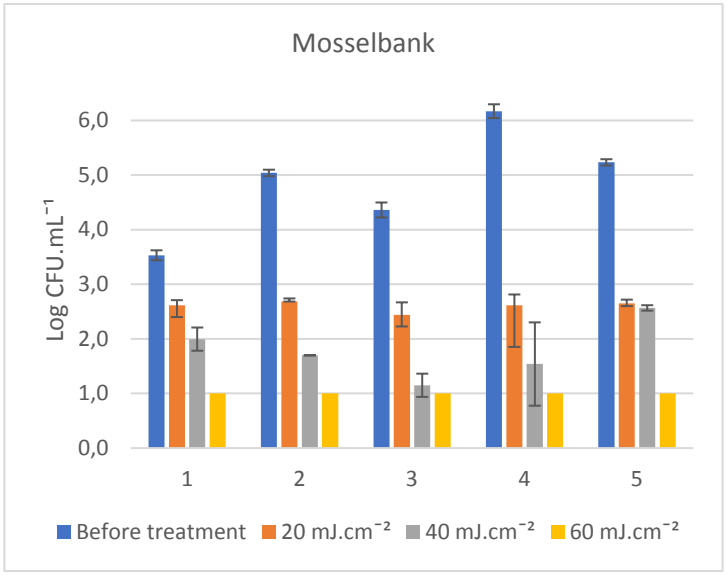
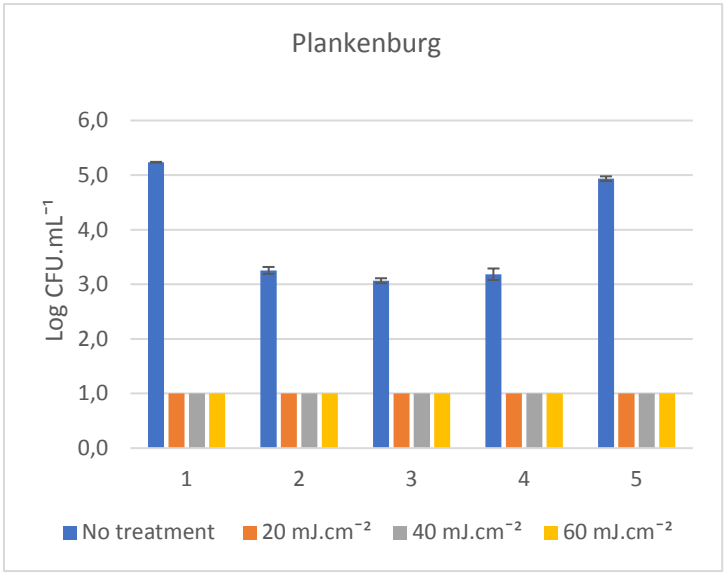
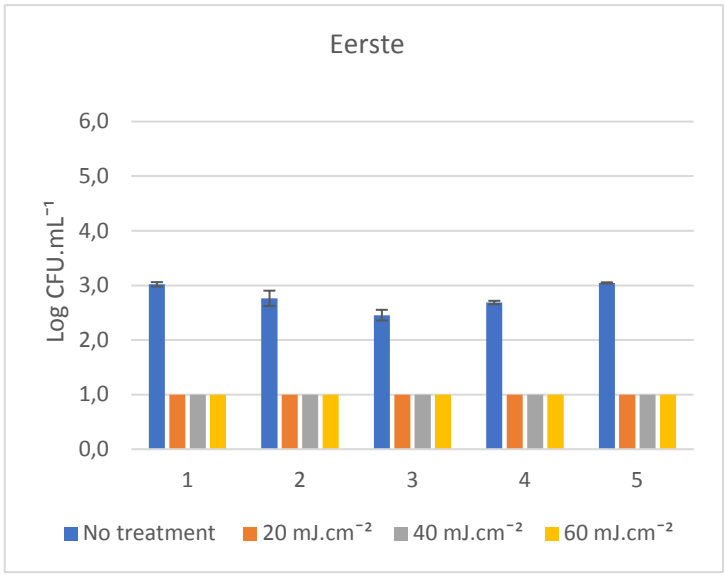
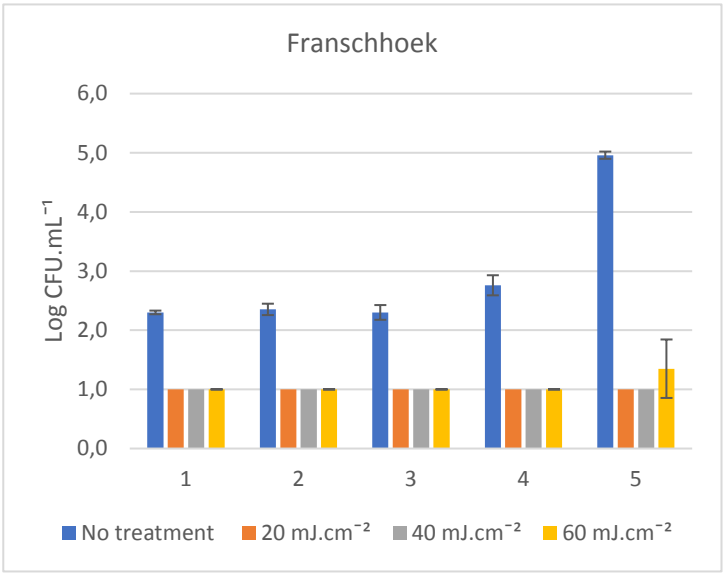


Figure 4.1.4 Heterotrophic Plate Counts (at 30°C) before and after three doses of UV radiation for the four selected rivers over five sampling occasions. Please note: Values between 0-1.0 Log CFU.mL^{-1} are theoretical representations as the detection limit for this method was 1.0 Log CFU.mL^{-1}.







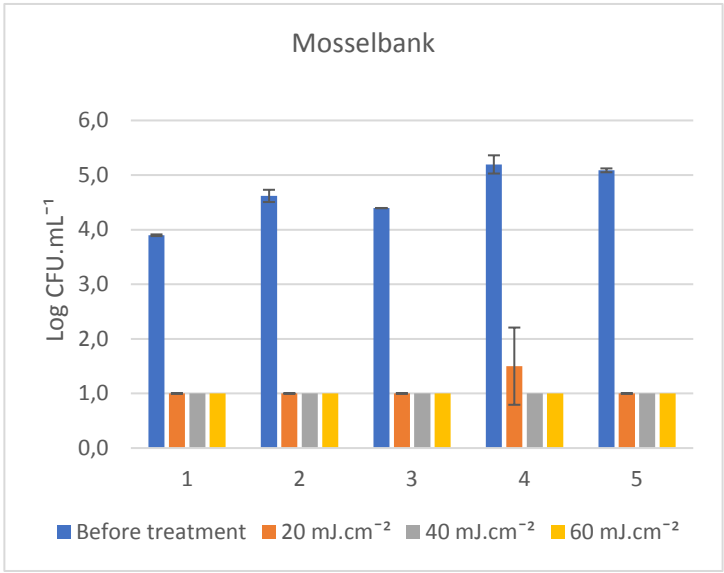


Figure 4.1.5 *Enterobacteriaceae* colony counts before and after three doses of UV radiation for the four rivers over five sampling occasions. Please note: Values between 0-1.0 Log CFU.mL^{-1} are theoretical representations as the detection limit for this method was 1.0 Log CFU.mL^{-1}.

Table 4.1.6 Presence/absence results of *Listeria monocytogenes* (LM) and *Salmonella* spp. (S) for four rivers, over five sampling repetitions after three UV radiation doses.

		Rep. 1				Rep. 2				Rep. 3				Rep. 4				Rep. 5			
River	Test	0	1	2	3	0	1	2	3	0	1	2	3	0	1	2	3	0	1	2	3
Plankenburg	*S*	+	-	-	-	+	-	-	-	-	-	-	-	+	-	-	-	+	-	-	-
	LM	+	-	-	-	+	+	-	-	+	-	-	-	+	-	-	-	+	-	-	-
Eerste	*S*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
	LM	+	-	-	-	+	-	-	-	+	-	-	-	+	-	-	-	-	-	-	-
Mosselbank	*S*	-	-	-	-	+	-	-	-	-	-	-	-	+	-	-	-	+	-	-	-
	LM	+	-	-	-	+	-	-	-	+	-	-	-	+	-	-	-	+	-	-	-
Franschhoek	*S*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
	LM	-	-	-	-	+	-	-	-	+	-	-	-	-	-	-	-	+	-	-	-

0 =No treatment, 1 = 20 mJ.cm^{-2}, 2 = 40 mJ.cm^{-2}, 3 = 60 mJ.cm^{-2} of UV radiation
LM – *Listeria monocytogenes,* S – *Salmonella* spp.

During the course of the study, microbial isolates were collected both before and after UV treatment. Bursey (2021) characterised these isolates extensively, and also tested for UV resistance of some isolates. Some of the data collected by Bursey is presented in Tables 4.1.7 and 4.1.8. UV resistance data for specific isolates are not included in this report, but Bursey's thesis can be accessed electronically (Bursey, 2021). The antimicrobial resistance testing of selected isolates was done against a limited collection of antibiotics, and the results are presented in Tables 4.1.9 and 4.1.10. Strain identification results obtained using MALDI-TOF analyses (for *E. coli* and *Salmonella* strains), and MALDI-TOF analysis as well as PCR (for *L. monocytogenes* strains), are also included. Results presented clearly indicate a number of multidrug resistant strains.

Listeria monocytogenes strains' lineage typing results using RFLP analysis are presented and discussed in detail by Bursey (2022) and is not extensively covered in this report. Analysis did reveal that all environmental *L. monocytogenes* strains belonged to lineage I.

Table 4.1.7 Bacterial isolates included in characterisation studies by Bursey (2021) including their source, known antimicrobial resistance (AMR) profiles and UV dose applied before isolation (Bursey, 2021). **Further characterisation:** Study **A – Lineage typing (*Listeria monocytogenes* strains) (Bursey, 2021); B – Antimicrobial resistance testing (Bursey, 2021); C – UV resistance testing of individual isolates (Bursey, 2021 – Results not included in this report)**

Isolate code	Source & isolation date	Known AMR	Treatment applied	Further characterisation
L. monocytogenes PR-03	Plankenburg River (22/01/2020)	None	20 $mJ.cm^{-2}$ UV	A & B
Listeria MEN09	MEN-09 (Lineage I – food production equipment)	tetracycline, erythromycin, gentamycin	None	A & B & C
Listeria MEN32	MEN-32 (Lineage II – raw beef)	tetracycline, erythromycin, gentamycin	None	A & B
ATCC 23074	ATCC 23074	None	None	B & C
L. monocytogenes FR-01	Franschhoek River (25/10/2019)	None	None	A & B
L. monocytogenes FR-02	Franschhoek River (25/10/2019)	None	None	A
L. monocytogenes FR-03	Franschhoek River (22/01/2020)	None	None	A
L. monocytogenes MR-01	Mosselbank River (25/10/2019)	None	None	A & B
L. monocytogenes MR-03	Mosselbank River (22/01/2020)	None	None	A & B & C
L. monocytogenes PR-01	Plankenburg River (16/10/2019)	None	None	A & B
L. monocytogenes PR-02	Plankenburg River (08/11/2019)	None	None	A

Table 4.1.8 Bacterial isolates included in characterisation studies by Bursey (2021), including their source, known antimicrobial resistance (AMR) profiles and UV dose applied before isolation. **Further characterisation: B – Antimicrobial resistance testing (Bursey, 2021); C – UV resistance testing (Bursey, 2021 – Results not included in this report)**

Isolate code	Source & isolation date	Known resistance	Treatment applied	Further characterisation
E. coli FR-01	Franschhoek River (16/10/2019)	None	20 mJ.cm^{-2} UV	B & C
E. coli FR-02	Franschhoek River (09/10/2019)	None	20 mJ.cm^{-2} UV	B & C
E. coli MR-01	Mosselbank River (22/01/2020)	None	20 mJ.cm^{-2} UV	B & C
E. coli CTX-TEM	Fresh produce	aminoglycosides, flouroquinolones, trimethoprim-sulfamethoxazole, gentamycin	None	B & C
E. coli BAA-2469	ATCC BAA-2469 (NDM +)	All – except nitrofurantoin & tigecycline	None	B & C
E. coli ATCC 35218	ATCC 35218	ampicillin, penicillin, Cephalosprins	None	B & C
Salmonella MR-02	Mosselbank River (22/01/2020)	None	None	B & C
Salmonella PR-02	Plankenburg River (22/01/2020)	None	None	B & C
Salmonella – poultry	Poultry source	None	None	B & C
S. braenderup	*S.* braenderup H9812 (clinical)	None	None	B & C

Table 4.1.9 Antimicrobial susceptibility testing results of *Enterobacteriaceae* isolates, and indication of the multi-drug resistance (MDR) of the isolates to the antimicrobials tested

Bacteria	Antimicrobial									MALDI-TOF ID confirmation
	AMP	STX	GM	C	TE	CIP	NA	S	MDR Yes/No	
***E. coli* ATCC 35218**	R	R	S	R	S	R	-	-	Yes	N/A
***E. coli* ATCC 29522**	S	S	S	S	S	S	-	-	No	N/A
***E. coli* FR-01**	R	R	S	S	R	S	-	-	Yes	Confirmed
***E. coli* FR-02**	R	R	R	S	R	I	-	-	Yes	Confirmed
***E. coli* MR-01**	R	R	S	S	R	R	-	-	Yes	Confirmed
***E. coli* BAA-2469**	R	R	R	R	R	R	-	-	Yes	N/A
***Salmonella* MR-02**	R	R	S	S	R	S	S	R	Yes	Confirmed
***Salmonella* PR-02**	R	R	S	S	R	S	R	R	Yes	Confirmed
***S. braenderup* BAA-664**	R	S	S	S	S	S	S	S	No	N/A
***Salmonella* poultry**	S	S	S	S	S	S	S	S	No	Confirmed

*MR – Mosselbank River, PR – Plankenburg River, FR – Franschhoek River **AMP – ampicillin, STX – trimethoprim sulfamethoxazole, GM – gentamycin, C – chloramphenicol, TE – tetracycline, CIP – ciprofloxacin, NA – nalidixic acid, S – streptomycin R – Resistant, S – Susceptible, I – Intermediate. N/A – not tested during MALDI-TOF analysis

Table 4.1.10 Antimicrobial susceptibility testing of *L. monocytogenes* isolates, and indication of the multi-drug resistance (MDR) of the isolates to the antimicrobials tested

Strain/ Source	Antimicrobial								
	AMP	STX	GM	P	MEM	E	**MDR** Yes/No	**MALDI-TOF ID confirmation**	**PCR ID confirmation**
***LM* MR-01**	R	R	R	S	S	R	Yes	Unconfirmed	Confirmed
***LM* MR-03**	R	R	R	R	S	R	Yes	Confirmed	Confirmed
***LM* FR-01**	S	R	S	R	S	S	No	Unconfirmed	Confirmed
***LM* PR-01**	R	R	S	R	R	R	Yes	Unconfirmed	Confirmed
***LM* PR-03**	R	R	S	R	S	R	Yes	Unconfirmed	Confirmed
***LM* MEN09**	R	R	R	R	R	R	Yes	N/A	N/A
***LM* MEN32**	R	R	R	R	R	R	Yes	N/A	N/A
***LM* ATCC 23074**	R	R	R	R	R	R	Yes	N/A	N/A
***S. pneumoniae* ATCC 49619**	R	R	R	R	S	R	Yes	N/A	-

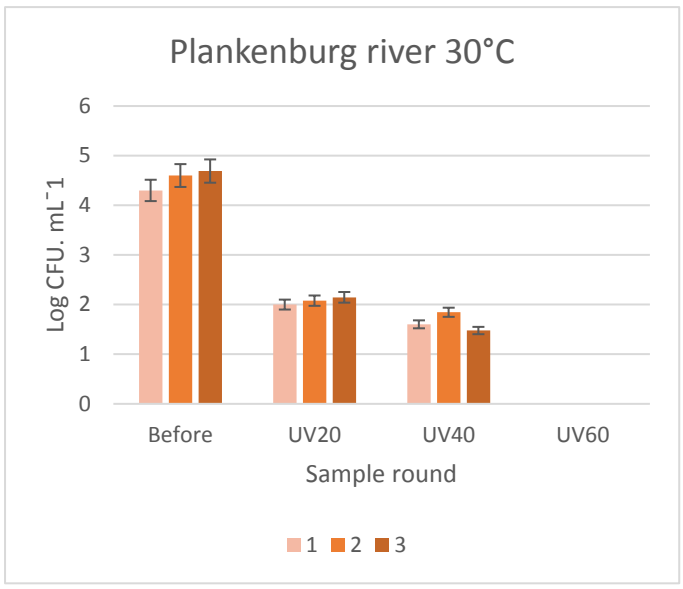
*MR – Mosselbank River, PR – Plankenburg River, FR – Franschhoek River ** AMP – ampicillin, STX – trimethoprim sulfamethoxazole, GM – gentamycin, P – penicillin, MEM – meropenem, E – erythromycin R – Resistant, S – Susceptible, I – Intermediate N/A – not tested during MALDI-TOF analysis. LM – *Listeria monocytogenes*

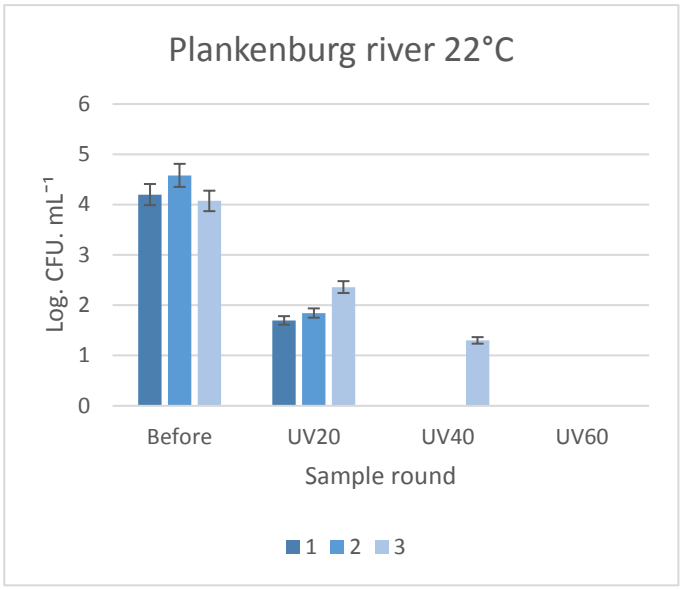
4.1.3.2 Study 2 (January 2021-March 2021) (Oosthuizen, 2022)

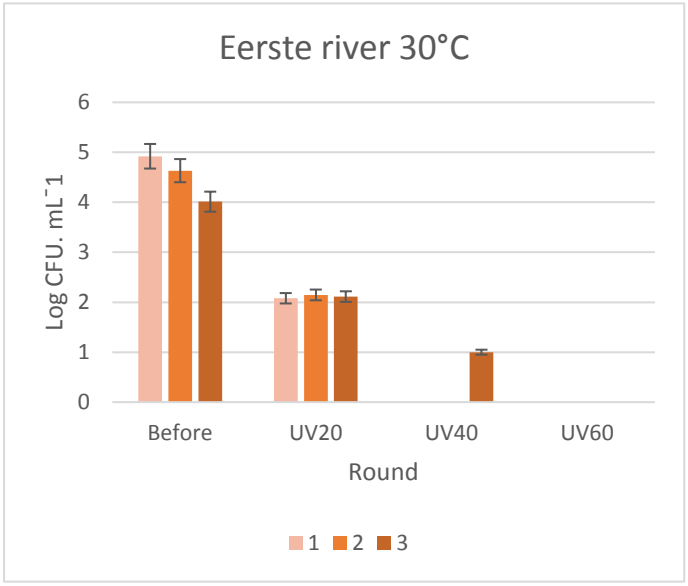
The four rivers investigated in Study 1, were sampled three times each in this study. The results of the total plate counts (HPC at 30°C and TPAC at 22 °C) before and after UV treatments are presented in Figure 4.1.6. A comparison of the log reductions observed in the four river water samples after the lowest UV dose (20 $mJ.cm^{-2}$) was applied, are presented in Table 4.1.11.

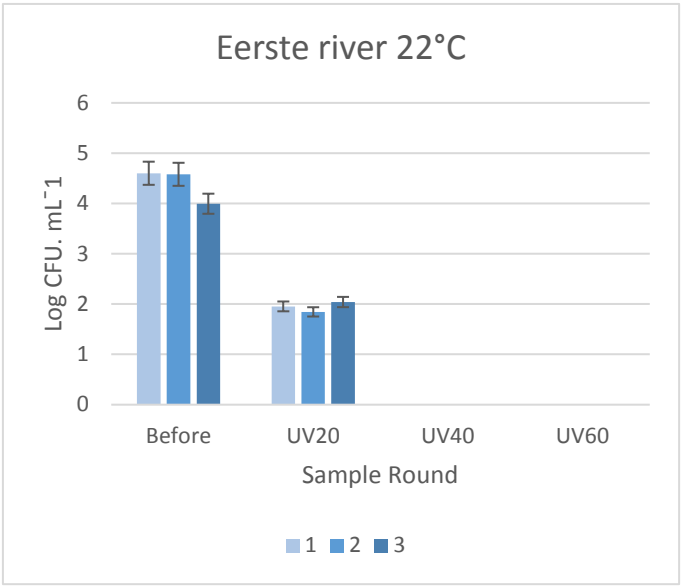
The physico-chemical analyses of each river were determined in duplicate for each sample and an average was calculated, which are presented in Table 4.1.12. Where possible, results were compared to the guideline limits provided in the Irrigation Water Guidelines (DWAF, 1996a) (see Section 3.1 in chapter 3).

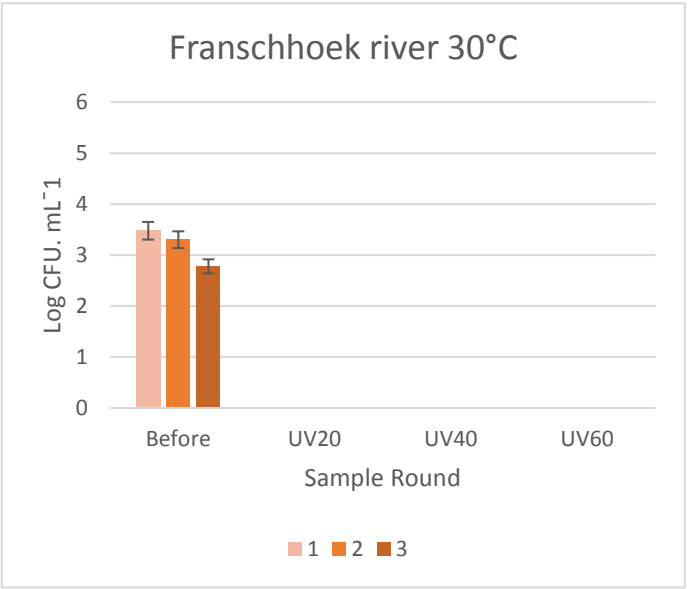
Bacterial isolates were prepared from the surviving HPC and TPC populations, after each of the three UV doses were applied, and stored at -80°C in 25% ($v.v^{-1}$) glycerol until further analyses and identification could occur. These isolates were then characterised in detail by Oosthuizen (2022), and a summary of the results are presented in Table 4.1.13.

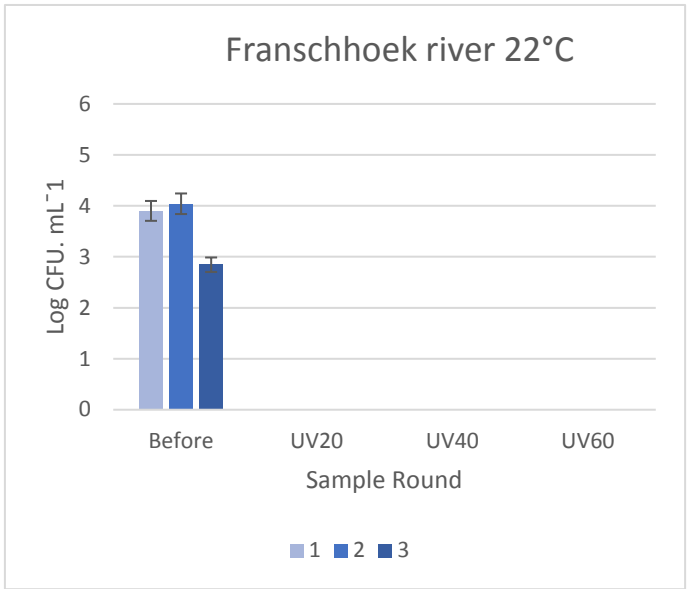

Plankenburg river 30°C
Log CFU. mL-1
0
1
2
3
4
5
6
Before
UV20
UV40
UV60
Sample round
1
2
3

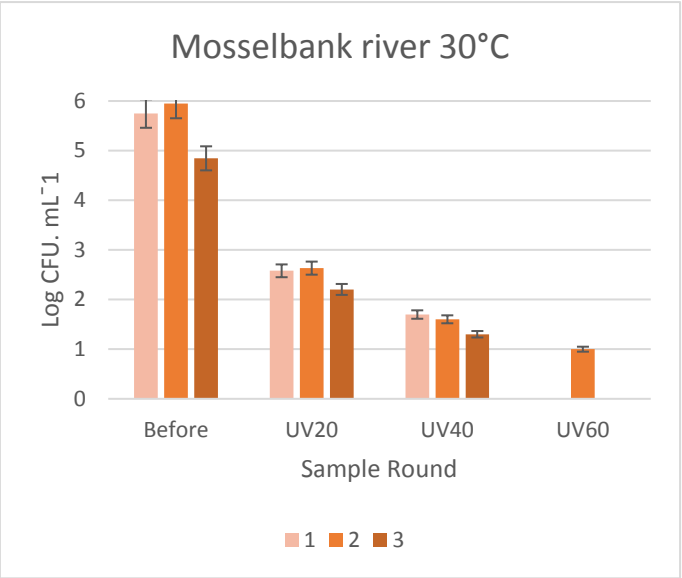

Plankenburg river 22°C
Log. CFU. mL-1
0
1
2
3
4
5
6
Before
UV20
UV40
UV60
Sample round
1
2
3


Eerste river 30°C
Log CFU. mL-1
0
1
2
3
4
5
6
Before
UV20
UV40
UV60
Round
1
2
3


Eerste river 22°C
Log CFU. mL-1
0
1
2
3
4
5
6
Before
UV20
UV40
UV60
Sample Round
1
2
3


Franschhoek river 30°C
Log CFU. mL-1
0
1
2
3
4
5
6
Before
UV20
UV40
UV60
Sample Round
1
2
3


Franschhoek river 22°C
Log CFU. mL-1
0
1
2
3
4
5
6
Before
UV20
UV40
UV60
Sample Round
1
2
3



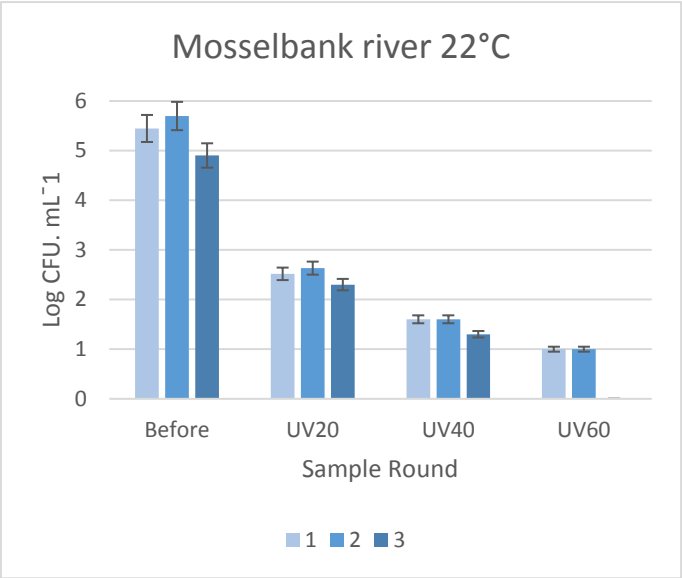


Figure 4.1.6 Results of indicator organism colony counts after the exposure of different UV doses (20, 40 & 60 mJ.cm^{-2}) and incubation temperatures (30°C & 22°C), expressed as log CFU.mL^{-1}, with standard deviation error bars included. The detection limit for this method was 1.0 Log CFU.mL^{-1}. Please note: only countable CFU's are indicated.

Table 4.1.11 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	>3.00*	>3.00*	3,04	2,87

*No growth detected

Table 4.1.12 Physico-chemical characteristics of four selected rivers used for irrigation purposes over three sampling occasions during Study 2 (January 2021-March 2021)

	Plankenburg river				Eerste river				Franschhoek river				Mosselbank river			
Characteristics	**1**	**2**	**3**	**Avg.**	**1**	**2**	**3**	**Avg.**	**1**	**2**	**3**	**Avg.**	**1**	**2**	**3**	**Avg.**
				SD				**SD**				**SD**				**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	28 0.34	3.3	3	3	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	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

Table 4.1.13 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.

Water from the four sites was also screened for the presence of STEC using the Pall GeneDisc STEC Top 7 molecular test and a summary of the results, presented in Table 4.1.14, indicates the presence and absence of targeted gene sequences (o-serotype, *stx* genes and virulence genes) prior to UV disinfection (0 mJ.cm^{-2}). Included in Table 4.1.14 are also the results from UV dose of 20 mJ.cm^{-2} of Eerste river. The results for the other three rivers obtained after a UV dose of 20 mJ.cm^{-2}, and after UV doses 40 mJ.cm^{-2} and 60 mJ.cm^{-2} of all four rivers, were not included in Table 4.1.14 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.

Table 4.1.14 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

In this study, the presence of ESBL-producing *Enterobacteriaceae* was determined on a presence/absence basis. Table 4.1.15 indicates the presumptive positive ESBL-producing *E. coli* and

other ESBL-producing *Enterobacteriaceae* colonies detected during sampling round three of this study, before and after specific UV doses were applied.

Table 4.1.15 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*	+	-	-	-	-	-	-	-	+	-	-	-	+	-	-	-

The presumptive positive ESBL-producing *E. coli* strains isolated from the four river sampling sites (before any UV doses were applied) 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 4.1.16 indicate the confirmed species identification results after the MALDI-TOF analyses, with individual spectra presented in Appendix B (B6-B30).

As all isolated strains were confirmed to be part of the *Enterobacteriaceae* family, each of the strains were prepared for the ESBL testing procedure. The test involved strains 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 4.1.16 indicate the confirmed ESBL-phenotype status of the isolates (detailed results presented in Appendix A)

Table 4.1.16 include the confirmed ESBL-phenotype status of the E coli, while a summary of the test results is presented in Appendix A for the five river water isolates tested (pre-UV treatment). All three river water strains isolated from the Plankenburg, Franschhoek and Mosselbank river were confirmed as ESBL producers.

Table 4.1.16 Confirmed ESBL-producing *E. coli* isolated from the CHROMagar ESBL plates during the third sampling round. Strain identification was done using MALDI-TOF analyses

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

[a] ESBL confirmation testing done according to standard methods (EUCAST, 2021) – results are summarised in Appendix A.

[b] 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.

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

4.1.4. DISCUSSION

In both studies four river sites (Eerste, Plankenburg, Mosselbank and Franschhoek rivers), were sampled during two summer irrigation periods (Study 1: October 2019-January 2020 & Study 2: January 2021-March 2021). During the summer season Western Cape farmers need to irrigate their crops as the province receives mainly winter rainfall. In both studies water samples were exposed to three selected UV doses (20, 40 & 60 mJ.cm^{-2}) and the microbial counts were recorded before treatment (0 mJ.cm^{-2}) and after each dose. Physio-chemical analyses were also conducted on all water samples and the results were compared to standard guidelines for irrigation water to determine the initial quality and study the effect on treatment efficacy.

Study 1 (October 2019-January 2020) (Bursey, 2021)

Physico-chemical analyses

The results of all physico-chemical tests that were conducted during Study 1 are presented in Table 4.1.3. Alkalinity provides an estimate of the ability of a water sample to resist pH changes (Sila, 2019). In this study, alkalinity values for all four rivers fell between 45 and 290 mg $CaCO_3.L^{-1}$ (Table 4.1.3) where 3% of samples (including all of the Mosselbank samples) had alkalinity values exceeding the limits provided by the DWAF (1996a) of < 120 mg $CaCO_3.L^{-1}$ (Table 3.3). The Mosselbank river had the highest alkalinity values overall. These significantly higher alkalinity values from the Mosselbank river could be due to the fact that this sampling location was situated downstream from a wastewater treatment plant. A study performed by Baharvand & Daneshvar (2019) on a wastewater treatment plant noted that raw sewage had alkalinity readings of 380-390 mg $CaCO_3.L^{-1}$, whereas the alkalinity values after the first treatment process dropped to 300-350 mg $CaCO_3.L^{-1}$. The impact of wastewater effluent from treatment facilities on the water quality profile of rivers used for irrigation purposes should be considered in future.

The other three rivers remained within the guideline limits for alkalinity on all sampling occasions, except for the Plankenburg and Franschhoek rivers on sampling occasion five. On this occasion, the water levels in the Franschhoek river were notably lower with a slower flow rate, and was coupled with increases in all other physico-chemical characteristics, most notably a 30% drop in UVT%, a quadrupled turbidity value and double the COD value – when comparing this sampling occasion to the previous four sampling ocassions. Similar findings were noted for the Plankenburg river on sampling occasion five.

Alkalinity is affected by dissolved salts and the oxidation of organic matter which wouldn't necessarily affect pH. It is possible to have a water sample that has a lower pH but still ranks high in alkalinity, therefore still has the capacity to buffer changes in acidity levels, while being acidic (USEPA,

2011). The pH value of a water sample, according to Abdelrahim *et al.* (2013), has an effect on the biological and chemical reactions that occur in water, as well as having an impact on the solubility of metal ions. An increased pH may result in dissolved metals precipitating out of solution, leading to an increase in turbidity, and a subsequent decreased UV inactivation efficacy (Farrell *et al.,* 2018). The specified pH range (DWAF, 1996a) for the irrigation water samples, 6.5-8.4, is relatively wide and it can be seen in Table 4.1.3 that all river water samples fell within this range.

The guideline limits (DWAF, 1996a) do not include a specification for the allowed turbidity for irrigation water. The Water Quality Guidelines for Domestic Use (DWAF, 1996c) states that for turbidity values exceeding 10 NTU, the water carries an associated risk of disease, and this guideline was then used to determine the water quality of the four selected rivers. Turbidity is determined by the amount of light that is scattered by particles within a water sample, but this does not include any settled solids (Perlman, 2014). Of the four rivers studied, the Plankenburg consistently had higher turbidity values (11.1-26.9 NTU), all of which exceeded the guideline limit. The other rivers all had values below 10 NTU (except on one occasion for the Mosselbank river) (Table 4.1.3). Interestingly, the Plankenburg river also had the most consistent presence of pathogenic microorganisms of all the rivers investigated (Table 4.1.5). Highly turbid water (with high turbidity and TSS values) can also rapidly clog of irrigation equipment.

Turbidity is one of the factors that has a notable influence on the efficacy of UV treatment of water, due to the inverse relationship between UVT % and turbidity (Gurol, 2005). Much like suspended solids, microbial aggregates can be enclosed within the particulates in turbid samples, which increases the resistance of microorganisms to UV penetration dramatically (Farrell *et al.*, 2018). According to Jones *et al.* (2014), the relationship between turbidity and UV treatment efficiency is, however, inconsistent. This is because the various factors that can contribute to turbidity show great variability in the ability to block or absorb UV radiation. Although there are seemingly conflicting opinions regarding the impact of turbidity on UV treatment efficacy, it is evident that as the turbidity value increases, the UVT % decrease, and it becomes more difficult to deliver a specific UV dose, which can influence UV irradiation efficacy.

The total solids are the sum of the suspended solids (TSS) and the dissolved solids (TDS) (USEPA, 1999). The TSS of a water sample includes sand, silt, clay, mineral precipitates as well as biological matter, which includes all particles that would be held back by a 2 µm filter (USEPA, 1999; Butler & Ford, 2018). The TDS refers to all dissolved matter, including dissolved salts. In this study it was observed that the Mosselbank river consistently had a very high dissolved solids (TDS) content (540-903 $mg.L^{-1}$) compared to the other three rivers (82-485 $mg.L^{-1}$) (Table 4.1.3). This was in contrast with the TSS values of the Mosselbank river (8.2-24.2 $mg.L^{-1}$), that were approximately in the same

range as those observed for the other three rivers combined (1.3-19.4 $mg.L^{-1}$) (Table 4.1.3). This points towards a higher concentration of dissolved salts and minerals, which could also explain the higher alkalinity values observed for this river. Once again, this could indicate the potential impact that the wastewater treatment plant could have had on the river water profile, as discussed previously.

A linear relationship between the turbidity and total suspended solids has been proposed by Hannouche *et al.* (2011). High turbidity, which is a reflection of high levels of TDS and TSS in the sample, implies a high level of pollution (Johnson *et al.,* 2010). The relationship between TDS and TSS is not considered constant or proportional over time (Butler & Ford, 2018). The variability in these two factors is, therefore, too great to allow for a direct relationship to be established. Higher TDS readings are noted in the Eerste river compared to the Plankenburg, and the opposite is reported for the TSS readings. This could mean that the dissolved solids in the Eerste river are not contributing in a manner that is significant to affect the UVT % (which remain relatively similar across the five sampling occasions of these two rivers). This could indicate that the suspended solids may play a greater role in the scattering of light for the Plankenburg river, or that organic matter is resulting in the scattering of light.

A report by Islam *et al.* (2017) states that the TDS of a water sample correlates positively with the electrical conductivity (EC) and directly affects the pH. As the TDS value increases, the electrical conductivity increases too and a more acidic pH is reported (Islam *et al.,* 2017). In this study, the high alkalinity values observed for the Mosselbank river generally correlated with slightly higher pH values (Table 4.1.3). Rusydi (2018) states that the relationship between electrical conductivity and total dissolved solids in water sources as one that correlates linearly ($R^2 = 0.97$). This can be calculated as:

$$TDS = 0.65 \times EC \text{ (where EC is } \mu S.m^{-1} \text{ and TDS is } mg.L^{-1}$$

When applying this calculation to the recorded values for EC and TDS (Table 4.1.3), it is evident that the correlation is correct. All rivers have electrical conductivity values within the specified limit (DWAF, 1996a). Lowered EC readings, according to Edokpayi *et al.* (2018), are most commonly reported in the rainy seasons of an area, which is as a result of the dilution effect from increased precipitation. Increased evaporation during the drier season can lead to increased levels of dissolved ions in river water (Edokpayi *et al.,* 2018). As the current study was performed in the drier season only, with limited rainfall, this correlation could not be observed.

Chemical Oxygen Demand (COD) is a measure of the total amount of oxygen required to oxidise all organic material into carbon dioxide and water (USEPA, 1999). There is currently no stipulation within the irrigation guidelines (DWAF, 1996a) in South Africa regarding the COD of a water

sample for either irrigation water or domestically used water. Therefore, the Guidelines for Industrial Use (DWAF, 1996b) were consulted instead. These guidelines state that acceptable limits for COD in irrigation water is < 75 $mgO_2.L^{-1}$ as irrigation water falls within Category 4 Utility Water of Industrial Processes, which allows for the discharge of an effluent without the clogging of equipment.

Relatively consistent values for COD content were reported for each river over the sampling occasions (Table 4.1.3). As discussed earlier, sampling occasion five were the exception for the Plankenburg and Franschhoek rivers, as a major increase in COD values were observed, compared to previous values. Wu *et al.* (2011) states that due to the fact that COD is predominantly an indicator of the organic pollution level in the water sample, and many of these pollutants absorb radiation in the UV region, it can significantly impact the UVT % in water, and therefore, ultimately influence UV treatment efficacy.

When observing the overall physico-chemical parameters of the four selected rivers, the Mosselbank river consistently had the lowest water quality (Table 4.1.3). This this river had the lowest UVT % (with a range of 22-30%), which could be attributed to the high TDS and low turbidity values. The impact of low UVT % on disinfection efficacy has been reported by Olivier (2015) and can influence the choice of pre-treatment steps as well as the UV dosage application. Of the four sites tested in this study, this river was labelled as the 'worst-case scenario' site for future treatment optimisation work (as part of the follow-up studies).

The Franschhoek river was consistently the river with the best physico-chemical water quality (Table 4.1.3). Both the Franschhoek and the Eerste rivers had the lowest indicator counts (Fig 4.1.1 and Fig 4.1.2) as well as pathogen presence (Table 4.1.5). The UVT% of this river had the highest average of 73.7% (Table 4.1.3). This river was thus labelled as the 'best-case scenario' site for the rest of the study.

Microbial analyses

The microbial quality of the four rivers over the five sampling events, before UV treatments were applied, are presented in Figure 4.1.1. The indicator counts exceeded the guideline recommendations for *E. coli* for all rivers on all sampling occasions. The Franschhoek and Eerste rivers consistently had the lowest *E. coli* counts (Fig 4.1.1), however, this was still above the guideline recommendation. The highest colony counts were noted in the Mosselbank river on sampling occasion four, where a count of log 5.45 CFU.mL^{-1}, which equates to 117 000 CFU.mL^{-1}, was recorded (Fig 4.1.1). Increased counts did, in some instances, correlate with worsening physico-chemical characteristics. For instance, a significant increase in indicator colony counts was noted between the fourth and fifth sampling occasions of the Franschhoek river (Fig 4.1.1). This correlated to a significant UVT % decrease, and all other physico-chemical results (except pH) almost doubled compared to the previous four sampling occasions (Table 4.1.3). Likewise, a similar trend was observed between sampling occasions four and five of the Plankenburg river (Fig 4.1.1, Table 4.1.3). It can therefore be noted that a tentative correlation could be made between the physico-chemical and microbial characteristics of the water sample.

The combined average of the microbial counts recorded over the five sampling events per river for each indicator population is presented in Figure 4.1.3. The Plankenburg and Mosselbank rivers were consistently the most contaminated rivers, based on the average microbial indicator counts (Fig 4.1.3). Even though the Eerste and Franschhoek rivers had *E. coli* counts that were lower than the other two rivers, these counts still markedly exceeded the water quality guidelines. Therefore, on the basis of microbial levels only, it was concluded that irrigation with any of the four rivers could result in microbial carry-over from irrigation water to produce.

A report by Sigge *et al.* (2016) has highlighted the possibility of a potential food safety risk that is associated with the presence of pathogens in irrigation water. Sigge *et al.* (2016) proposed setting limits for important food pathogens in addition to *E. coli,* including *L. monocytogenes,* and *Salmonella* spp. in irrigation water to limit the risk of foodborne outbreaks associated with fresh produce. At present, knowledge gaps exist with regard to the prevalence of these pathogens in South African surface waters, and also about how effectively UV disinfection can be used to reduce pathogen levels under South African conditions. The aim of this study, and the larger project, is thus to generate information on the incidence of these pathogens in river water, and to determine how best to treat them using UV irradiation.

The results of the pathogen detection tests can be seen in Table 4.1.5. The Plankenburg and Mosselbank rivers, had the most consistent pathogen presence over the five sampling occasions. The Eerste river was the only river to have typical *Salmonella* colonies present on one sampling occasion

only. *L. monocytogenes* colonies was present in all but one sampling occasion of the Eerste river. The Franschhoek river had the lowest prevalence of *Salmonella* spp. and *L. monocytogenes* type colonies.

Vasquez-Boland *et al.* (2001) states that *Listeria* spp. are saprophytic organisms, that feed on dead organic matter in the environment and are ubiquitous in nature. The presence of *Listeria* species in general in river water is thus not surprising. Unfortunately, *Listeria monocytogenes* in particular carry pathogenic genes that it can activate the moment it enters the human body (Vasquez-Boland *et al*., 2001). The need to control this organism in an industry that involves minimally processed foods is therefore of great importance to ensure consumer safety.

Ultra-violet radiation results

As discussed, poor physico-chemical characteristics of a water sample may negatively impact the efficacy of UV disinfection. These factors should be taken into account when determining UV dosages. Water that has a lower turbidity and suspended solids content, ultimately has higher UVT % readings and therefore, improved disinfection efficacy can be noted. This is due to the fact that microorganism aggregates can be enclosed within particulates in the turbid samples, which increases the microorganism's resistance to UV penetration dramatically (Farrell *et al.*, 2018). Liu (2005) reported that an increase in turbidity from 1 to 10 NTU would, for instance reduce the average dosage by between 5 and 33%.

Table 4.1.4 provides an example of how the exposure times in the bench top collimated beam UV device needed to be adjusted between water samples with varying UVT % values to enable the application of the three UV doses in this study. As the UVT % decreased, the exposure time to deliver the required dose increased. As the volume of water sample in the test beaker decreased with each increasing dose (as described in methods section), the exposure time decreased.

The results of the microbial reductions after UV radiation can be seen in Figures 4.1.3-4.1.5 as well as Table 4.1.6. The *E. coli* colony counts (Fig 4.1.3) all fell well within the guideline (DWAF,1996a) after the first dose of UV radiation (20 $mJ.cm^{-2}$). The irradiated water could thus be considered acceptable for irrigation . Figure 4.1.4 indicates the microbial reductions for the Heterotrophic Plate Count test after UV radiation. It can be noted that a more gradual reduction in the HPC population after UV irradiation occurred when compared to what was observed for the *Enterobacteriaceae* and *E. coli* populations, particularly in the Mosselbank river (Figures 4.1.3-4.1.5).

The HPC population typically represents a broad spectrum of aerobic microorganisms, both gram positive and gram negative, that can grow on Plate Count Agar (Oxoid, South Africa) at 30°C. When observing the HPC counts of the Mosselbank river, the prevalence of microorganisms after 20 and 40 $mJ.cm^{-2}$ of UV radiation was much higher than observed for any other river. These results could

have several explanations. The overall initial microbial concentration in the Mosselbank river was higher than the other rivers (Fig 4.1.2), therefore, even after several log reductions following UV irradiation, the microbial load was still higher than the other rivers. Another possible explanation is that the type of microorganisms present in the HPC population of this river could show greater resistance to UV radiation than the organisms present in the other rivers, and therefore, result in their presence in the Mosselbank sample after 40 $mJ.cm^{-2}$ (Fig 4.1.4). Lastly, the physico-chemical composition of the Mosselbank river was also the worst of the four rivers, with average UVT % around 27% (Table 4.1.3). It can thus be argued that UV efficacy of the applied doses was not the same in this river sample than in the other rivers with better water quality. Whether the surviving HPC microbes a threat to fresh produce safety is not clear. It is thus suggested that isolates are collected for further identification and characterisation in Study 2.

The *Enterobacteriaceae* results before and after UV treatment (Fig 4.1.5), indicate the efficacy of UV radiation against this microbial population. Overall, the significant drop in indicator microbial counts after a UV dose of just 20 $mJ.cm^{-2}$ indicate a high treatment efficacy against this population in all the rivers, in spite of the varying water qualities. According to Gayán *et al.* (2012), UV-C irradiation is effective against for Gram-positive organisms, yeasts, spores, viruses and moulds. Gram-negative organisms show even less resistance than Gram-positive organisms to this treatment method, due to structural differences. The resistances and lethal dosages vary widely amongst different organisms and strain-to-strain. Therefore, it is of great importance to determine the microbial type and load present within the water source in order to apply an effective dose to achieve the required result.

Table 4.1.6 shows the presence/absence results of the pathogen tests, before and after UV irradiation at the specified doses. It is interesting to note that, on all but one occasion, the *L. monocytogenes* and *Salmonella* type colonies were no longer present after the lowest dose of UV radiation was applied. The presence of *L. monocytogenes* after 20 $mJ.cm^{-2}$ in the Plankenburg river on the second sampling occasion might be attributed to a higher initial concentration of this organism in the river on this occasion. This is, however, a speculative observation as the detection methods used for both pathogens in this study are enrichment methods that do not allow for the determination of the actual microbial levels prior to enrichment. When observing the physico-chemical results on this day, there is no outlying factor or significantly differing characteristic that could have contributed to *Listeria* survival. Due to the fact that the pathogens were no longer present after the 20 $mJ.cm^{-2}$ UV dose was applied (Table 4.1.6) on other sampling occasions of this river, as well as the other three rivers, it was concluded that this dose was mostly effective against the prevalent levels of pathogens in the rivers at the time of testing.

According to Gayán *et al.* (2014), pathogenic bacterial strains are more resistant to UV irradiation than non-pathogenic strains. A study performed by Gayán *et al.* (2012) on the effect of UV irradiation on different *Salmonella* species with regard to their individual resistances and noted that *Salmonella typhimurium* STCC 878 was the most resistant strain, requiring 18.03 $mJ.cm^{-2}$ to achieve a 4-log reduction. In the current study, it can be noted from the outlying result in the Plankenburg river, that when deciding on an effective dose to ensure complete microbial disinfection and water safety, that a dose of >20 $mJ.cm^{-2}$ may be required if these pathogens are present at higher initial levels.

Characterisation of environmental isolates (Bursey, 2021)

Listeria monocytogenes is a saprophytic organisms and Pirone-Davies *et al.* (2018) reports that infection from *L. monocytogenes* is difficult to control due to the widespread dissemination of this organism in the natural environment. Chen *et al.* (2017) also reported that *L. monocytogenes* is capable of surviving normal as well as severe environmental conditions, which adds to the complexity of this organism. *L. monocytogenes* has been isolated from a wide variety of environmental sources, including food products, river water, industrial effluent, soil, human faeces and animals (Chen *et al.,* 2017).

As discussed, the results of the identification confirmation (PCR and MALDI-TOF) (Table 4.1.10), and the subsequent lineage typing of the *Listeria monocytogenes* environmental strains (n=5) revealed that all river water isolates obtained by Bursey (2021) from the Plankenburg, Franschhoek and Mosselbank rivers, were from lineage I. Of these strains, it was only the Franschhoek river strain that were not classified as multi drug resistant (Table 4.1.10).

L. monocytogenes strains can be divided into four phylogenetic lineages, which vary in their evolutionary, ecological and virulence characteristics. The determination of lineage type provides an indication of the source of contamination (Chen *et al.,* 2017). Dreyer *et al.* (2016) state that lineage I strains are most commonly reported in clinical infections and are linked to animals, whereas lineage II is associated with the environment as well as foodborne outbreaks. Lineages III and IV are isolated very rarely, but according to Dreyer *et al.* (2016), are associated predominantly with animals. Pirone-Davies *et al.* (2018) reported that *L. monocytogenes* isolates from lineage I are more virulent, on average, than lineage II.

Kayode *et al.* (2019) performed a study which characterised listeriosis outbreaks in the Southern African region. This study gathered information regarding all studies performed in Southern Africa that included the detection of *L. monocytogenes* from different sources. Most notably, 11 reports of *L. monocytogenes* prevalence in wastewater and river water sources were included from various locations in South Africa. According to Kayode *et al.* (2019), the presence of *L. monocytogenes* in water could be attributed to food-processing factory effluent, sewer discharge, vegetation and run-

off from land or dump sites. This consistent presence of *L. monocytogenes* in river water all over country indicates that this pathogen is capable of surviving adverse environmental conditions and is resilient to treatment processes designed to disinfect it (Kayode *et al.,* 2019).

A study performed on *L. monocytogenes* isolates from soil and surface water in Austria noted that 33.3% of isolates were associated with lineage I serotypes and 66.67% were associated with lineage II serotypes (Linke *et al.,* 2014). A study performed by Lyautey *et al.* (2007) reported that lineage I isolates dominated (61%) during the summer, whereas lineage II dominated (77%) during autumn in Canadian river water isolates. It is evident from these previously mentioned studies, that lineage I isolates are commonly found in river water, and the findings in the current study correlate with previous findings.

The presence of *Listeria monocytogenes* in all the rivers during most of the sampling events (Table 4.1.5), with some specific isolates being confirmed as MDR strains (Table 4.1.10), can be a risk to consumer health, and justifies the need for irrigation water treatment prior to application. The same can be said of *Salmonella* (Table 4.1.9). Although it was less frequently detected in untreated river water than *Listeria* (Table 4.1.5), the two specific *Salmonella* strains tested in this study were also classified as MDR strains. No *L monocytogenes* or *Salmonella* could be detected after UV treatment (Table 4.1.6), which indicates that the UV treatment applied as part of this study was effective in eliminating these pathogens at the levels it was present prior to enrichment (for presence/absence testing procedures applied as part of this study).

The three *E. coli* strains characterised were all isolated from the surviving populations observed for some of the sampled water from the Franschhoek and Mosselbank rivers (Figure 4.1.3). Although the pathogenic status of these strains was not established during this study, all were classified as MDR strains.

Antimicrobial resistance is not only a concern in the human health industry but is dramatically affecting the food industry around the world. A study performed on antimicrobial resistance profiles of microbial isolates from bovine carcasses in Spain and Croatia found that 54.5-55.6% of *Salmonella* isolates were multi-drug resistant. A total of 66% of *Salmonella* isolates from poultry and pork carcasses were multidrug resistant in Thailand (Kidsley *et al.,* 2018). A study was performed on the evolution of drug resistance profiles of *E. coli* isolates obtained from clinical sources as well as animal meat between the years 1950-2002 in America (Tadesse *et al.,* 2012). A 7.2% multidrug resistance was noted in *E. coli* isolates in the years 1950-1959, which is in stark contrast with the alarming statistic that 63.6% of isolates obtained from 2000-2002 were resistant to multiple antimicrobials. When considering the increase in resistance per antimicrobial, the resistance of *E. coli* isolates from animal sources to chloramphenicol was reported to have an average of 0.3% increase per year. According to

Tadesse *et al.* (2012), gentamycin resistance in human isolates was only reported from the late 1990's and onwards, with 0% of isolates showing resistance in 1970-1979 to 28.1% of isolates showing resistance in 2000-2002. A 1.28% increase in resistance per year was reported for isolates against gentamycin.

Blaak *et al.* (2015) reports that surface water is a major vehicle for the dissemination of antimicrobial resistant microorganisms in the environment. Contamination occurs as a result of the pollution of water by animal faeces, agricultural runoff, or through the discharge of improperly treated sewage wastewater into surrounding rivers and dams. Through recreational activities as well as the irrigation of crops with antimicrobial resistant microorganisms, humans may be exposed to these bacteria. The ingestion of microorganisms that have resistance to antimicrobials holds both direct and indirect risks for humans. The direct risk is the introduction of infections that are difficult to treat. Indirectly, the ingestion of resistant microorganisms that are harmless to a healthy individual, are able to colonise the gut or skin even in healthy humans, and results in an asymptomatic carriage of that bacteria. This may result in gene transfer from the resistant bacteria to the naturally-occurring commensal bacteria in the body, which could cause opportunistic infections in immunocompromised individuals (Blaak *et al.,* 2015).

The results of this study confirm the efficacy of UV irradiation as a treatment method at laboratory-scale, as is evident from the significant log-reductions observed in the Gram-negative indicator populations' colony counts after the lowest dose applied, as well as effective pathogen disinfection. A follow-up study is, however, proposed to study the effect of UV irradiation on different HPC populations (mesophilic and psychrotrophic), and to isolate and identify colonies that survive UV irradiation. A more in-depth look at the AMR-status of UV resistant isolates is also warranted, considering the potential threat to consumer health.

Study 2 (January 2021-March 2021) (Oosthuizen, 2022)

Physico-chemical analyses

Study 2 was conducted a year later than Study 1 during the summer (January 2021 to March 2021). The physico-chemical characteristics that were observed for the four river sites during this time are presented in Table 4.1.12. Overall, the results indicated that the Mosselbank river could still be considered the 'worst case scenario' site, compared to the other three sites. This is despite the fact that the UVT % of the Mosselbank improved from an average of 27% (Study 1, Table 4.1.3) to 40% (this study, Table 4.1.12). The TDS levels of the Mosselbank were still very high at an average of 792 $mg.L^{-1}$ (average of 789 $mg.L^{-1}$ in Study 1), with COD and turbidity levels that remained mostly

unchanged from the previous season (Tables 4.1.3 and 4.1.12). A decrease in conductivity (from 0.93 to 0.7 $mS.m^{-1}$) was the only other improvement observed, other than the higher UVT %. These results suggest that the source of contamination (that contributed to the extremely high TDS values observed at the Mosselbank site) was a constant and ongoing cause of pollution, as would be typical of a wastewater treatment plant. It could thus have a continuing impact on the food safety risks associated with fresh produce cultivated in surrounding areas.

The Franschhoek river could still be considered the 'best case scenario' river based on the fact that it had the lowest COD, conductivity and TSS levels, combined with an average UVT% above 73%. It did, however, show a notable increase in the TDS content, from 123 $mg.L^{-1}$ in Study 1, to 176 $mg.L^{-1}$ in this study, which coincided with an increase in alkalinity of 40 mg.L $CaCO_3^{-1}$ (Tables 4.1.3 and 4.1.12). These increases are in contrast with the great improvement that was observed overall in the physico-chemical profile of the Plankenburg river. The latter showed a significant increase in average UVT % (from 55% to 73%), which was probably a direct result of the lowered levels of TDS, TSS, Turbidity and COD observed in this study compared to Study 1 (Tables 4.1.3 and 4.1.12). An 8% improvement in UVT % (from 59 to 67%) was also observed in the Eerste river samples, despite increases in TDS, COD and alkalinity. The reason for these improvements is, however not known, and it can thus not be concluded whether it is of a seasonal or long-term nature.

Microbial loads

The microbial analyses conducted as part of Study 2 focussed on the more UV resistant HPC population present in the rivers (initially observed during Study 1). The prevalence of STEC as well as ESBL-producing *Enterobacteriaceae* before and after UV treatment were also evaluated in this study.

Overall, it is clear from the HPC and TPAC counts presented in Figure 4.1.6 that although these populations form a significant part of any river ecosystem, UV resistance of these populations differs between rivers. It can be argued that microbial numbers do play a part in UV resistance (considering the lower initial levels in the Franschhoek river that coincided with no observed survival after UV treatment). This observation does, however, not rule out the fact that inherent differences in population composition can influence UV resistance (considering that the initial levels in the Eerste and Plankenburg rivers were in the same range, but more resistance to UV treatment was observed at the latter site).

As was observed for the Gram-negative indicators in Study 1, the highest initial numbers of HPC and TPAC were observed in the Mosselbank river ('worst case scenario' site), while the Franschhoek river ('best case scenario' site) had the lowest numbers (Figure 4.1.6). During Study 1, HPC counts for the Mosselbank river were in the range of about 3.5-6 log $CFU.mL^{-1}$, compared to the

higher average range observed in Study 2 (4.8-6 log CFU.mL^{-1}) (Figures 4.1.2 and 4.1.6). Like Study 1, the HPC population of the Mosselbank during Study 2 were also resilient enough to survive UV doses above 40 mJ.cm^{-2}. Further identification of the surviving HPC genera is necessary to determine the food safety risk associated with these survivors. As observed for the physico-chemical parameters for this site during both seasons (Tables 4.1.3 and 4.1.12), these counts could be indicative of the constant and long-term impact of the wastewater treatment plant upstream. Overall, no significant difference in counts were observed for the HPC count at 30°C and the TPAC count at 22°C. Whether differences in population composition exist in the UV resistant mesophilic and psychrotrophic fractions of the Mosselbank, is not clear.

Although the physico-chemical profile and UVT % of the Plankenburg river in study 2 were better than the parameters observed in Study 1, the initial HPC numbers were still more or less in the same range: 4-5 log CFU.mL^{-1} for Study 2, compared to 4-5.2 CFU.mL^{-1} for Study 1 (Figures 4.1.2 and 4.1.6). During Study 2 the Plankenburg river was the only river, other than the Mosselbank site, which HPC population could survive consistently on all three sampling occasions after a UV dose of 40 mJ.cm^{-2} was applied. The Plankenburg HPC population also showed a more consistent survival after the 40 mJ.cm^{-2} UV dose than the Plankenburg TPAC population (Figure 4.1.6), which might be indicative of a more resilient mesophilic population compared to the psychrotrophic population.

As with Study 1, it was concluded that the physicochemical profile of a river might correlate with the size of the microbial populations observed, with higher numbers being associated with a more unfavourable physicochemical profile (including lower UVT% values). This would suggest that although UV treatment efficacy would be challenged at typical 'worst case scenario' sites that have lower UVT % values, it is also these sites that are most in need of disinfection prior to irrigation to reduce potential health risks.

Identification of HPC and TPAC isolates

The results presented in Table 4.1.13 suggest that the UV resistant 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 from various point and non-point pollution sources, including industrial and domestic wastewater effluents (Table 4.1.2). This could have contributed chemical contaminants, including heavy metals, which could have added selection pressures that influenced microbial population composition. In addition, Nascimento et al. (2020) indicated that *B. megaterium* can grow at temperatures between 7°C and 45°C. This 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 4.1.13). 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 are common carriers 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 *B. cereus* can 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 4.13). 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.

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 genus 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). 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 4.1.13). 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 4.1.13). 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.

Molecular detection and UV dose response of STEC

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 downstream. 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. (2001), only a small number of STEC bacteria, less than 100 organisms, can cause illness.

The results obtained using the Pall GeneDisc top 7 molecular detection system (Table 4.1.14) indicated that STEC-associated gene sequences were present in Plankenburg, Eerste and Mosselbank river water samples 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 genes (*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 4.1.14). 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 4.1.14). 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 *E coli* strains with 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. According to Oosthuizen (2022), the Plankenburg river results corresponded with results obtained four months earlier at the same site (results not included in this report). Oosthuizen concluded that there might be a long-term presence of non-O157 STEC at this site.

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 4.1.14 & Fig. B2 in Appendix B). 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).

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 4.1.14 & Fig B4). All other virulence genes, apart from O145-O157, have also been detected (Table 4.1.14). 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 by Oosthuizen (2022) in this study were 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 4.1.14 & Fig. B3). During the course of the study, the Franschhoek river consistently had the best physico-chemical and microbial profiles. As discussed , the Franschhoek site had the lowest HPC and TPAC population counts (Fig 4.1.6), 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.

Detection of ESBL-producers

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). Because of the widespread disposal of various wastewaters into various surface waters, rivers can act as a contact and exchange location for environmental and human microorganisms, which can be both 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).

The results presented in Table 4.1.15 indicate that both ESBL-producing *E. coli* and *Enterobacteriaceae* were phenotypically detected, using CHROMagar ESBL (MediaMage), in water from the Plankenburg, Franschhoek and Mosselbank rivers prior to UV treatment. No ESBL-producing strains were observed in water from the Eerste river site. The results also clearly indicate that 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} (Table 4.1.15). As discussed in Results (Table 4.1.16) and presented in Appendix A, the ESBL status of *E coli* isolates from three of the rivers (Plankenburg, Franschhoek and Mosselbank) were confirmed by Oosthuizen (2022) using the standard disc diffusion method described by EUCAST (2021) .

The fact that the *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, UV treatment applied in this study proved effective, as the lowest UV dose of 20 mJ.cm^{-2} inactivated ESBL producers detected in river water prior to UV treatment.

4.1.5 CONCLUSIONS

The results of the indicator and pathogen testing in Study 1 indicated that the river water examined as part of the study might be sources of high-risk irrigation water, depending on their application. The indicator counts, specifically *E. coli,* notably exceeded the guideline limits on all sampling occasions. This means that transfer of *E. coli* from the water to fresh produce would be highly probable during agricultural irrigation. Treatment before use is thus recommended.

The baseline microbial and physico-chemical profiles of the rivers that were determined over the course of both Study 1 and 2 revealed that a correlation could exist between the physico-chemical characteristics of river water and microbial population levels, where poorer physico-chemical characteristics were coupled with higher microbial loads. It could also be a tentative indicator of the potential survival of microbial populations after UV treatment, although individual differences in microbial population composition between different rivers sites need to be considered.

The Mosselbank river consistently had the poorest physico-chemical characteristics as well as the highest indicator population counts in both studies. All the pathogens tested for (*Salmonella, L. monocytogenes* and STEC) were also present. Based on its stable (but poor) physico-chemical profile as well as its persistent high HPC loads over the course of both studies, this river site can be considered a ‘worst-case scenario’ site that can be utilised for future UV treatment optimisation at pilot-scale as part of the ongoing project. This is based on the assumption that, if the UV dose requirements are optimised to ensure that microbial levels in UV-treated water from this river falls within the guideline

limits, there is a high probability that water from the other selected rivers will also be of acceptable standard (if UV treatment based on the same parameters are applied).

The efficacy of UV radiation in the disinfection of Gram-negative microorganisms present in river water was confirmed in Study 1, where even at the lowest dose applied, the *E. coli* counts fell well within guideline limits for all sites. The recommended UV dose to ensure sufficient microbial disinfection for indicator organisms in rivers with similar profiles as those included in this study can thus be suggested to be between 20-40 $mJ.cm^{-2}$.

The pathogen results of Study 1 and 2 indicate the efficacy of UV irradiation against *Salmonella* spp., *L. monocytogenes* and STEC. Considering the results observed for in the Plankenburg river (Study 1), where *L. monocytogenes* was present after a UV dose of 20 $mJ.cm^{-2}$, the dose recommended for microbial disinfection of these pathogens is between 20-40 $mJ.cm^{-2}$. These recommendations, based on the results of Study 1 and 2, correspond with USEPA (1999) recommendations that a UV dose between 21-36 $mJ.cm^{-2}$ should be sufficient in the inactivation of bacterial and viral pathogens depending on water quality parameters such as turbidity and COD. It is, however acknowledged that initial concentration of pathogens might have a significant influence on their survival patterns. This aspect needs to be explored, as well as any microbial recovery that may occur after UV irradiation treatment.

Heterotrophic Plate Count results indicated a slower drop in microbial loads in both Study 1 and 2 – specifically in the Mosselbank river – which is indicative of diverse and possibly more resistant microbial populations. The potential risk that the HPC and TPAC populations may present to fresh produce safety was investigated further by identifying environmental strains using MALDI-TOF analysis. The species identified during this study included *B. cereus*, *A. hydrophilia*, *B. megaterium* and *Exiguobacterium* genus (Table 4.1.13). In addition to these findings, it was noted that the proportion of Gram-positive bacteria were significantly higher compared to Gram-negative bacteria (Table 4.1.13). The results obtained, indicated that 80% of the isolates found after UV disinfection were Gram-positive bacteria (Table 4.1.13). 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.

The occurrence of antimicrobial resistant strains was also investigated in both studies. Study 1 reported the presence of several MDR strains, including *E coli*, *Salmonella* and *Listeria monocytogenes*, while study 2 showed the presence ESBL-producing *E. coli* strains. Although no guideline limits exist regarding the presence or resistant bacteria in irrigation water, the long-term negative implications that this may have for consumer health can not be ignored.

Overall, the rivers examined in this study proved to be highly diverse considering their physico-chemical and microbial profiles. The results of the UV irradiation trials indicated this treatment

method is effective in ensuring microbial disinfection of river water. It can therefore be concluded that UV radiation is effective at laboratory-scale and can be utilised to ensure satisfactory disinfection of Gram-negative indicator organisms as well as certain pathogens present.

4.2 Laboratory-scale collimated beam UV treatment study 2: Recovery potential of selected indicators and specific food pathogens post-UV irradiation

Please note: This chapter contain extracts from the MSc theses of Oosthuizen (2022) and Jankowitz (In press)

4.2.1 BACKGROUND & AIM

4.2.1.1 Background

During the UV irradiation process, UV light is absorbed by DNA causing the formation of cyclobutane pyrimidine dimers (CPD) and pyrimidine-pyrimidone 6-4 photoproducts (6-4PP) which prevent DNA replication and can lead to cell death if left unrepaired (Friedberg *et al.,* 1995; Rastogi et al., 2010). These mutagenic lesions are due to neighbouring pyrimidine nucleotides within the microorganism's DNA forming covalent bonds (Maclean et al., 2008). Photoreactivation is one of the mechanisms used by microorganisms to break these bonds and convert the thymine dimer back to two normal thymines through utilisation of a photolyase enzyme and energy from blue light (300-500 nm) (light dependant repair) (Coohill & Sagripanti, 2008; Sancar, 2016). Another recovery mechanism that microbes use is nucleotide excision repair, which can be divided into transcription coupled repair and global genome repair, which is both light independent mechanisms (Johann to Berens & Molinier, 2020). These repair processes therefore enable the microbes to repair DNA damage after UV exposure and start replicating again, which compromises the efficacy of the UV treatment.

The occurrence and extent of photoreactivation post UV exposure has been widely studied with specific focus on *Escherichia coli* spp. (Wang *et al.*, 2021). Various factors influencing photoreactivation were identified including time (Moreno-Andres *et al.*, 2019; Giannakis *et al.*, 2014), temperature (Sanders *et al.,* 2005; Salcedo *et al*., 2007; Li *et al*., 2017a; Wen *et al*., 2019a), water characteristics (Mao *et al.*, 2018; Shafaei *et al.*, 2017; Giannakis *et al.,* 2014) and light conditions (Wen *et al*., 2019b; Li *et al*., 2017b; Giannakis *et al*., 2015; Hallmich & Gehr, 2010). Drinking water, effluent and wastewater are the water sources that were studied, whereas lake water and seawater have mostly been used only to compare water matrices (Moreno-Andrés *et al.,* 2019; Shekoohiyan *et al.,* 2019). Olivier (2015) evaluated the sole use of UV treatment to decrease the microbial load of irrigation water and found that photo-repair of microorganisms post UV treatment might be a research area requiring additional attention.

Hoyer (1998) established that the minimum UV dose to achieve 4-log10 reduction of *Escherichia coli* ATCC 11229, considering possible photoreactivation, was 30 $mJ.cm^{-2}$. In the absence of photoreactivation, he found that a UV dose of only about 10 $mJ.cm^{-2}$ was sufficient. Beltrán & Jiménez (2008) found that a UV dose of 30 $mJ.cm^{-2}$ resulted in a maximum post-UV increase of 1.9

logs for *Salmonella* Typhi under light conditions, while the light-independent excision repair mechanisms were ineffective even after lower UV doses. Contradicting data have been reported for the photoreactivation of *Listeria innocua* (surrogate for *Listeria monocytogenes):* Fitzhenry *et al*.'s study in 2021 concluded no light or dark repair was observed when the medium used was water, whereas Kramer et al. (2015) observed photoreactivation rates of between 10^2 and 10^6 CFU.mL^{-1} after 24 h of lamp exposure on tryptic soy agar at 37°C on the same variant and different UV doses. Olivier's (2015) results indicated that the rate of recovery observed in poor quality river water was much lower following a double, but lower, dose of UV radiation (2 x 20 mJ.cm^{-2} = 40 mJ.cm^{-2} – applied by implementing a water recirculation step), than a single high dose (1 x 40 mJ.cm^{-2} – applied once-off, in-line). As the water quality of rivers can vary, it is important to study photoreactivation of food safety related microbes in irrigation water post-UV treatment in more depth to establish an effective disinfection strategy.

4.2.1.2 Aim

The aim of this study was to determine the recovery potential of specific food pathogens and microbial populations in water after UV-C treatment. This was done to establish which UV dose would decrease the recovery ability of the selected microbial populations most.

Specific objectives included:

- Comparison of UV susceptibility of six environmental isolates and reference strains to three UV doses in a collimated beam device.
- Comparison of microbial recovery, under light and dark conditions, of selected strains after three different UV doses in three different sterile water matrices in a collimated beam device.
- Comparison of microbial recovery, under light and dark conditions, of certain microbial populations (naturally present in river water) after UV treatment in a collimated beam device.

4.2.2 MATERIALS & METHODS

4.2.2.1 Study Design

Two experimental approaches were implemented to determine the microbial recovery of specific microbial populations and food pathogens after UV-C treatment in a collimated beam device. In Studies A1 and A2, the effect of UV treatment and microbial recovery were investigated using pure strains and different sterile water matrices. In Study B, the effects of UV treatment on the recovery of microbial populations naturally present in river water were investigated:

Study A1 – *UV susceptibility of selected food pathogens in distilled water*

In Study A1, six bacterial strains, representing three different species (*E. coli, Salmonella* spp. and *L. monocytogenes*), were treated with three different UV-C doses (1x20; 2x20; 3x20 $mJ.cm^{-2}$) in sterile distilled water to determine if UV susceptibility.

Study A2 – *Recovery potential of selected food pathogens in different water matrices post UV treatment*

In Study A2, bacterial inoculums were separately prepared from three strains – *Salmonella enterica* ATCC 14028, an environmental *E. coli* strain previously isolated from the Plankenburg river, and an environmental *L. monocytogenes* strain previously isolated from the Franschhoek river. These inoculums were separately subjected to UV treatment in sterile distilled water, as well as sterilised river water from the Plankenburg and Jonkershoek rivers. After UV treatment, treated samples were subjected to a recovery procedure for three hours in a light box, which was described in previous literature (Olivier, 2015). After recovery, microbial counts were performed using standard plate count methods.

Study B – *Recovery of microbial populations and food pathogens in river water post UV treatment*

In Study B, river water samples were collected from Mosselbank, Plankenburg and Franschhoek rivers, respectively. After UV treatment, the same light box was used to determine recovery of *E. coli*, *Salmonella spp*., *L. monocytogenes*, Coliforms and Heterotrophic bacteria (HPC). Bacterial populations (*E. coli*, Coliforms and HPC) were enumerated using standard plate count methods, while the presence/absence of *Salmonella spp*. and *L. monocytogenes* were determined using standard enrichment methods.

4.2.2.2 General methods for STUDIES A1 and A2

Preparation of microbial cultures for UV radiation

The microbial strains used in this study are listed in Table 4.2.1. All strains used were previously stored at -80°C in 25% glycerol ($v.v^{-1}$). Each organism was defrosted, after which 0.1 mL was transferred to either 5 mL Tryptone Soy Broth (for *E. coli* strains) or Brain Heart Infusion Broth (for *L. monocytogenes* and *S. enterica* strains). Inoculums were incubated at 37°C for 24 hours. The strain viability and identity were confirmed by streaking the broth on Brilliance Chromogenic E. coli/ Coliform Agar (Oxoid, South Africa) for *E.coli*, Xylose Lysine Deoxycholate Agar (Oxoid, South Africa) for *S. enterica* and Rapid' L. mono Agar Plates (BioRad, South Africa) for *L. monocytogenes* before incubating at 37°C for 24 hours.

After incubation, colonies were streaked on Violet Red Bile Glucose (VRBG) Agar ((Oxoid, South Africa) for *E. coli* and *S. enterica* and Brain Heart Infusion (BHI) Agar (Oxoid, South Africa) for *L. monocytogenes*. All strains were subjected to Gram-staining and strain identities were confirmed using the VITEK® automated identification system according to the instructions of the manufacturer. In this study the VITEK® GN identification cards (for the *E. coli* and *Salmonella* spp. isolates in this study) as well as the VITEK® GP cards (for the *L. monocytogenes* strains in this study) were used as part of the automated process.

After strain identification confirmation, colonies were streaked on multiple plates of VRBG Agar (Oxoid, South Africa) for *E. coli* and *S. enterica* and BHI Agar (Oxoid, South Africa) for *L. monocytogenes*. For each strain, an inoculum was prepared by aseptically transferring colonies from the non-selective agar plates to sterilised distilled water or autoclaved river water until an approximate cell density equivalent to an 0.5 McFarland standard was reached.

Table 4.2.1 Environmental and reference strains used in Studies A1 and A2

Isolate strain	**Source**
Salmonella enterica	ATCC 14028
Salmonella spp.	Mosselbank river – August 2021
E. coli	ATCC 35218
ESBL-producing *E. coli*	Plankenburg river – May 2021
L. monocytogenes	ATCC 7644
L. monocytogenes	Franschhoek river – September 2021

Ultraviolet treatment of microbial inoculums

For this study, UV doses of 20 mJ.cm^{-2} each were applied once, twice or three times consecutively to a single sample using a collimated beam device (Fig. 3.1) according to the method described in section 3.5.1 in Chapter 3 'General methods'. In this study, a "sample" consisted of a sterile 600 mL beaker contained 350 mL bacterial inoculum, which was measured with a sterile measuring cylinder. Sterilised magnetic stirrer bars were used to stir the sample during UV treatment. After the first UV treatment (1 x 20 mJ.cm^{-2}) was applied, 100 mL of the UV-treated inoculum was removed for the recovery procedures, followed by the plate count methods. The remaining sample was covered in foil and placed in a dark cupboard for a 10-minute waiting period before the second dose of 20 mJ.cm^{-2} was applied. This was done to emulate real-life delays between doses in practice when only one pilot-scale UV device is available. The same procedure was followed for the second and third UV doses of 20 mJ.cm^{-2}.

Escherichia coli detection and enumeration

Before and after each *E. coli* sample was exposed to the individual UV doses, 2 x 1 mL of the suspension was directly plated in duplicate, and 1 mL suspension removed from the beaker and used to prepare a dilution series up to 1 x 10^{-8} in Buffered Peptone Water (BPW). One mL of each dilution was then plated, in duplicate, using the pour plate technique, with Violet Red Bile Glucose Agar (Oxoid, South Africa). After incubation at 37°C for 24 hours (SANS Method 4832 – SANS, 2007a), bright pink colonies (*E. coli*) between 1 and 300 were counted. The entire procedure from UV treatment to reactivation and counting was done in triplicate.

Salmonella enterica ATCC 14028 detection and enumeration

Before and after each *S. enterica* sample was exposed to the individual UV doses, 2 x 1 mL of the suspension was directly plated in duplicate, and 1 mL suspension removed from the beaker and used to prepare a dilution series up to 1 x 10^{-8} in BPW. One mL of each dilution was then plated, in duplicate, using the pour plate technique, with Violet Red Bile Glucose Agar (Oxoid, South Africa). After incubation at 37°C for 24 hours, bright pink colonies (*S. enterica*) between 1 and 300 were counted. The entire procedure from UV treatment to reactivation and counting was done in triplicate.

Listeria monocytogenes detection and enumeration

Before and after each *L. monocytogenes* sample was exposed to the individual UV doses, 2 x 1 mL of the suspension was directly plated in duplicate, and 1 mL suspension removed from the beaker and used to prepare a dilution series up to 1 x 10^{-8} in BPW. One mL of each dilution was then plated, in duplicate, using the pour plate technique, with Brain Heart Infusion Agar (Oxoid, South Africa). After incubation at 37°C for 24 hours, cream-coloured colonies (*L. monocytogenes*) between 1 and 300 were counted. The entire procedure from UV treatment to reactivation and counting was done in triplicate.

Microbial recovery determination

To facilitate photoreactivation, each 100 mL of UV-treated inoculum that was removed from samples during the UV treatment procedures described above was split equally into two sterile 250 mL beakers using a sterile measuring cylinder after a temperature reading was taken. As represented in Figure 4.2.1, one beaker (with magnetic stirrer bar) was placed in a light box (Fig. 4.2.2) and the second (without magnetic stirrer bar) in a dark box. The light intensity measurement was taken at the surface of the sample in the light box (Figure 4.2.2) with the ILT1400 radiometer (International Light Technologies, USA) coupled with a XRL140T254 detector (International Light Technologies, USA). The samples were left in the respective boxes (Figure 4.2.2) for three hours, based on recommendations

from a previous study (Olivier, 2015), before a second temperature reading was taken. One millilitre of the suspension was directly plated, and 1 mL suspension removed from the beaker in the light box and used to prepare a dilution series up to 1 x 10^{-6} in BPW. One millilitre of each dilution was then plated, in duplicate, using the pour plate technique, with the respective agars as per organism mentioned above. After incubation at 37°C for 24 hours the colonies between 1 and 300 were counted. The same procedure was followed for the sample placed in the dark box. The microbial loads of the light box samples were then compared with the corresponding dark box samples to serve as indicators of potential photoreactivation and/or dark repair.

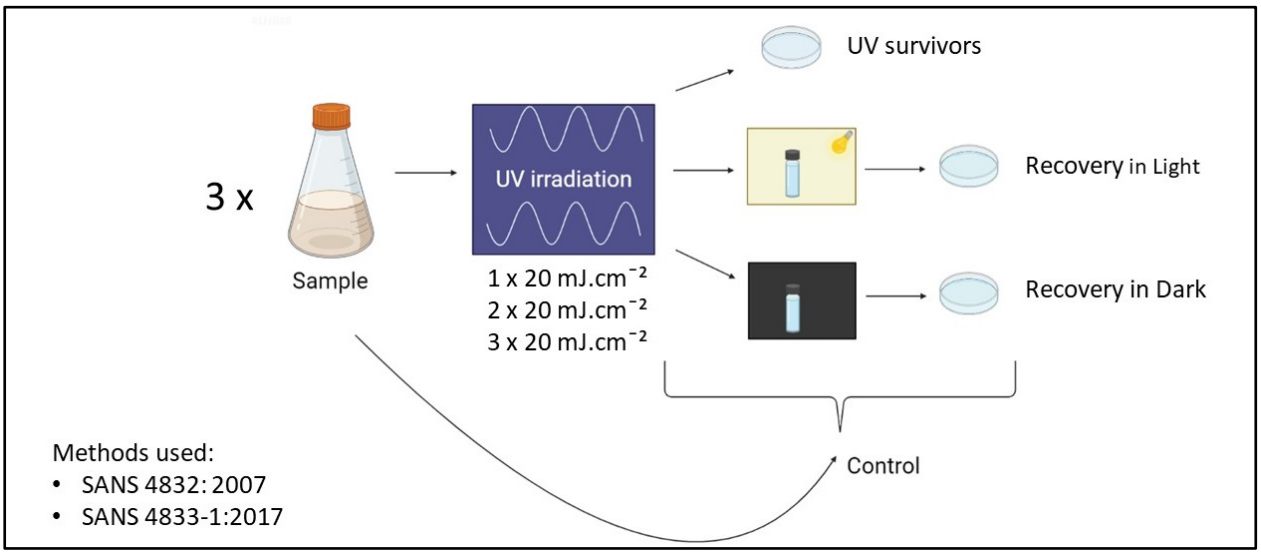


Figure 4.2.1 Schematic representation of experimental procedure followed in both studies A2 and B

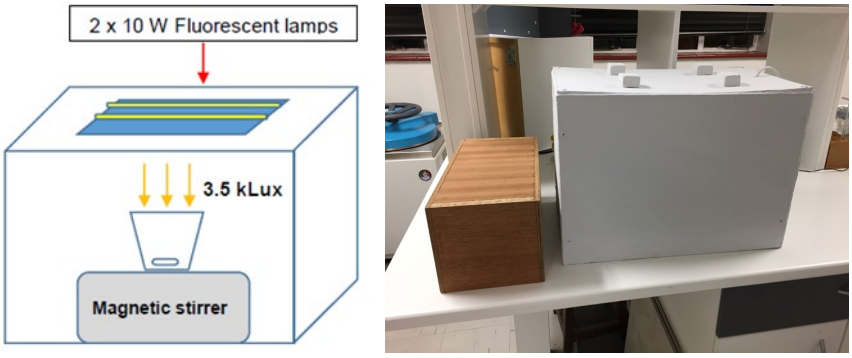


Figure 4.2.2 Representation of the box unit that was used in this study to facilitate photo recovery of UV-C treated water samples (Olivier, 2015)

4.2.2.3 General methods for STUDY B

River water sampling and *physico-chemical analysis of river water* were done according to the methods described in sections 3.2 and 3.3 in Chapter 3 'General methods'. Results were compared to the irrigation water guidelines summarised in Section 3.1 in Chapter 3.

Microbial analysis of river water samples

Following sampling at each river, water batches were analysed in triplicate for the presence of indicators and pathogens. For each of the triplicate water samples a standard dilution series were prepared as described in Section 3.4.1, after which the enumeration of Coliforms, *E. coli* and HPC populations followed, before and after UV treatment. Dilutions were transferred to the relevant agars using standard pour plate methods as described in Sections 3.4.2 (HPC) and 3.4.3 (*E. coli*/coliforms). Colony numbers <300 CFU per plate were recorded for all indicator organisms. Microbial enrichments were also prepared for the presence/absence testing of the pathogens *L. monocytogenes* and *Salmonella* spp. as described in Sections 3.4.5 (*Salmonella*) and 3.4.6 (*L. monocytogenes*).

Ultraviolet treatment of river water samples

The same UV irradiation procedure was followed as described in Study A using the collimated beam bench-scale UV irradiation device (Fig. 3.1). Each sterile 250 mL beaker with sterile magnetic stirrer bar contained 50 mL sample which was measured with a sterile measuring cylinder. The Mosselbank and Plankenburg river samples each received either a single UV dose (1 x 17 $mJ.cm^{-2}$) or double dose (2 x 17 $mJ.cm^{-2}$ = 34 $mJ.cm^{-2}$, administered sequentially). These doses were similar to the maximum doses that could be obtained in the pilot plant UV facility at the time for these water samples*.

Franschhoek river water samples received either a single (1 x 20 $mJ.cm^{-2}$) or double UV dose (2 x 20 $mJ.cm^{-2}$ = 40 $mJ.cm^{-2}$), similar to the maximum doses that could be obtained in the pilot plant UV facility at the time)*. (*PLEASE NOTE: The results of the pilot plant UV treatments will follow in the next chapter)

Microbial recovery determination

The reactivation testing procedure described in Study A as part of Section 4.2.2.2 (including Figure 4.2.2) was also followed in Study B. Reactivation was followed with the enumeration procedures for HPC and *E. coli* described in the previous sections. For *L. monocytogenes* and *Salmonella spp*. detection after recovery, the samples from the three triplicate UV treatments (after being exposed to either the light box or dark box) were pooled together, respectively, before pathogen enrichment commenced (as described in previous sections).

4.2.3 RESULTS

The results in this chapter are divided into two parts, based on the experimental approaches. Work done in the first part (Studies A1 and A2) focussed on examining UV treatment and post-UV recovery of pure cultures in different water matrices. In the second part of the chapter, UV treatment and post UV recovery of river water populations were studied.

4.2.3.1 Study A1

The colony counts before and after UV treatment of the six bacterial isolates (Table 4.2.1) in sterile distilled water are presented in Fig 4.2.3. The three UV doses that were applied were multiples of 20 $mJ.cm^{-2}$ (including 1 x 20; 2 x 20; and 3 x 20 $mJ.cm^{-2}$). The strains tested represented three important food safety related genera (*E. coli, L. monocytogenes* and *Salmonella* spp.), and included reference ATCC strains as well as environmental strains isolated as part of previous work (Bursey, 2021; Oosthuizen, 2022; chapter 4.1 of this report). For the purpose of this study all surviving CFU's at the lowest dilution plated were counted after UV treatment, even if there were <10 colonies, to give an indication of the efficacy of the UV process. It is, however, acknowledged that the statistical variation could be high between experimental repeats if counts of 1-10 CFU (with 1 $CFU.mL^{-1}$ = 0 log $CFU.mL^{-1}$, and 10 $CFU.mL^{-1}$ = 1 log $CFU.mL^{-1}$ at the lowest dilution plated) are included. It is also acknowledged that if no CFU's were observed, it does not rule out the possibility that surviving cells could still be present, albeit at concentrations below the countable detection limit of the colony counting methodology applied in this study.

The results (Fig 4.2.3.) indicated that the initial UV dose of 1 x 20 mJ.cm^{-2} reduced the microbial load of all isolates significantly by approximately six logs (from counts > 1x10^{8} CFU.mL^{-1} to counts < 100 CFU.mL^{-1}). Individual differences between different strains could however be observed if surviving counts (between 1-10 CFU.mL^{-1}) after higher doses (2x20 and 3x20 mJ.cm^{-2}) are considered. In this water matrix, the *E. coli* ATCC 35218 and the environmental *Salmonella* isolate (Table 4.2.1) were more UV sensitive and had no surviving colonies after the higher UV doses (2 x 20 and 3 x 20 mJ.cm^{-2}) were applied. The *Salmonella* ATCC 14028 strain, which had observable colonies after the highest UV dose (3 x 20 mJ.cm^{-2}), was slightly more UV resistant than the environmental *E. coli* strain, with the latter indicating the presence of a countable small surviving fraction after the second UV dose (2 x 20 mJ.cm^{-2}), but not after the highest dose (3x20 mJ.cm^{-2}). Similar to the *Salmonella* ATCC 14028 strain, surviving fractions after the highest dose were also observed for both the *L. monocytogenes* strains tested (Fig 4.2.3). Based on these results, *Salmonella* ATCC 14028, as well as the environmental *L. monocytogenes and E. coli* strains (Table 4.2.1) were selected for further investigation in different water matrices as part of Study A2.

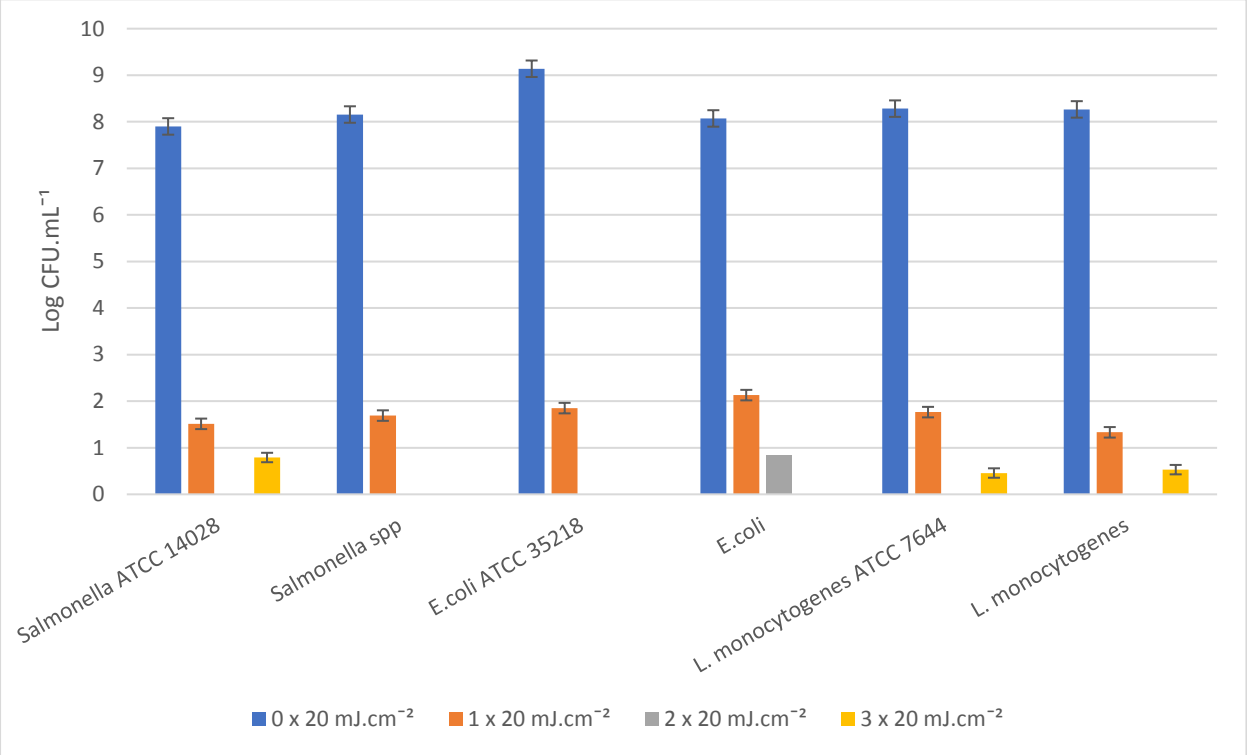


Figure 4.2.3 Average colony counts observed for six different isolates in sterile distilled water done in triplicate, indicated as Log CFU.mL^{-1} with error bars representing standard error. The detection limit for this method was 1.0 Log CFU.mL^{-1}. Legend indicates UV doses administered post inoculation.

4.2.3.2 Study A2

Sterilised distilled water, as well as two batches of autoclaved river water (from two different river sites located along the Plankenburg and Jonkershoek rivers, respectively) were used to investigate the effect of different water matrices on bacterial survival of three strains after UV treatment. The survival directly after UV treatment, without taking recovery into account, of each organism in the different water matrices is presented in Figure 4.2.4. As was observed for Study A1, a large initial decrease in CFU numbers of between 5-6 log was observed following the first UV dose of 1 x 20 mJ.cm^{-2} for all three organisms in all three water matrices tested. This was followed by highly variable decreases in CFU numbers at lower levels, which appeared to be influenced by both the type of organism and the water matrix in which it was exposed to UV.

No significant pattern of difference could be observed between organisms or their surviving fractions after any of the three UV doses applied in distilled water, where surviving colonies, albeit few, were observed for all three isolates. Significant differences ($p < 0.01$ – indicated as different small letters in Fig 4.2.4) between the three different organisms' survival were only observed in the river water matrices.

In the sterile Jonkershoek River water, no significant difference could be observed between the three UV doses tested for the *Salmonella* ATCC 14028, for which surviving colonies were observed after all UV doses. The *Salmonella* isolate, did however, show higher surviving numbers than *E. coli* and *L. monocytogenes* at higher UV doses (2 x 20 and 3 x 20 mJ.cm^{-2}) (Fig 4.2.4). A similar trend was observed for the *Salmonella* isolate in the sterile Plankenburg River water, although only at the highest UV dose tested (3 x 20 mJ.cm^{-2}) (Fig 4.2.4). For the *E. coli* and *L. monocytogenes* strains tested no significant difference could be observed in survival trends at higher UV doses (2 x 20 and 3 x 20 mJ.cm^{-2}).

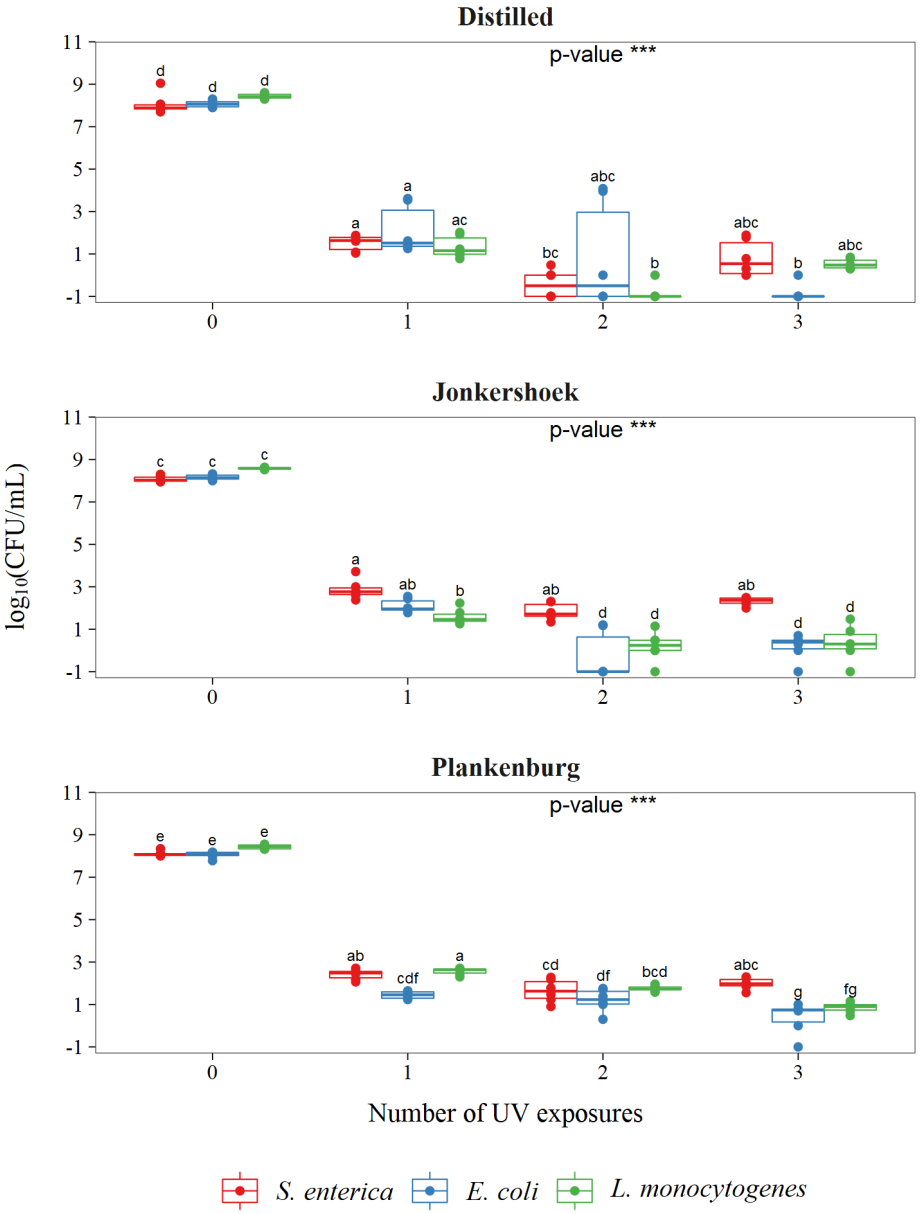


Figure 4.2.4 Comparison of different organisms' survival in similar water matrix post multiple exposure to 20 mJ.cm^{-2} UV doses (1 x 20; 2 x 20; 3 x 20). A log value of -1 was assigned when no growth was observed, with values between log 0-log 1 representing CFU numbers between 1-10 CFU.mL^{-1}. Significant differences ($p < 0.01$) are indicated by different letters. The box is drawn from the first quartile to the third quartile and the horizontal line through the box indicates the median with each dot representing a data point to show distribution.

Table 4.2.2 indicates the physio-chemical characteristics of the two river water batches used after it was sterilised. Important to note that, due to the autoclaving process, the pH values are higher than normal due to the autoclaving process, which could have contributed to various chemical reactions (such as reduction, hydrolysis, condensation, precipitation, depolymerisation), most of which produced or consumed protons (Vacin & Went, 1949). (Normal pH values for the Plankenburg are usually between 6.48 and 7.55 [Tables 4.1.3 and 4.1.12 in chapter 4.1] and for the Jonkershoek usually between 6.72 and 7.21 [Oosthuizen, 2022]). Total dissolved solids, total soluble solids and alkalinity were the characteristics which showed the highest variability between the autoclaved Plankenburg and Jonkershoek river water batches. Most parameters were within the required limits for irrigation water (specified in Section 3.1 of Chapter 3 'General methods') except the following: the TDS content and pH of the Plankenburg river, and the alkalinity value of the Jonkershoek River water (Table 4.2.2).

It should be noted that for both batches the UVT% values were comparable to the averages reported for three of the rivers (excluding the Mosselbank) analysed as part of the previous chapter (Tables 4.1.3 and 4.1.12 in Chapter 4.1). The Jonkershoek River batch, in particular, had a UVT% (74.5%) which was in the same range as the data presented for the 'best scenario' Franschhoek river in the previous chapter (Tables 4.1.3 and 4.1.12 in Chapter 4.1). Differences observed between the physico-chemical profile of the river water batches, as well as between the river water and the distilled water (which had no physico-chemical content) could account for the different survival patterns observed for *Salmonella* in the different water matrices (Fig 4.2.4).

Table 4.2.2 Physico-chemical characteristics of sterilised river water used as matrix for pure strains

Characteristics	Plankenburg River	Jonkershoek River
UVT%	62.1	74.5
TDS ($mg.L^{-1}$)	356	64
TSS ($mg.L^{-1}$)	38	5
COD ($mg\ O_2\ L^{-1}$)	12	8
pH	9.19	8.35
Turbidity (NTU)	7.2	6.27
EC ($mS.m^{-1}$)	0.47	0.08
Alkalinity ($mg\ CaCO_3.L^{-1}$)	70	465

The results obtained as part of the post-UV recovery observations for *E. coli, Salmonella* ATCC 14028 and *L. monocytogenes* in Study A2, are presented in Fig 4.2.5. Differences were observed between the effect of UV doses (1 x 20, 2 x 20 and 3 x 20 $mJ.cm^{-2}$) after time (3h) was allowed for recovery of the

UV-injured inoculums in both light and dark conditions. The number of surviving colonies observed varied based on species as well as type of water matrix, and UV dose. This was expected given the inherent differences between the organisms, as well as the nutrient-poor nature of distilled water and the complex composition variations between different rivers (Table 4.2.2).

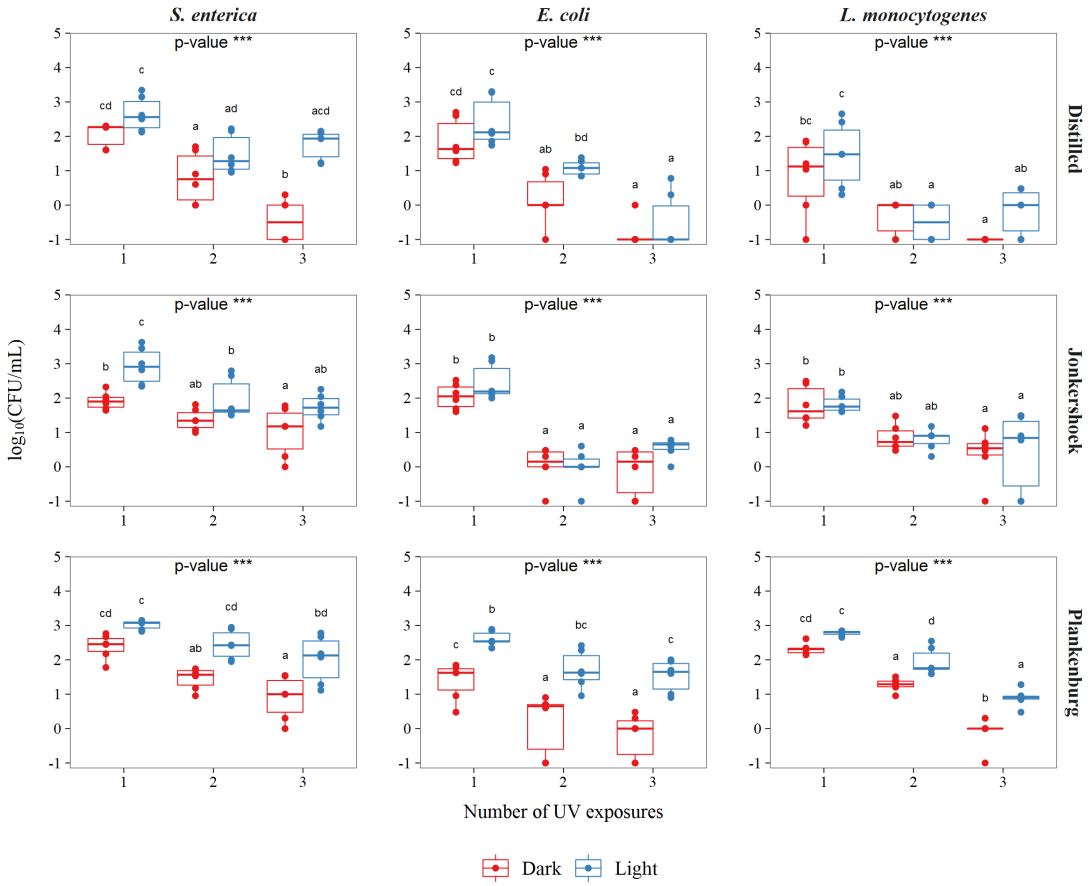


Figure 4.2.5 Comparison of microbial recovery under light and dark conditions in different water matrix following multiple exposures to 20 mJ.cm^{-2} UV doses (1 x 20; 2 x 20; 3 x 20 mJ.cm^{-2}). A log value of -1 was assigned when no growth was observed. Significant differences ($p < 0.01$) are indicated by different small letters. The box is drawn from the first quartile to the third quartile and the vertical line through the box indicates the median with each dot representing a data point to show distribution.

Overall, it should be noted that even with the time (3h) allowed for recovery, CFU numbers did not rapidly increase to average levels above 3 log (1 000 CFU.mL^{-1}) during the recovery period (Fig 3.6), which was also the maximum average levels observed directly after UV treatment (Fig 4.2.4). Although not significant for all doses, overall, there was also a trend that better survival of the UV injured inoculums was observed in the presence of light (Fig 4.2.5). Another trend, not surprisingly, was also that better recovery was also observed after treatment with lower initial UV doses (Fig 4.2.5).

The organism which showed the best capacity for recovery in this study, regardless of dose, water matrix or the presence of light/darkness, was *Salmonella*.

4.2.3.3 Study B

The results obtained as part of the UV treatment and post-UV recovery observations for the Mosselbank river in Study B, are presented in Fig 4.2.6. The results indicate that both the doses applied as part of this study were effective to reduce *E. coli* levels present in this river to below detectable limits directly after UV treatment. No post-UV recovery was furthermore observed for the *E. coli* population under light or dark conditions.

Both the Coliform and HPC populations survived UV treatment. Although no significant differences in colony counts were observed between the two doses directly after UV treatment, lower levels of recovery were observed after three hours for HPC and Coliforms following the double dose (2 x 17 mJ.cm^{-2} = 34 mJ.cm^{-2}). HPC levels were reduced more permanently following the higher UV dose. At the lower dose the HPC count dropped with 2 log CFU.mL^{-1} directly after UV and then increased more during the recovery period in the light box compared to the dark conditions (Fig 4.2.6).

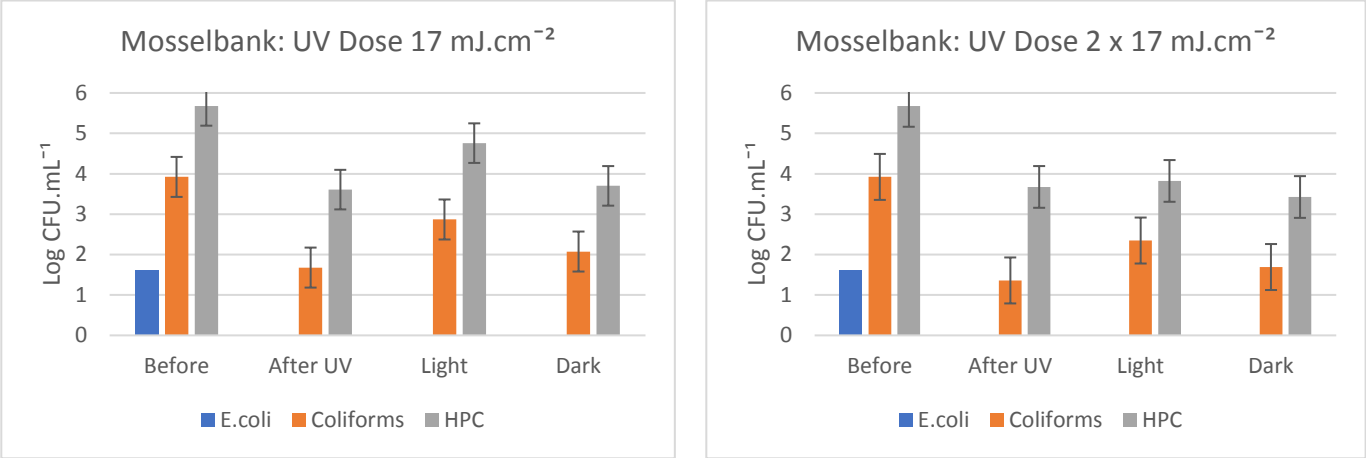


Figure 4.2.6 Average colony counts tested across three sampling occasions from Mosselbank river, indicated as Log CFU.mL^{-1} with error bars representing standard deviation. The detection limit for this method was 1.0 Log CFU.mL^{-1}.

The results obtained as part of the UV treatment and post-UV recovery observations for the Plankenburg river in Study B are presented in Fig 4.2.7. This river had a much higher *E. coli* count than the Mosselbank river, although the total Coliform levels were in the same range. Both UV doses effectively reduced *E. coli* and Coliform levels to below the detection limit, but only Coliform levels

recovered slightly after the recovery period of 3h in the light box (Fig 4.2.7) that followed both doses. The HPC population proved to be more resilient compared to the HPC population of the Mosselbank (Fig 4.2.6), as the CFU levels after recovery under the light conditions – following both UV doses (1 x 17 and 2 x 17 mJ.cm^{-2}) – were higher than what was observed directly after UV treatment before the 3h recovery period (Fig 4.2.7).

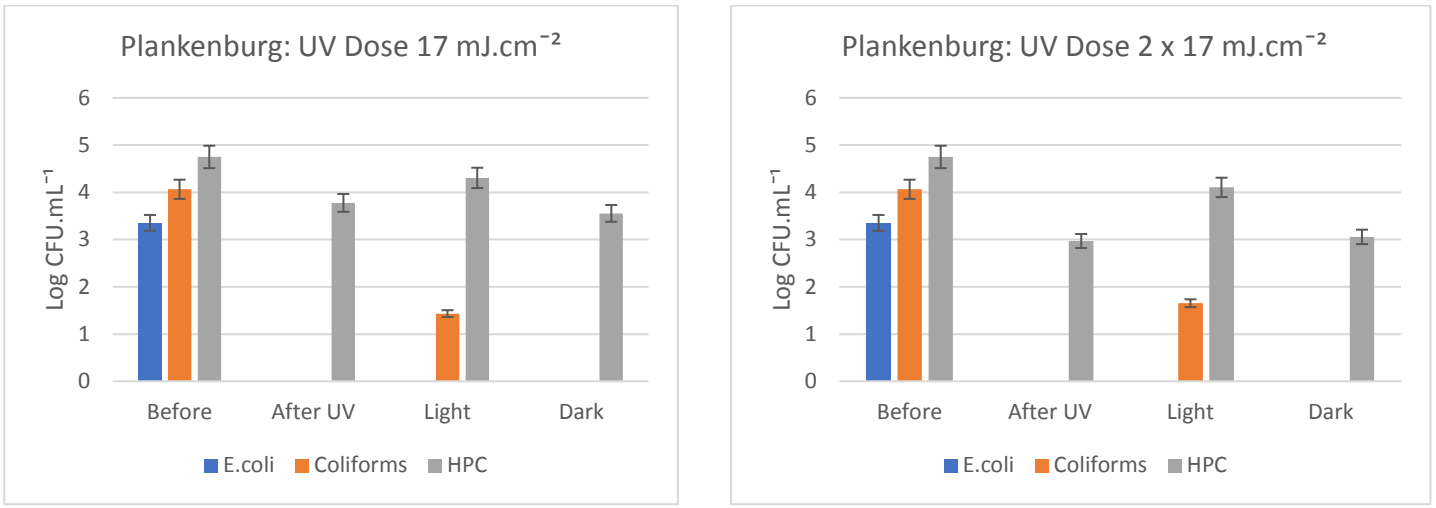
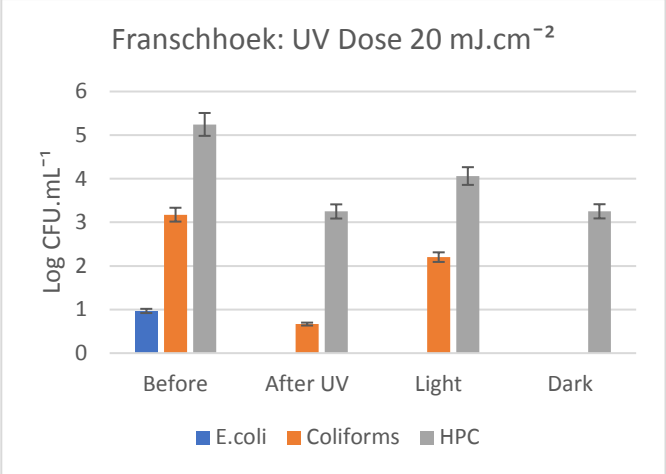


Figure 4.2.7 Average colony counts tested across three sampling occasions from Plankenburg river, indicated as Log CFU.mL^{-1} with error bars representing standard deviation. The detection limit for this method was 1.0 Log CFU.mL^{-1}.

The results obtained as part of the UV treatment and reactivation observations for the Franschhoek river in Study B are presented in Fig 4.2.8. This river had the lowest initial *E. coli* and Coliform levels of all three rivers tested. Like the previous two rivers, no *E. coli* was detected after UV treatment and recovery. Interestingly, the Coliform population recovered after UV treatment under light conditions and decreased under dark conditions to below detectable limits. The degree of recovery in the light box was more prominent after the lower UV dose was applied (Fig 4.2.8). The HPC population was reduced after UV but remained at levels above 3 log CFU.mL^{-1}. Slight recoveries in HPC levels were only observed after light exposure and not in the control incubated in the dark box.



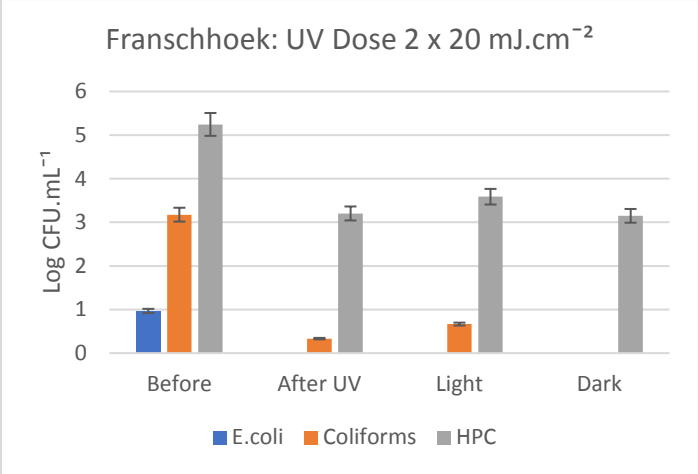


Figure 4.2.8 Average colony counts tested across three sampling occasions from Franschhoek river, indicated as Log CFU.mL^{-1} with standard deviation bars included for error across sampling occasions. The detection limit for this method was 1.0 Log CFU.mL^{-1}.

The results for the physico-chemical analyses of the three rivers are presented in Table 4.2.3. Plankenburg had the highest turbidity value and lowest UVT% value. The TDS content of Mosselbank was the highest, and outside the guideline limit for irrigation water (<260 mg.L^{-1}). The alkalinity values determined for Plankenburg and Mosselbank, were also both outside the guideline limit (<120 mg $CaCO_3$.L^{-1}). Franschhoek had the highest UVT% value which coincided with the lowest values of total TDS and TSS.

Table 4.2.3 Physico-chemical characteristics of sampled river water.

Characteristics	**River location**		
	Mosselbank	Plankenburg	Franschhoek
UVT%	40.1	13.3	69.0
TDS (mg.L^{-1})	701	247	89
TSS (mg.L^{-1})	14	32	6
COD (mg O_2.L^{-1})	47	52	<10
pH	7.21	7.67	6.96
Turbidity (NTU)	6.88	38.4	5.38
EC (mS.m^{-1})	0.76	1.30	0.15
Alkalinity (mg $CaCO_3$.L^{-1})	186	149	65

The results for the pathogen detection methods are presented in Table 4.2.4. These indicate that *L. monocytogenes* was present in the Mosselbank and Franschhoek river water batches, but the UV treatment was sufficient in reducing the pathogen to below detectable limits. No *Salmonella spp*. could be detected in any of the rivers at the times sampled as part of this study.

Table 4.2.4 Presence and absence results of *Salmonella* spp. and *L. monocytogenes* in river water samples, prior to the application of UV radiation, after UV radiation, after light box and after dark box. Positive test results are indicated by a "+" sign and negative tests are indicated by a "-" sign for the specific organism.

River	Pathogen tested	Before UV	After single UV dose	After Light	After Dark	After double UV dose	After Light	After Dark
Mosselbank	*Salmonella* spp.	-	-	-	-	-	-	-
	L. monocytogenes	**+**	-	-	-	-	-	-
Plankenburg	*Salmonella* spp.	-	-	-	-	-	-	-
	L. monocytogenes	-	-	-	-	-	-	-
Franschhoek	*Salmonella* spp.	-	-	-	-	-	-	-
	L. monocytogenes	**+**	-	-	-	-	-	-

4.2.4 DISCUSSION

In studies A1 and A2 the recovery potential of pure bacterial strains (*E.coli*, *L. monocytogenes* and *Salmonella* spp.) were studied in three different water matrices, including distilled water as well as autoclaved river water obtained from two different rivers. This was followed by Study B where the presence and recovery potential of various indigenous microbial populations (including *E. coli*, Coliforms, HPC and the pathogens *L. monocytogenes* and *Salmonella* spp.) in three different rivers (Plankenburg, Mosselbank and Franschhoek) were studied.

4.2.4.1 Studies A1 & A2

Prior to the UV treatments that were applied as part of these studies, bacterial inoculums of the pure strains (Table 4.2.1) were intentionally prepared to have very high initial CFU.mL^{-1} concentrations between 8-9 log CFU.mL^{-1}, which are significantly higher than any of the environmental *E. coli* levels measured as part of the river water analyses in previous deliverables. As part of this study, colony counting strategies were also employed for the *L. monocytogenes* and *Salmonella* spp strains (opposed to the standard presence/absence detection methods done as part of the previous chapter). This was done in an attempt to get a more accurate indication of the impact of UV dose on microbial recovery after UV treatment of *L. monocytogenes* and *Salmonella* specifically.

As mentioned, all growth (defined as CFU.mL^{-1}) after UV treatment and recovery were recorded, although it is acknowledged that statistical error increases (as a percent of the colony average) when plate counts are below 25 CFU.mL^{-1} (Sutton, 2006). In spite of the high statistical variations, colony counts below 25 CFU.mL^{-1} at the lowest dilutions plated were still considered to be indicative of microbial survival and recovery after UV treatment.

As reported in the results section, the initial UV dose of 1 x 20 mJ.cm^{-2} resulted in a minimum reduction of 6 log CFU.mL^{-1} to levels below 3 log CFU.mL^{-1}, regardless of the microbial species or the water matrix in which the UV treatment was applied (Figures 4.2.3 & 4.2.4). After the rapid decrease in CFU numbers after the 1 x 20 mJ.cm^{-2} UV dose was applied, CFU numbers stabilised at very low levels and overall, the decreases that were observed after the higher UV doses were applied were not always significant (Fig 4.2.4). A similar “tailing effect” that followed a large initial decrease in CFU numbers was reported by Zhang et al. (2017), who exposed several *E. coli* strains (suspended in BPW) to increasing UV doses. Interestingly, Zhang et al. (2017) also reported that the “tailing effect” was observed after a UV dose of 20 mJ.cm^{-2} was applied, and that no significant differences in CFU decrease tempo were observed between different *E. coli* strains once the tailing phase started. Our

study has thus indicated that the "tailing effect" was also observed for *L. monocytogenes* and *Salmonella* spp in addition to *E. coli*, and that this phenomenon occurs despite the water matrix used.

Between the different species studied, there were minimal significant differences ($p < 0.01$) observed (Fig 4.2.4). *S. enterica* exhibited the least difference between UV doses irrespective of the water matrix, while the *E. coli* and *L. monocytogenes strains* followed similar UV dose response trends. When comparing the low-pressure UV dose required for a 5-log reduction, without taking recovery into account, *E.coli* requires 1.3-15 $mJ.cm^{-2}$ (dependant on strain), *Salmonella spp*. 14 $mJ.cm^{-2}$ and *L. monocytogenes* 4.6 $mJ.cm^{-2}$ (Malayeri et al., 2016). These dose ranges support the results presented in Fig 4.2.4.

In contrast, some studies have reported that it is the fact that *L. monocytogenes* is Gram-positive that causes it to be more UV resistant (Shin *et al.,* 2016). However, the studies cited by Shin et al. (2016) studied the UV sensitivity of this strain in matrices such as BPW and Apple juice (Gabriel & Nakano, 2009). In addition, it has also been reported that, even without UV treatment, survival over time of Gram-positive strains such as *L. monocytogenes* suspended in minimal media such as distilled water is less than in phosphate buffered saline, compared to Gram-negative strains (Liao & Shollenberger, 2003). Considering this, it might be argued that the liquid matrix in which *L. monocytogenes* is suspended when it is subjected to UV treatment, might affect the capacity of the organism to survive UV treatment.

When recovery under light and dark conditions were taken into consideration, the effective log reduction after storage in light (Fig 4.2.5) remained similar to that observed directly after UV treatment (Fig 4.2.4). This is interesting as numerous previous studies found that photoreactivation was significant (Wang *et al.,* 2021; Sivhute, 2019; Oliver, 2015). One of the explanations for this finding could be the nutrients and ideal conditions the organisms plated directly after UV treatment was subjected to. Kollu & Örmeci (2015) demonstrated that availability of nutrients has a major effect on regrowth of bacterial cells and lysis of cells deactivated by UV light could also add nutrients to a given environment.

On closer inspection of dark and light conditions (Fig 4.2.5), it is notable that in some circumstances a significant difference ($p < 0.01$) is observed as was the case with all strains in Plankenburg river water after the second and third UV dose where there was a bigger decline under dark conditions than light. It seems the physico-chemical characteristics of the water matrix influenced the difference between recovery under light and dark conditions as Jonkershoek River water and distilled water had minimal differences between light and dark recovery. A possible reason could be that UV radiation was more effective in the latter water matrices and therefore less cells were still in a state to recover. Wen et al. (2019b) observed something similar with fungal spores that exhibited

higher photoreactivation in groundwater than phosphate buffer solution (PBS) after a higher UV dose was required in groundwater than PBS to produce a 2-log inactivation.

Salmonella displayed the most constant recovery between water matrices and UV dose with colony counts resulting in 2 log CFU.mL^{-1} even after being radiated with 3 x 20 mJ.cm^{-2} UV light. It is therefore considered the most resistant strain tested in the river water batches included in this study.

This finding has two implications. Firstly, *Salmonella* is pathogenic, has a low infective dose and is frequently linked to produce-related food-borne disease outbreaks. It has also been detected on a sporadic basis in the rivers tested as part of the Chapter 4.1. In this study, the *Salmonella* ATCC 14028 strain was also resistant to increasing UV doses, and showed the ability to survive post-UV, even though levels remained low. Since the presence of *Salmonella*, even at low levels, could thus still pose a health threat to consumers, it is therefore recommended that standard presence/absence monitoring for this pathogen be included on a regular basis in surface waters that is subjected to UV treatment before produce irrigation.

Secondly, considering the survival post-UV observed in this study, the application of the resistant strain (*Salmonella* ATCC 14028) as a "challenge microorganism" (USEPA, 2006, p D-3) to determine optimal UV dosing (to prevent post UV recovery) for the target food pathogens *E. coli*, *L. monocytogenes,* and other *Salmonella* spp. could be explored further. It is, however, recommended that presence/absence testing be included for the detection of *Salmonella*, *E. coli* and *L. monocytogenes*, in addition to CFU counting methods, to compensate for the high statistical CFU variations observed at low log values.

In terms of the physico-chemical guideline limits for irrigation water (Section 3.1, Chapter 3), it should be noted that these limits primarily relate to the impact certain water properties might have on the irrigation process and the soil and plant quality. These guidelines should thus not be viewed as requirements to ensure UV process efficacy, but it is acknowledged that water of which the parameters measured fall within the guideline limits presented in Section 3.1 (Chapter 3), can be treated very effectively with UV disinfection technologies. It has been reported by various authors that the physico-chemical characteristics of water affects UV treatment efficiency (Reddy & Krishnamurthy, 2020). TSS and TDS are, for instance, known to absorb UV radiation or scatter the light and shield microorganisms present in water (Carré *et al.*, 2018; Christensen & Linden, 2003; Ong *et al.*, 2019). The upper recommended limit for TSS content for effective UV treatment is 30 mg.L^{-1} (USEPA, 1999), which is lower than the 50 mg.L^{-1} guideline limit for general irrigation water (Table 3.2). Two other very important parameters to consider in the application of UV technologies is the UVT% and the turbidity of the water subjected to treatment. Neither of these parameters are included in the general guideline limits (Section 3.1, Chapter 3). UVT% has a direct impact on UV dose delivery

and is a critical parameter to consider (USEPA, 2006). Turbidity (measured in NTU) can furthermore affect the accuracy of UVT measurements and lead to an underestimation of UVT% values in water with turbidity values above 10 NTU (although some authors reported that the upper limit can be as low as 3 NTU) (USEPA, 2006).

Based on these considerations, the TSS levels observed in the Plankenburg River as part of this deliverable (38 $mg.L^{-1}$ in Table 4.2.2 and 32 $mg.L^{-1}$ Table 4.2.3), could thus be considered as just out of range for optimal UV treatment. The small difference between these TSS readings (6 $mg.L^{-1}$), is however in contrast with the significant differences observed in UVT% values (62% in Table 4.2.2 and 13% in Table 4.2.3) and Alkalinity values (70 mg $CaCO_3.L^{-1}$ in Table 4.2.2 and 149 mg $CaCO_3.L^{-1}$ in Table 4.2.3). A large difference was also observed in turbidity values (7 NTU in Table 4.2.2 and 38.5 NTU in Table 4.2.3). To what degree the latter influenced the underestimation the UVT% measurement in studies A2 and B is not clear, as the Plankenburg's turbidity (Table 4.2.3) was above the recommended 10 NTU, and all the rivers had turbidity values (Table 4.2.3) above 3 NTU (USEPA, 2006). Overall, it can be concluded that a variety of factors can influence the physico-chemical profile of river water, and these should be considered during the design of large-scale installations to compensate for high physico-chemical variability and ensure optimal long-term UV treatment efficacy.

4.2.4.2 Study B

The physico-chemical characteristics in Table 4.2.3 confirm that every river had a different physico-chemical profile, which can, in turn, affect UV irradiation efficacy and photoreactivation (Mao et al., 2018; Shafaei et al., 2017; Giannakis et al., 2014). When compared with the guideline limits (Section 3.1, Chapter 3), Mosselbank's total dissolved solids and alkalinity were above the recommended specification with 441 $mg.L^{-1}$ and 66 $mgCaCO_3.L^{-1}$, respectively. Plankenburg had an alkalinity above 120 mg $CaCO_3.L^{-1}$ and although the guideline limits (DWAF, 1996a) do not include a specification for the allowed turbidity for irrigation water, The Water Quality Guidelines for Domestic Use (DWAF, 1996c) state that for turbidity values exceeding 10 NTU, the water carries an associated risk of disease. The fact that Plankenburg had a turbidity value of 38.4 NTU, which was markedly higher than the other two rivers, could indicate potential risk. Based on physico-chemical characteristics only Franschhoek River was within the guideline limits. There is, however, still a microbiological risk as Franschhoek did test positive for *L. monocytogenes* (Table 4.2.4) and *E. coli* (Fig 4.2.8).

Plankenburg had the highest *E. coli* counts compared to the other rivers (Figures 4.2.6-4.2.8), which might be indicative of higher faecal contamination (Ashbolt *et al.*, 2001). This could be due to human activities upstream including informal settlements with minimal sewage infrastructure and an industrial area. These types of activities have been linked to faecal contamination of the environment

before (Bridgemohan *et al.*, 2020; Santo Domingo & Edge, 2010). The coliform counts were quite similar for Mosselbank (Fig 4.2.6) and Plankenburg (Fig 4.2.7), with Franschhoek indicating 0.8 log CFU.mL^{-1} less (Fig 4.2.8). The Irrigation Water Guidelines (DWAF, 1996a) specify only an *E. coli* limit of 1 000 CFU.100 mL^{-1} for agricultural irrigation water, which is similar to the detection limit in this study of 1 log CFU. mL^{-1}. The results indicate that both the Mosselbank (Fig 4.2.6) and the Plankenburg (Fig 4.2.7) exceeded this guideline limit by a significant margin before UV treatment.

UV treatment was effective at reducing the *E. coli* levels to below the guideline limits for all doses applied, with no recovery observed in any of the samples (Fig 7-9). Some coliforms did however survive and demonstrate potential photo-repair abilities, with a higher level of reactivation after a single UV dose in comparison with a double dose (Figures 4.2.6-4.2.8).

Although there is no limit specified for the HPC in the Irrigation Water Guidelines (DWAF, 1996a), all of the rivers had high initial HPC counts between 4.5 and 5.7 log CFU.mL^{-1} (Figures 4.2.6-4.2.8). HPC measurements are generally used as an efficacy indicator of water treatment processes (DWAF, 1996c) and after both UV dose treatments for all the river samples the HPC count was still high. Horn *et al.* (2016) found evidence suggesting that HPC bacteria may be hazardous to human health with *Bacillus cereus* identified as the highest pathogenicity risk from this group of bacteria. Only Plankenburg's results (Fig 4.2.7) showed a bigger initial decrease after 2 x 17 mJ.cm^{-2} UV dose in comparison with 1 x 17 mJ.cm^{-2}. The results of both Mosselbank and Franschhoek (Figures 4.2.6 and 4.2.8) indicated no significant difference between the reduction of HPC after a single dose or double dose of UV radiation.

Photoreactivation of the HPC population (Figures 4.2.6-4.2.8) was observed for most doses in all three the rivers, with dark repair showing no significant increase after UV radiation. When the photo-repair is considered the nett reduction after a single dose of UV radiation was 0.9 log CFU.mL^{-1}, 0.4 log CFU.mL^{-1} and 1.2 log CFU.mL^{-1} for Mosselbank, Plankenburg and Franschhoek, respectively. After a double UV dose and photoreactivation, the decrease in HPC counts were 1.9 log CFU.mL^{-1}, 0.6 log CFU.mL^{-1} and 1.7 log CFU.mL^{-1} for Mosselbank, Plankenburg and Franschhoek, respectively. Therefore, it was concluded that in this study the higher UV dose (administered as a double the original UV dose given to each sample individually) was slightly more effective than a single, lower UV dose.

Both Mosselbank (Fig 4.2.6) and Franschhoek (Fig 4.2.8) rivers follow the trend of a larger decrease in reactivation after a double (higher) dose, when compared to recoveries observed after a single dose. In contrast, the Plankenburg results (Fig 4.2.7) indicated a higher level of reactivation after the double (higher) dose as opposed to a single dose. A possible reason for this could be linked to the extremely low UVT% observed in this water (Table 4.2.3), which might have resulted in unpredictable UV treatment impacts. It has been reported that high turbidity values (which was also observed in this

river – Table 4.2.3) can decrease the UVT% (Gurol, 2005). Microbial aggregates can be protected from UV penetration by the particulates in turbid samples (Farrell et al., 2018), which might lead to more superficial damage easily fixed by photoreactivation.

Although no *Salmonella* spp could be detected in this study (Table 4.2.4), the sporadic presence of this genus in river water has been reported as part of previous deliverables. Since the pure *Salmonella* strains tested as part of Study A1 and A2, seemed more resistant to UV treatment than the other strains, monitoring the presence of this pathogen in irrigation water should be considered.

It was, however, encouraging that *L. monocytogenes,* which was detected initially in both the Mosselbank and Franschhoek rivers, did not survive UV treatment and recovery (Table 4.2.4). This is a saprophytic organism which is widely distributed in nature (Vasquez-Boland *et al.,* 2001). The presence of *Listeria* species in general in river water is thus not surprising. Unfortunately, *L. monocytogenes* could pose a threat to fresh produce consumers because of its pathogenic infective potential (Vasquez-Boland *et al*., 2001). The need to control this organism in minimally processed foods is therefore of great importance to ensure consumer safety.

4.2.5 CONCLUSIONS

Studies A1 and A2 verified the recovery potential of various *E. coli, Salmonella spp.* and *L. monocytogenes* strains under light and dark conditions following low-pressure UV irradiation. It was confirmed that the type of water matrix and UV treatment plays a role in the recovery of these isolates. Of the three species tested, *Salmonella* was the least affected by the type of water matrix in which UV treatment was applied.

In Study B, three rivers with different physico-chemical characteristics were assessed using the same reactivation method and box design. It was found that high turbidity could affect the level of reactivation as it decreases the efficiency of the UV radiation to destroy the microorganisms. In this study E. *coli* and *L. monocytogenes* were successfully eliminated by doses between 17-40 $mJ.cm^{-2}$, applied as either single or cumulative doses of UV radiation, regardless of the river water source. Coliforms (excluding *E. coli*) indicated great potential for reactivation. In addition, UV treatment did not impact HPC counts significantly. Further investigation should be conducted on isolated strains of Coliforms and HPC bacteria to determine whether the UV-surviving populations include any pathogens which could pose a health risk to consumers.

4.3 Pilot scale UV treatment dose responses of selected indicators and specific food pathogens present in various irrigation water sources (including impact of microbial recovery)

Please note: Data presented as part of this chapter are extracts from the MSc theses of Oosthuizen (2022) and Jankowitz (In press)

4.3.1 BACKGROUND & AIM

4.3.1.1 Background

The application of UV irradiation as a water treatment method for large volumes of water, is a rapidly developing technological field of study. The efficacy of the process can depend on various design factors, including the type of lamp chosen as UV source. However, for the purpose of treating larger volumes of water in this project, a medium-pressure (MP) lamp-based device was used, which was similar to the installation used in the pilot-scale studies of Olivier (2015). A detailed description of the installation used in this project is given in Section 3.5.2 in Chapter 3 'General Methods'.

Various practical factors should be considered when transitioning from a laboratory-scale low-pressure (LP) UV collimated beam system to pilot-scale MP 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. Jones *et al*. (2014) also stressed that a study simulating a water treatment process in a real agricultural setting, with the use of significantly larger water volumes, is of immense importance to the test the UV-C efficiency.

Along with the transition from laboratory-scale water volumes to pilot-scale water volumes, the implementation of MP UV lamps instead of LP UV lamps might add additional treatment variability (Zimmer-Thomas et al., 2007). Firstly, the LP UV system used at lab-scale emits UV light at a monochromatic wavelength of only 253.7 nm (Zimmer-Thomas et al., 2007). The MP UV lamp emits polychromatic wavelengths, with a broader range from 185 to 1 367 nm (Mofidi *et al*., 2002). According to Ijpelaar *et al*. (2010), MP UV may be more advantageous for a pilot-scale water disinfection systems as the output energy and power density are significantly higher, enabling the construction of a compact system. In addition, the broader wavelength range may potentially target a variety of microbial structures, resulting in irreversible physiological damage in microorganisms (Zimmer & Slawson, 2002).

As discussed, the ability of bacteria to recover after UV irradiation using various light-dependent and light independent repair processes, can be another process limiting factor to consider (Johann to Berens & Molinier, 2020). These abilities have developed because bacteria are exposed to

UV in nature, causing many environmental bacteria to live in a delicate balance between UV damage and repair (Calkins & Thordardottir, 1980; Hanawalt, 1989; Mitchell & Karentz, 1993). The presence of microbial repair mechanisms – in food pathogens in particular – should therefore be considered when determining the UV dose required to achieve the desired log reduction. To implement UV-treatment of irrigation water successfully at farm-level, it is important to determine the sufficient UV dose applicable to ensure that microbial DNA is injured beyond repair.

4.3.1.2 Aim

The aim of this study was to determine the disinfection efficiency of a pilot-scale, medium pressure UV-C system treating larger volumes of river water (from different sources), with a single UV radiation dose (1 x 20 $mJ.cm^{-2}$), as well as double (2 x 20 $mJ.cm^{-2}$) or triple (3 x 20 $mJ.cm^{-2}$) UV radiation dose.

- The first objective was to establish the efficacy of the UV system by comparing microbial loads present before and directly after each of the UV treatments.
- The second objective was to determine the recovery potential of microbial populations naturally present in river water by comparing microbial loads directly after UV treatment with samples that had time to recover for three hours after different UV treatments. Both objectives were tested as part of two studies (Study 1 and Study 2) conducted in 2021 and 2022.
- The third objective was to isolate and identify surviving colonies after UV treatment AND recovery.

4.3.2 MATERIALS & METHODS

4.3.2.1 Study design

Two experimental studies (Study 1 and Study 2) were conducted on river water from different sampling sites to determine the disinfection efficacy of a UV-C pilot plant (Objective 1), followed by a reactivation procedure to determine the degree of recovery of specific microbial populations and food pathogens present in river water (Objective 2). Colonies that were present after UV treatment and recovery were isolated for further identification (Objective 3)

Study 1: Plankenburg, Mosselbank & Franschhoek rivers (August-October 2021) (Oosthuizen, 2022)

For this study river water was sourced from three different rivers in 1 000 L volumes. The choice of the three rivers that served as water sources in this study was based on the results presented as part of Chapter 4.1 of this report (Bursey, 2021; Oosthuizen, 2022). Sites chosen included the Plankenburg river (poorest microbial characteristics), the Mosselbank river (poorest physico-chemical

characteristics), and the Franschhoek river (best microbial and physico-chemical characteristics). 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 presented in Appendix D, included at the end of this chapter).

As part of Objective 1, standard physico-chemical analysis was performed on samples of all three rivers after bag filter filtration before UV treatment. 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}), were applied using the medium-pressure pilot-scale UV system (Berson, The Netherlands). Microbial analyses were performed before and after UV radiation and included Heterotrophic Plate Count (HPC), *E. coli* and Total Coliform (TC) counts, as well as the detection of *Listeria monocytogenes* and *Salmonella*. In addition, microbial colonies that survived UV radiation were identified by MALDI-TOF analysis.

For Objective 2, the recovery potential (including photoreactivation and dark repair) after UV treatment was determined for the of *E. coli*, *Salmonella spp*., *Listeria monocytogenes*, coliforms, and heterotrophic bacteria populations (HPC) present in the river water.

As part of objective 3, UV surviving colonies were isolated and identified using MALDI-TOF analysis.

Study 2: Farms A, B and C situated along the Eerste and Berg Rivers (June 2022) (Jankowitz, *In press*)

For this study water was sourced from three different farms where river water is used for irrigation. The rivers in question were the Eerste River, and the Berg River system at two different locations. The volumes sampled were again 1 000 L, which was pumped through a bag filter prior to UV radiation at doses of (2 x 20 mJ.cm^{-2} = 40 mJ.cm^{-2}) and (3 x 20 mJ.cm^{-2} = 60 mJ.cm^{-2}) . These respective doses were chosen due to survival and reactivation observed at lower doses in Study 1. Again, to address Objective 1, HPC, *E.coli* and Total Coliform counts counts were determined, as well as detection *of Listeria monocytogenes* and *Salmonella*. In addition, *E.coli* detection after an enrichment step was also done to determine survival below the colony count detection limits. As in Study 1, the recovery potential of the microbial populations (under light and dark conditions) was also investigated to address Objective 2. In order to address Objective 3 in this study, surviving colonies after both UV and Recovery were isolated and identified using VITEK-2 analysis.

4.3.2.2 General methods for Studies 1 and 2

Site selection

Study 1:

Three rivers in the Western Cape were selected based on their use as irrigation water sources, and their known microbial and physico-chemical profiles that were established as part of previous work (results presented as part of Chapter 4.1 of this report; Bursey, 2021; Oosthuizen, 2022). The site descriptions and coordinates are presented in Table 4.3.1. The Plankenburg river, which is in Stellenbosch, acted as a study control as previous studies have indicated very high microbial loads (Bursey, 2021; Sivhute, 2019). The Plankenburg river has also previously been identified as the 'worst-case scenario river' in terms of microbiological characteristics (Bursey, 2021).

The Mosselbank river site is in the Kraaifontein area and is situated close to a wastewater treatment plant (WWTP). This river acts as the irrigation water source for large-scale commercial farmers further downstream (Bursey, 2021). When compared to the Plankenburg river, the Mosselbank river has previously been identified as the 'worst-case scenario river' in terms of physico-chemical characteristics (Bursey, 2021).

The last river, the Franschhoek river in Franschhoek 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, 2021). Previous analyses conducted at this site have indicated that this river has some of the best physico-chemical and microbial characteristics, compared to the other two rivers (Bursey, 2021).

Study 2:

Three farms in the Western Cape were selected based on accessibility and the active irrigation of produce from the river on the farm. No previous water analyses were conducted at these locations and the description and coordinates are summarised in Table 4.3.1.

Farm A is a research farm located on the banks of the Berg River just off the R45. They use water from the river to irrigate guavas, grapes and stone fruit trees. This is located closer to the origin of the Berg River before it enters Paarl.

Farm B is a commercial farm that cultivates peppers and aubergines via irrigation from the Berg River that flows past their lands. This farm is located after Paarl on the outskirts of Wellington and was sampled after heavy rainfall.

Farm C is a well-known commercial wine farm, which also cultivates vegetables for food outreach programmes and cattle for Karan Beef products. They only irrigate grass that serves as cattle

fodder directly from the Eerste river that flows through their lands, as they have previously found the water is not always of a high enough standard for the irrigation of their vegetable patches.

Table 4.3.1 Sampling locations and descriptions of the river sites used in Study 1 and Study 2.

River location	Location description	Coordinates
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
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
Farm A (Berg River)	Located in Franschhoek off the R45 and used for agricultural research on stone fruit, guavas and grapes.	33°50'23.4" S 18°59'04.8" E
Farm B (Berg River)	Located between Paarl and Wellington where the R44 and the R45 cross. Irrigates peppers and aubergines from the Berg River.	33°39'02.2" S 18°58'03.4" E
Farm C (Eerste River)	Located off the R310 in Stellenbosch and irrigates feeding pastures for cattle from the Eerste River.	33°58'21.3" S 18°47'12.2" E

River water collection method

A utility vehicle with a 1 000 L mobile water tank was used to collect a 1 000 L river water batch per sampling 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. At each of the three river sites (Mosselbank, Franschhoek and Plankenburg river) 1 000 L of water was collected once. Water samples for further analyses were taken in triplicate at the pilot plant setup at each sampling point: 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. Physico-chemical tests were performed the next day, within 24 hours of the sampling.

Pilot plant operating procedure and UV dosing

In Figure 4.3.1 an illustration of the pilot plant system is presented. 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 (chosen based on the results presented in Appendix D). 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.

Before UV disinfection commenced using the MP-UV system described in Section 3.5.2 in Chapter 3, 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 ($mJ.cm^{-2}$) 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 1 L Schott bottles at taps situated directly before and after the UV chamber (Figure 4.3.1). Triplicate water samples (n=3) were taken before and after UV radiation, for each 1 000 L batch of river water collected in Study 1. For Study 2, three additional water samples (n=6) were analysed after UV radiation to increase the sample size.

Two UV doses were applied in both Studies 1 and 2. It was decided to apply a single and double UV dose of 20 $mJ.cm^{-2}$ (1x20 = 20 $mJ.cm^{-2}$ and 2x20 = 40 $mJ.cm^{-2}$) for Study 1, and a double and triple dose of 20 $mJ.cm^{-2}$ (2x20 = 40 $mJ.cm^{-2}$ and 3x20 = 60 $mJ.cm^{-2}$) for Study 2. As mentioned, the maximum UV dose that can be applied at a time can be affected by the physico-chemical profile of the river water, which in turn influences the UVT% of the water. It has previously been observed that if the UVT% of river water is very low, the Berson EC tronic Ω medium-pressure UV system is not always able to produce the required output energy necessary to deliver a single high UV dose (e.g. 1 x 40 $mJ.cm^{-2}$) as water flows through the system. To ensure proper UV dosing, treated water was,

therefore, recirculated and lower doses were repeated (e.g. 2 x 20 mJ.cm^{-2} = 40 mJ.cm^{-2}) instead of applying only one high dose.

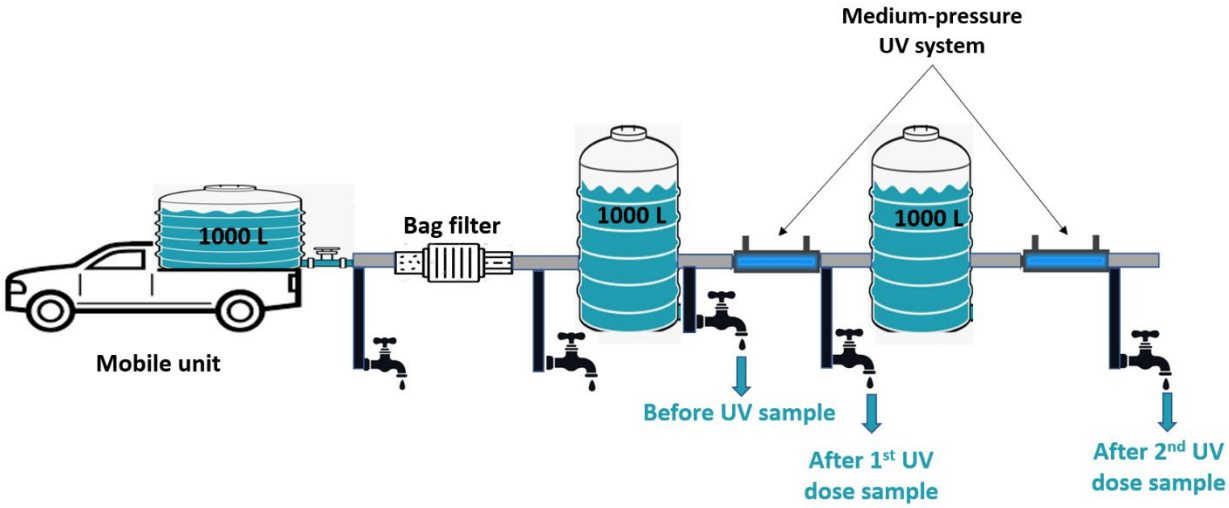


Figure 4.3.1 Visual illustration of the pilot plant system that includes bag filtration and UV disinfection. (Sampling points, where water samples were withdrawn for further analysis, are indicated) (Oosthuizen, 2021)

Pilot plant cleaning procedure

After use, the mobile unit along with all holding tanks and water pipes were disinfected as described in section 3.5.3 in Chapter 3.

Recovery of UV-treated river water samples

After UV treatment of water samples, time for microbial recovery in the presence of light and darkness was allowed according to the method described as part of section 4.2.2.2, including figures 4.2.1 and 4.2.2 in Chapter 4.2.

Physico-chemical analysis of river water samples

All physico-chemical tests were performed in triplicate (Study 1) or in duplicate (Study 2) for each of 1 000 L batches of river water collected, based on standard methods. The analyses methods used were discussed in 'Section 3 General methods'. Results were compared to the Water Quality Guidelines for Irrigation Water (DWAF, 1996a) included in Section 3.1 in Chapter 3. The percentage ultraviolet transmission (UVT%) is not stipulated in the guidelines but might affect UV radiation efficiency and was therefore still tested.

Anion and cation analysis – Only Study 1

In addition to the standard physico-chemical analyses included in this project throughout all research chapters, anion and cation analyses on river water samples was done in Study 1. Triplicate water samples, withdrawn before UV, were pooled 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) (Personal communication: Dr Colling, J. 2021, Manager of Vibrational Spectroscopy, BIOGRIP Node for Soil and Water Analysis, Central Analytical Facility, Stellenbosch University, Stellenbosch).

Microbial analysis of river water samples

The microbial analyses included monitoring the *E. coli*, coliforms and HPC population counts before and after UV treatment. For each 1 000 L of river water collected in Study 1, 3 x 'before UV' samples, 3 x 'after first UV' samples, and 3 x 'after second UV' samples were taken for microbiological analysis. In Study 2 three additional samples were analysed after each UV radiation, with 3 x 'before UV' samples, 6 x 'after first UV' samples and 6 x 'after second UV' samples. After microbial analysis was conducted, all the 'after UV' samples were also subjected to the photoreactivation test procedure described further on. Furthermore, one dilution series (10^{-1} to 10^{-6}) was prepared per water sample, where each dilution was plated out twice.

Standard dilution series were prepared as described in Section 3.4.1, after which the dilutions were transferred to the relevant agars using standard pour plate methods as described in Sections 3.4.2 (HPC) and 3.4.3 (*E. coli*/coliforms). After incubation colony numbers <300 CFU per plate were recorded for all indicator organisms. Although lower limits for the range of countable colonies were adhered to for all water samples analysed before UV, as specified by each of the respective standard counting methods, all surviving colonies were counted for the 'after UV' water samples. It is acknowledged that, generally, using a lower limit (between 10-30 CFU per plate) and an upper limit (between 250-300) 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 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 a standard method's limits. Considering this, all CFU below 300 that were present after UV at the lowest dilution plated (10^{-1} in study 1 and 10^{0} in Study 2) were counted and indicated in the results.

The presence of *Salmonella* species and *Listeria monocytogenes* were also determined before UV, after the first UV dose and after the second UV dose, as well as after the photoreactivation procedure. Here the water samples (collected as indicated above), were pooled together prior to preparing duplicate enrichments, as required for the presence/absence testing of the pathogens *L. monocytogenes* and *Salmonella* spp. as described in Sections 3.4.5 (*Salmonella*) and 3.4.6 (*L. monocytogenes*).

Escherichia coli enrichment – Only Study 2

An additional enrichment step was added to determine the presence/absence of *E. coli* after UV treatment and recovery, as the detection limit of the cell counting method was considered a limitation in Study 1. The same incubated Buffered Peptone Water sample, as prepared for *Salmonella* identification, was used to transfer 1 mL to 9 mL of autoclaved EC Broth (Oxoid, South Africa), and incubated at 35°C for 24 hours. Using a sterile loop, each sample was streaked on Brilliance Coliform/*E. coli* Selective Agar (Oxoid, South Africa) plates, and incubated at 35°C for 24 hours. The presence of purple colonies was considered to be indicative of the presence of *E. coli*.

Identification of bacterial strains using MALDI-TOF spectroscopy (Biotyper) – Only Study 1 (Oosthuizen, 2022)

Environmental isolates were prepared for MALDI-TOF identification according to standard procedures (Zvezdanova *et al*., 2020) and identified with MALDI-TOF as described in Section 3.4.7. in Chapter 3.

Identification of bacterial strains using Vitek® 2 compact – Only Study 2 (Jankowitz, In press)

The Vitek® 2 compact system is an automated microbiology system utilizing growth-based technology (Michalik, 2017). Following the isolation and characterisation procedures (including Gram-staining), pure environmental strains were prepared for Vitek identification. Each culture, no older than 24 hours, was used to inoculate a 3 mL saline solution to a McFarland turbidity range between 0.50 and 0.63 respectively. The barcode scanning system was employed, and each inoculation received an identification card (GN or GP) according to its gram stain results. Each cassette contained six isolate samples before being placed in the Vitek® 2 compact system for processing and analysis.

4.3.3 RESULTS

4.3.3.1 Study 1 (August-October 2021) (Oosthuizen, 2022)

Physico chemical analysis

The results for the physico-chemical analyses of the three rivers are presented in Table 4.3.2. The Mosselbank river had the highest total dissolved solids (TDS), total suspended solids (TSS), alkalinity, conductivity and COD values, which probably all contributed to this river also having the lowest ultraviolet transmission percentage. The Franschhoek river had the highest ultraviolet transmission percentage, which coincided with the lowest values of total dissolved solids, COD, and alkalinity. The Plankenburg river had the lowest total suspended solids.

Table 4.3.2 Physico-chemical characteristics of three selected rivers, analysed in triplicate per 1 000 L water trial

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

	Mosselbank river				Franschhoek river				Plankenburg river			
Characteristics	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

The BIOGRIP elemental analysis (Table 4.3.3) revealed that the Mosselbank river had the highest concentrations of all the elements tested, which could explain the extremely high levels of dissolved solids observed for this river (Table 4.3.2). The Mosselbank had notably high levels of chloride, sodium, and sulphate as well as calcium, which might be linked to the fact that the sampling site was downstream of a WWTP.

Table 4.3.3 BIOGRIP elemental analysis results obtained for pooled water samples from the three rivers

Characteristics	River location		
	Mosselbank river	Franschhoek river	Plankenburg river
Fluorine ($mg.L^{-1}$)	0.48	0.08	0.14
Chloride ($mg.L^{-1}$)	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

Microbial analysis

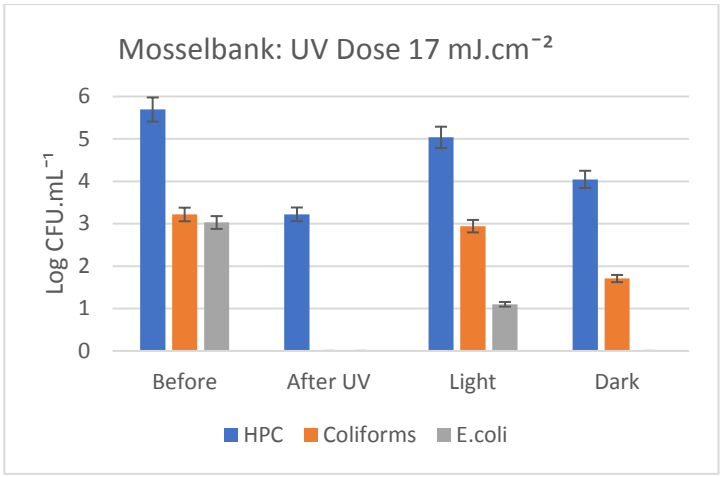
The microbial results obtained as part of the UV treatment and reactivation observations for the Mosselbank, Plankenburg and Franschhoek rivers are presented in Figures 4.3.2 4.3.3 and 4.3.4, respectively. The results indicate that the initial levels of *E. coli* were above guideline limits (1 log $CFU.mL^{-1}$) (DWAF, 1996a) (Table 3.1 chapter 3) for both the Mosselbank and Plankenburg rivers. The results also indicate that both the doses applied as part of this study were effective to reduce coliforms and *E. coli* levels present in all three rivers to below detectable limits directly after UV treatment. There was, however, post-UV recovery observed for the coliform and *E. coli* populations under light and dark conditions following both doses. For all rivers larger log recoveries were observed for the coliform population when compared to the *E. coli* population. For both Plankenburg and Franschhoek rivers specifically no *E. coli* recoveries were observed following treatment with the double dose (2 x 20 $mJ.cm^{-1}$). Smaller log recoveries were also observed for the coliform populations in these two rivers following the double dose UV treatment.

When comparing light and dark recovery of the *E. coli* and coliform populations, it was observed that recovery in the presence of light was higher than what was observed for the samples kept in darkness for all river/UV dose combinations except the double UV dose treatment of the Mosselbank river water. It should be noted, following the double dose UV treatment, that in none of

the rivers *E. coli* could reach levels above guideline limit of 1 log cfu.mL^{-1} (DWAF, 1996a) after time was allowed for recovery. The fact that recoveries slightly above the guideline limit of 1 log cfu.mL^{-1} (DWAF, 1996a) was observed in the Mosselbank and Franschhoek rivers after the single UV dose might suggests that, as a general rule to prevent potential reactivation, doses > 20 mJ.cm^2 should be considered when treating river water of unpredictable quality. In this study, the double dose strategy of (2 x 20 mJ.cm^2 = 40 mJ.cm^2) proved efficient, although the CFU counting method becomes statistically unreliable at levels below 1 Log (or <10 CFU.mL^{-1}). Future studies might consider including presence/absence enrichment testing for *E. coli* to determine if it is still present after UV treatment at levels below the detection limit.

The HPC populations present in all three the rivers (Figures 4.3.2, 4.3.3 and 4.3.4) proved to be much more UV resistant than the respective *E. coli*/coliform populations. Although reductions of about 2 log cfu.mL^{-1} were observed in all rivers directly after UV treatment, no significant differences were observed in colony counts between the two doses directly after UV treatment. For all rivers the HPC counts increased significantly during the three-hour recovery period following UV treatment, with slightly higher levels of recovery observed in the presence of light. Since UV did not prove to be very effective at reducing the HPC levels, and the potential risk this may have for the consumer is not known, the need to identify and characterise the surviving HPC population became apparent.

In addition to the microbial results presented in Figures 4.3.2 4.3.3 and 4.3.4, 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 Gram staining as well as catalase and oxidase testing, according to standard methods (Tarrand & Gröschel, 1982; Chester, 1979; Smith & Hussey, 2005). The isolates were further identified using the MALDI-TOF/Biotyper® system. The results are summarised in Table 4.3.4, while detailed MALDI-TOF spectra are provided in Appendix C, Figure C.1.



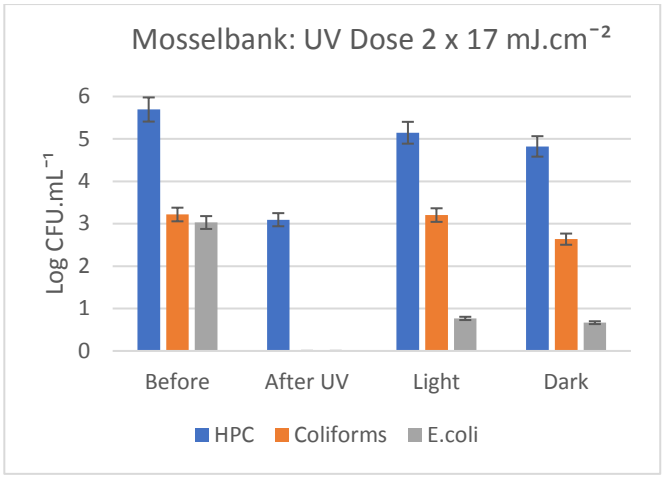
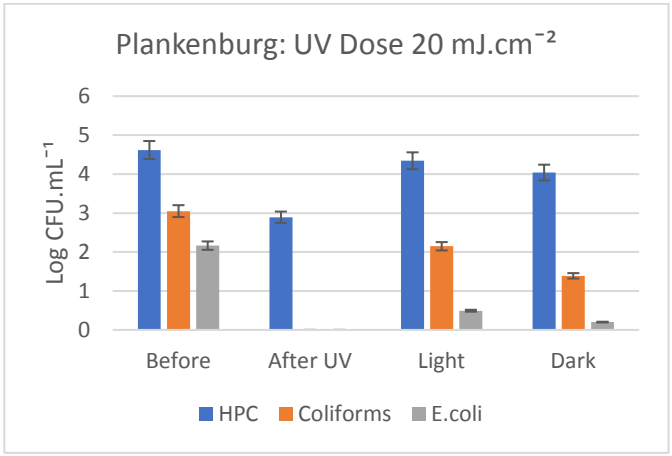


Figure 4.3.2 Average colony counts obtained for triplicate water samples from the Mosselbank river, expressed in log CFU.mL^{-1}, with error bars representing standard deviation**.**



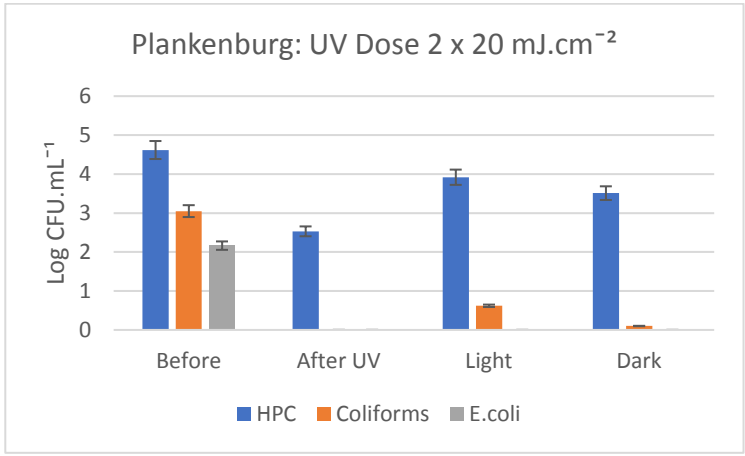
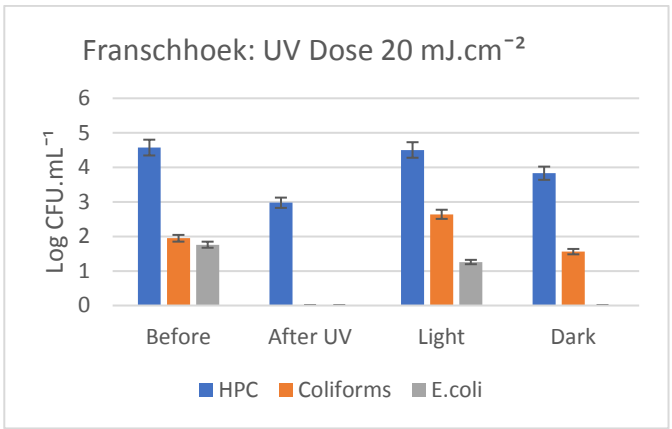


Figure 4.3.3 Average colony counts obtained for triplicate water samples from the Plankenburg river, expressed in log CFU.mL^{-1}, with error bars representing standard deviation.



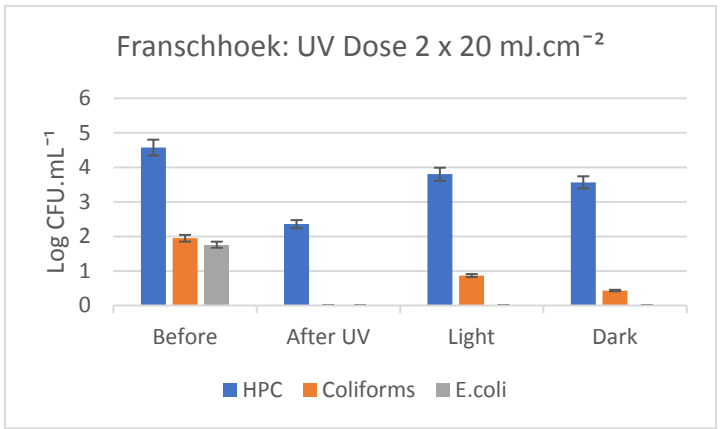


Figure 4.3.4 Average colony counts obtained for triplicate water samples from the Plankenburg river, expressed in log CFU.mL^{-1}, with error bars representing standard deviation.

Table 4.3.4 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, Figure C.1 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.

The results for the pathogen detection methods are presented in Table 4.3.5. These indicate that *L. monocytogenes* was present in both the Mosselbank and Franschhoek rivers prior to UV, but that the UV treatment was sufficient in reducing the pathogen to below detectable limits, even after time was allowed for recovery. No *Salmonella* spp. could be detected prior to UV in any of the rivers sampled.

Table 4.3.5 Presence/absence test results of *Salmonella* spp. and *L. monocytogenes* in river water samples, prior to the application of UV radiation, after UV radiation, after light box and after dark box. Positive test results are indicated by a "+" sign and negative tests are indicated by a "-" sign.

River	Pathogen tested	Before UV	After single UV dose	After Light	After Dark	After double UV dose	After Light	After Dark
Mosselbank	*Salmonella* spp.	-	-	-	-	-	-	-
	L. monocytogenes	+	-	-	-	-	-	-
Plankenburg	*Salmonella* spp.	-	-	-	-	-	-	-
	L. monocytogenes	-	-	-	-	-	-	-
Franschhoek	*Salmonella* spp.	-	-	-	-	-	-	-
	L. monocytogenes	+	-	-	-	-	-	-

4.3.3.2 Study 2 (June 2022) (Jankowitz, *In press*)

Physico chemical analysis

In Table 4.3.6, the physico-chemical results for the three sites sampled during Study 2, are presented. Farm C had the highest TDS values recorded and Farm A the best UVT%, despite also having the highest COD values compared to the other two sites. Farm B was sampled two days after a significant rainy spell, which could have contributed to increased river sediment stir-up. This might, in turn, have contributed to the higher Turbidity and TSS levels, as well as the lower UVT% values observed at Farm B. Geographical distance, and other activities along the Berg river could, however, also have contributed to the river water quality differences observed between the two Berg river sites, as Farm A is far upstream from Farm B (Table 4.3.1).

Table 4.3.6 Physico-chemical characteristics of three selected rivers, analysed in duplicate per 1 000 L water trial.

	Farm A – Berg river			**Farm B – Berg river**			**Farm C – Eerste river**		
Characteristics	1	2	**Avg.**	1	2	**Avg.**	1	2	**Avg.**
UVT%	79.6	80.4	**80**	59.8	59.6	**59.7**	76.2	75.8	**76**
TDS ($mg.L^{-1}$)	50	49	**49.5**	133	137	**135**	364	362	**363.5**
TSS ($mg.L^{-1}$)	10	10	**10**	14	18	**16**	2	3	**2.5**
COD ($mg\ O_2.L^{-1}$)	122	126	**124**	106	95	**101**	94	92	**93**
pH	7.3	7.5		7.6	7.58		7.87	7.9	
Turbidity (NTU)	1.99	2.05	**2.02**	7.19	7.21	**7.2**	3.02	3.14	**3.08**
EC ($mS.m^{-1}$)	0.05	0.07	**0.06**	0.15	0.17	**0.16**	0.44	0.42	**0.43**
Alkalinity ($mg\ CaCO_3.L^{-1}$)	104	105	**104.5**	102	102	**102**	101	102	**101.5**

UVT – Ultra-violet Transmittance; TDS – Total Dissolved Solids; TSS – Total Suspended Solids; COD – Chemical Oxygen Demand; EC – Electrical Conductivity

Compared to the physico-chemical analysis results presented for Study 1 (Table 4.3.2) it was observed that the water sampled at Farm A and Farm C had, on average, higher UVT% values (76-80%) (Table 4.3.6) than what was observed during Study 1. The lowest UVT% average for Study 2 was observed at Farm B, which had an average UVT% around 60%. This was, however, significantly higher than the worst UVT% value observed during Study 1 (UVT%=40 for the Mosselbank river) (Table 4.3.2). This was in spite of the fact that all three sites tested in Study 2 had significantly higher COD content values (Table 4.3.6) than what was observed during Study 1. As observed for Study 1, the parameter which

showed the highest variability between the three sites tested in Study 2, was TDS, which ranged between 50-364 ($mg.L^{-1}$)(Table 4.3.6).

When the results (Table 4.3.6) are compared with the suggested guideline limits (Table 3.2) it can be concluded that the TDS values observed at the Farm C site were above the guidelines for agricultural irrigation. All three sites had COD values above the chosen limit for this study (<75 mg $O_2.L^{-1}$), although this limit applies to land irrigation for industrial use, and not for agricultural purposes (Table 3.3).

Microbial analysis

The microbial results representing the UV dose response of the Total Coliform and HPC populations are presented in Fig 4.3.5. Both UV doses applied with the pilot plant UV system were effective at reducing colony counts of the Total Coliform populations (Fig 4.3.5), irrespective of sampling point.

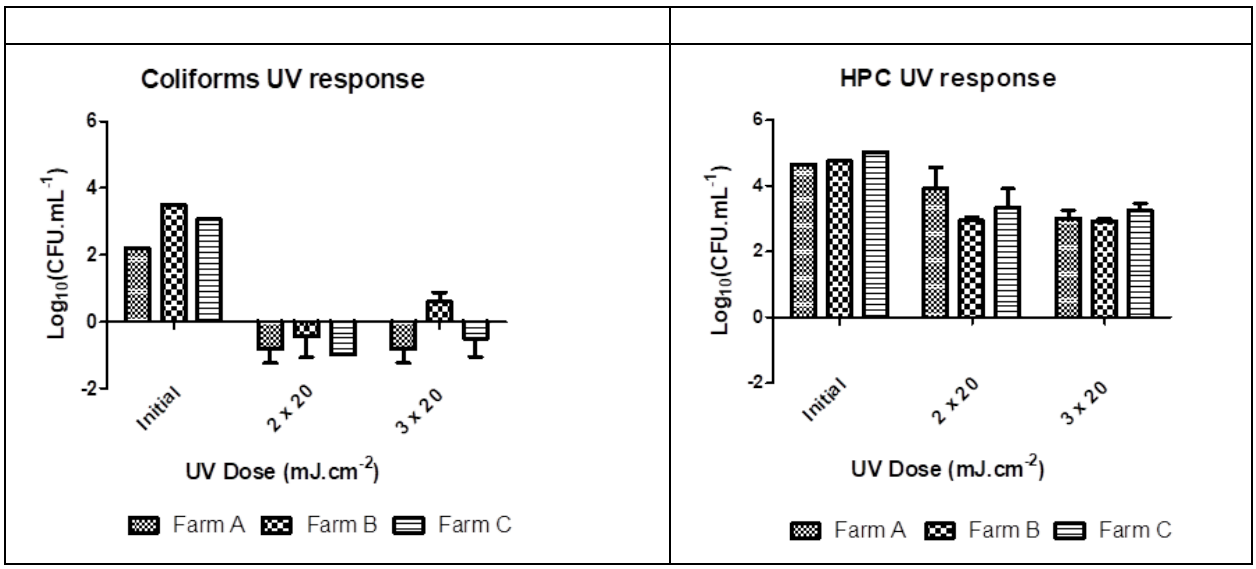


Figure 4.3.5 Average colony counts (n=6) obtained for river locations, expressed in log $CFU.mL^{-1}$ before and after UV treatment. Error bars represent standard deviation. When no growth was observed a log value of -1 was assigned.

The HPC levels showed more resistance to UV treatment than the Coliform populations (Fig 4.3.5), similar to what was reported in previous chapters and in study 1. The HPC numbers never decreased below 3 log, irrespective of the highest UV dose (3x20 $mJ.cm^2$ = 60 $mJ.cm^{-2}$) applied in this study.

The colony counts for *E. coli* after UV treatment, which were derived using the standard plate count methodology to determine the "Before UV count" in this study, was very low (1 colony per plate) and inconsistent (not detected on all triplicates plated) for the two Berg River sites. The *E. coli*

enrichment strategy employed, did, however, indicate that *E. coli* was present in the water sampled from both sites (Table 4.3.7).

Considering these limitations encountered at the Berg River sites in this study, Membrane filtration (MF) (USEPA, 2002) with Brilliance Coliform/E. coli Selective Agar (Oxoid, South Africa) was used to determine *E. coli*/coliform levels present per 100 mL water at Farm C before UV treatment commenced. This was done in addition to the standard plate counts method analysis (testing 1 mL water). The MF results for Farm C indicated a total coliform count of 4.97 log CFU. 100 mL^{-1} (or 2.97 log cfu.mL^{-1}) and an *E. coli* count of 3.3 log CFU.100 mL^{-1} (or 1.3 log CFU.mL^{-1}).

The Total Coliform levels determined with the MF method (2.97 log cfu.mL^{-1}) correlated well with the average log CFU obtained for Total Coliforms with standard plate count methods (3.06 log cfu.mL^{-1}) represented in Fig 4.3.5. (*E. coli* counts with standard plate count methods were once again too low and inconsistent to be considered reliable). This indicates that the standard plate count methods (used to analyse 1 mL samples before and after UV treatment) are a reliable indication of microbial levels if population numbers are high enough to provide statistically accurate results (i.e. above the minimum detection limits for statistically reliable results on the lowest dilution plated). The statistical limitations linked to plate count methods also confirm the value of presence/absence enrichment testing for *E. coli* (Table 4.3.7). Enrichment testing in this study indicated that *E. coli* was present at all sites before UV treatment and remained viable after UV treatment and recovery in the water from all three sites.

The MF values at Farm C were slightly lower than what was found previously for sites further upstream by Oosthuizen (2021), who reported coliform counts ranging from 3.4-5.6 log CFU.mL^{-1}, and *E. coli* levels from 2.3-3.9 log CFU.mL^{-1}. Oosthuizen (2021) did, however, sample the upstream river sites in a different season. Another reason for the lower counts could also be that Farm C is situated after the confluence point of the Eerste river with the Blaauwklippen river (while the other sites were before the confluence point). This could indicate that the Blaauwklippen had a dilution effect on the *E. coli*/coliform levels observed in this study.

The *Salmonella* spp. and *L. monocytogenes* presence/absence detection results are presented in Table 4.3.7. No *L. monocytogenes* was detected at any of the sites. Farm B (sampled two days after heavy rains) tested positive for *Salmonella* spp. and revealed better recovery under dark conditions than light conditions.

Table 4.3.7 Presence/absence enrichment detection results of *Salmonella* spp*., L. monocytogenes and E. coli* in river water samples, prior to the application of UV radiation, after UV radiation, after light box and after dark box. Positive test results are indicated by a "+" sign and negative tests are indicated by a "-" sign.

River	Species	Before UV	After double UV dose	After Light	After Dark	After triple UV dose	After Light	After Dark
Berg river	*Salmonella spp.*	-	-	-	-	-	-	-
(Farm A)	*L. monocytogenes*	-	-	-	-	-	-	-
	E. coli	**+**	-	**+**	**+**	**+**	**+**	-
Berg river	*Salmonella spp.*	**+**	-	-	**+**	-	-	**+**
(Farm B)	*L. monocytogenes*	-	-	-	-	-	-	-
	E. coli	**+**	-	**+**	-	**+**	**+**	**+**
Eerste river	*Salmonella spp.*	-	-	-	-	-	-	-
(Farm C)	*L. monocytogenes*	-	-	-	-	-	-	-
	E. coli	**+**	**+**	**+**	**+**	**+**	**+**	**+**

To address Objective 2, the recovery of the Total Coliforms and HPC populations after UV treatment is presented in Fig 4.3.6 and Fig 4.3.7. Recovery of Coliforms appeared to be more obvious under light conditions than dark (Fig. 4.4.6) over both UV doses tested in study 2. A similar trend was also observed for the three sites tested in Study 1 (Figures 4.3.2, 4.3.3 and 4.3.4).

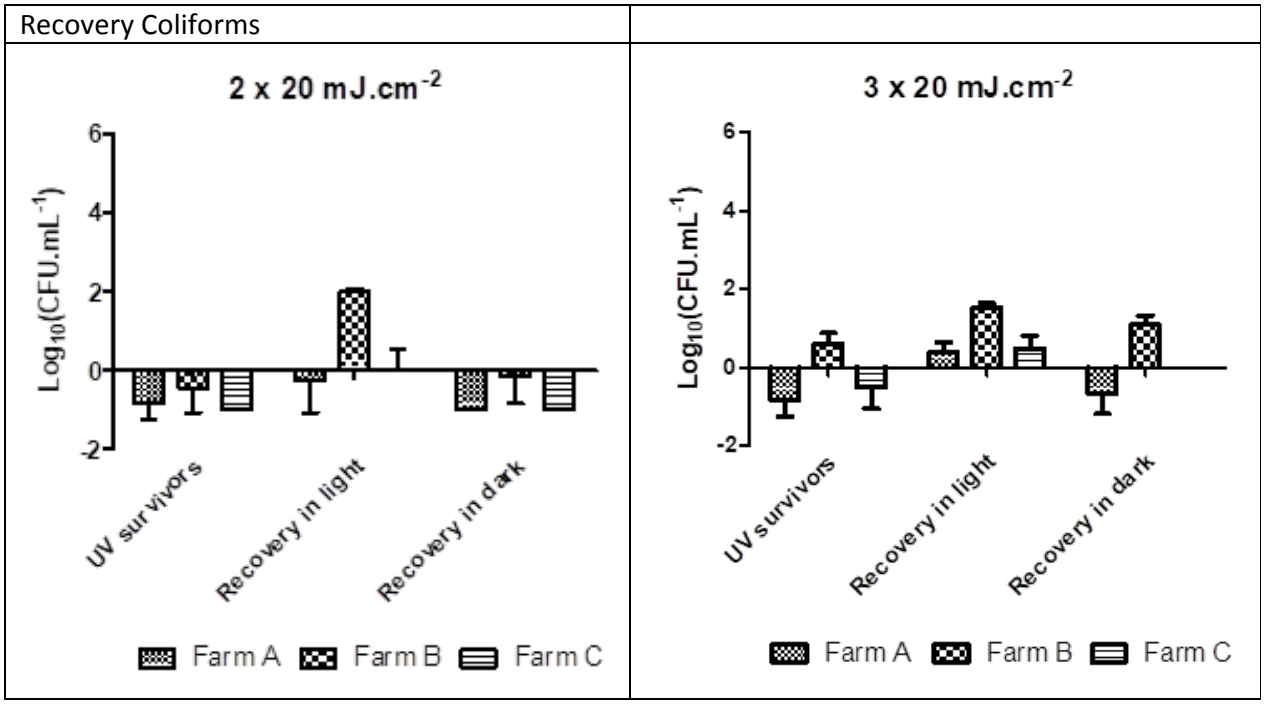


Figure 4.3.6 Average Total Coliform counts (n=6) obtained for river locations before and after recovery under light or dark conditions, expressed in log CFU.mL^{-1}. Error bars represent standard deviation. When no growth was observed a log value of -1 was assigned.

HPC levels after UV treatment (3 x 20 mJ.cm^{-2} = 60 mJ.cm^{-2}) and recovery, stabilised at lower levels (<4 log) compared to UV treatment (2 x 20 mJ.cm^{-2} = 40 mJ.cm^{-2}) and recovery (Fig 4.3.7). The CFU counts also did not exceed the initial UV survivor counts post light or dark treatment which indicates no significant recovery after the highest dose (3 x 20 mJ.cm^{-2} = 60 mJ.cm^{-2}) was applied. An increase in HPC numbers under light conditions was observed for the HPC count at Farm B after the double UV dose treatment (2 x 20 mJ.cm^{-2} = 40 mJ.cm^{-2}). A similar trend was also observed for the three sites tested in Study 1 (Figures 4.3.2, 4.3.3 and 4.3.4), after the (2 x 20 mJ.cm^{-2} = 40 mJ.cm^{-2}) dose. In contrast with Study 2, smaller recoveries were also observed in HPC numbers under dark conditions in Study 1 (Figures 4.3.2, 4.3.3 and 4.3.4).

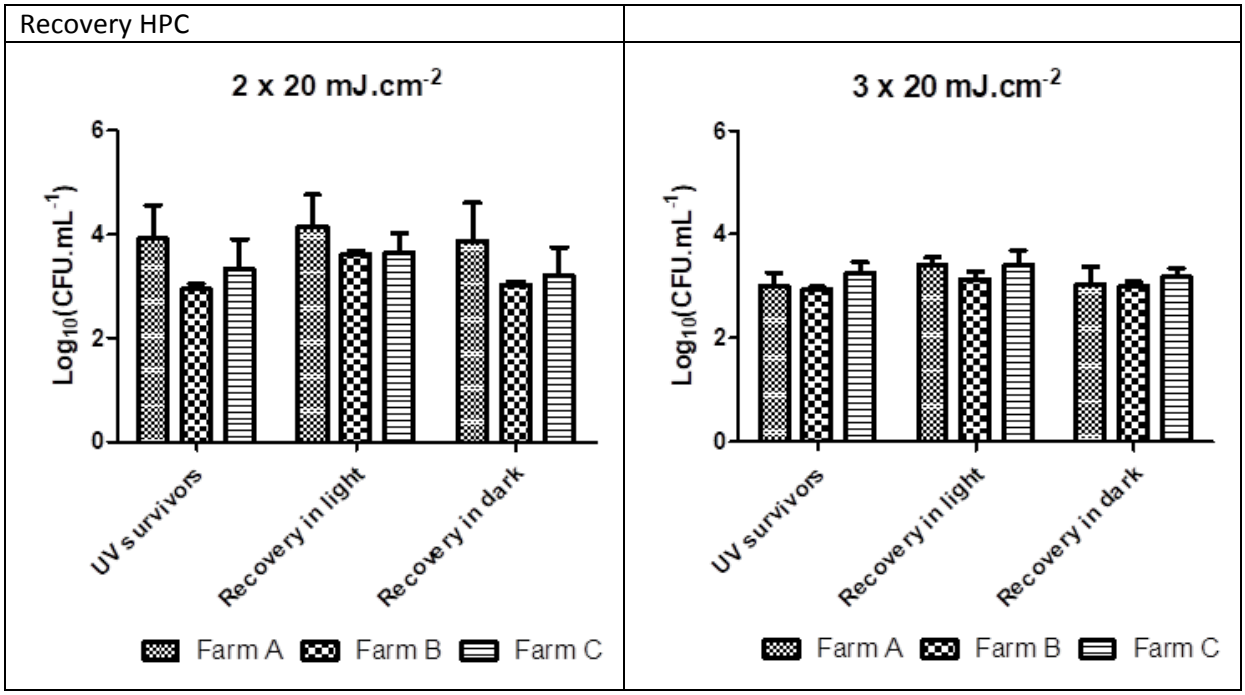


Figure 4.3.7 Average HPC numbers (n=6) obtained for river locations before and after recovery under light or dark conditions, expressed in log CFU.mL^{-1}. Error bars represent standard deviation. When no growth was observed a log value of -1 was assigned.

Following UV treatment of river water on a pilot plant scale with medium pressure UV lamps, regrowth was observed for Coliform and HPC populations as well as *E. coli* as identified with selective agar. To clarify the risk associated with surviving microbes in these populations after UV recovery under light or dark conditions, colonies were isolated and cultured on Tryptic soy agar (TSA). Gram staining was applied to cultures no older than 24 hours. Once Gram status (GN or GP) has been confirmed the isolates were subjected to the Vitek® 2 ID procedure. The identification results of the isolated strains are presented in Table 4.3.8.

Table 4.3.8 Each isolate identified with the Vitek® 2 system is listed below along with information on the sample it was isolated from following the experimental treatments applied as specified.

Presumed Organism*	Sample Date	Location	River	Treatment**	Conditions	Gram stain	Vitek®2 Result
Salmonella	18-6-2022	Farm B	Berg	UV: 3 x 20 mJ.cm^{-2} 3 h dark recovery	Isolated from Hektoen following RV broth	-	*Citro. youngae*
Salmonella	18-6-2022	Farm B	Berg	UV: 3 x 20 mJ.cm^{-2} 3 h dark recovery	Isolated from XLD following RV broth	-	*Citro. youngae*
E. coli	10-6-2022	Farm C	Eerste	UV: 3 x 20 mJ.cm^{-2} 3 h dark recovery	Post EC broth enrichment of original sample	-	*E. coli*
Coliform	10-6-2022	Farm C	Eerste	UV: 3 x 20 mJ.cm^{-2} 3 h dark recovery	Post EC broth enrichment of original sample	-	*Ent. cloacae* complex
Coliform	10-6-2022	Farm C	Eerste	UV: 3 x 20 mJ.cm^{-2} 3 h light recovery	E coli/Coliform counts plate (no enrichment)	-	*Ent. cloacae* complex
Coliform	10-6-2022	Farm C	Eerste	UV: 3 x 20 mJ.cm^{-2} 3 h light recovery	E coli/Coliform counts plate (no enrichment)	-	*Ent. cloacae* complex
E. coli	10-6-2022	Farm C	Eerste	UV: 3 x 20 mJ.cm^{-2} 3 h light recovery	E coli/Coliform counts plate (no enrichment)	-	*E. coli*
Coliform	10-6-2022	Farm C	Eerste	UV: 3 x 20 mJ.cm^{-2} 3 h light recovery	Post EC broth enrichment of original sample	-	*K. oxytoca*
E. coli	10-6-2022	Farm C	Eerste	UV: 3 x 20 mJ.cm^{-2} 3 h light recovery	E coli/Coliform counts plate (no enrichment)	-	*E. coli*
E. coli	10-6-2022	Farm C	Eerste	UV: 3 x 20 mJ.cm^{-2} 3 h dark recovery	Post EC broth enrichment of original sample	-	*E. coli*
Coliform	18-6-2022	Farm B	Berg	UV: 3 x 20 mJ.cm^{-2} 3 h light recovery	E coli/Coliform counts plate (no enrichment)	-	*Raou. planticola*
Coliform	18-6-2022	Farm B	Berg	UV: 3 x 20 mJ.cm^{-2} 3 h dark recovery	E coli/Coliform counts plate (no enrichment)	-	*Raou. planticola*
E. coli	18-6-2022	Farm B	Berg	UV: 3 x 20 mJ.cm^{-2} 3 h dark recovery	E coli/Coliform counts plate (no enrichment)	-	*E. coli*

Coliform	3-6-2022	Farm A	Berg	UV: 3 x 20 mJ.cm^{-2} 3 h light recovery	Post EC broth enrichment of original sample	-	*Citro. freundii*
E. coli	3-6-2022	Farm A	Berg	UV: 3 x 20 mJ.cm^{-2} 3 h light recovery	Post EC broth enrichment of original sample	-	*E. coli*
E. coli	3-6-2022	Farm A	Berg	UV: 3 x 20 mJ.cm^{-2} 3 h dark recovery	Post EC broth enrichment of original sample	-	*E. coli*
Coliform	3-6-2022	Farm A	Berg	UV: 3 x 20 mJ.cm^{-2} 3 h dark recovery	Post EC broth enrichment of original sample	-	*Ent. cloacae* complex
E. coli	3-6-2022	Farm A	Berg	UV: 3 x 20 mJ.cm^{-2} 3 h light recovery	Post EC broth enrichment of original sample	-	*E. coli*
Coliform	3-6-2022	Farm A	Berg	UV: 3 x 20 mJ.cm^{-2} 3 h dark recovery	Post EC broth enrichment of original sample	-	*Ent. cloacae* complex
Coliform	3-6-2022	Farm A	Berg	UV: 3 x 20 mJ.cm^{-2} 3 h light recovery	Post EC broth enrichment of original sample	-	*K. pneum. pneumoni*
HPC	18-6-2022	Farm B	Berg	UV: 3 x 20 mJ.cm^{-2} 3 h light recovery	PCA counts plate	-	*Ent. cloacae* complex
HPC	18-6-2022	Farm B	Berg	UV: 3 x 20 mJ.cm^{-2} 3 h light recovery	PCA counts plate	-	*Ent. cloacae* complex
HPC	18-6-2022	Farm B	Berg	UV: 3 x 20 mJ.cm^{-2} 3 h light recovery	PCA counts plate	-	*Ent. cloacae* complex
HPC	18-6-2022	Farm B	Berg	UV: 3 x 20 mJ.cm^{-2} 3 h dark recovery	PCA counts plate	+	*Staph. aureus*
Coliform	18-6-2022	Farm B	Berg	UV: 3 x 20 mJ.cm^{-2} 3 h light treatment	Post EC broth enrichment of original sample	-	*Ps. aeruginosa*
E. coli	18-6-2022	Farm B	Berg	UV: 3 x 20 mJ.cm^{-2} 3 h dark recovery	Post EC broth enrichment of original sample	-	*E. coli*
Coliform	18-6-2022	Farm B	Berg	UV: 3 x 20 mJ.cm^{-2} 3 h dark recovery	Post EC broth enrichment of original sample	-	*Raou. planticola*
Coliform	18-6-2022	Farm B	Berg	UV: 3 x 20 mJ.cm^{-2} 3 h dark recovery	Post EC broth enrichment of original sample	-	*K. pneum. pneumoni*

*As indicated on selective agar used.

**Survivors were isolated after these water treatment steps were completed. All UV treatments were applied with the MP UV pilot plant installation.

4.3.4 DISCUSSION

In this study the disinfection efficacy of a medium-pressure pilot-scale UV system, with a 5 µm bag filter as pre-treatment, was evaluated for the treatment of significantly larger river water volumes (1 000 L), compared to the laboratory scale UV treatments that was used before (Chapters 4.1 and 4.2).

In Study 1, three rivers were chosen based on their known river water profiles from previous work (Bursey, 2021; Oosthuizen, 2022; Chapter 4.2). These included: the Plankenburg river (previously shown to have very poor microbial characteristics); the Mosselbank river (previously shown to have poor physico-chemical characteristics); and the Franschhoek river (previously shown to have good microbial and physico-chemical characteristics) (Bursey, 2021; Oosthuizen, 2022; Chapter 4.2). The physico-chemical analysis (Table 4.3.2) as well as the microbial analyses conducted prior to UV treatment in Study 1 (Figures 4.3.2, 4.3.3, and 4.3.4) confirmed that all rivers still exhibited the same characteristics as was previously reported; with the Plankenburg and the Mosselbank having several parameters outside the guideline limits (DWAF, 1996a) (Section 3.1, Chapter 3) for acceptable and safe agricultural irrigation water.

In Study 2 sampling sites were chosen on specific farms. Two of the sampling sites Farm A and Farm B) were on the banks of the Berg River, although geographically distinct (Table 4.3.1). The Berg river system have not previously been evaluated in this study. Farm C, which is located next to the Eerste river, forms part of the Plankenburg river system and is downstream from other sites previously investigated by Oosthuizen (2022). This site (Farm C) is also situated downstream from the Eerste river's confluence point with the Blaauwklippen river. It is, however, upstream from the Eerste river site sampled as part of Bursey's study (Bursey, 2021; Chapter 4.1) in December 2019-January 2020, and Oosthuizen's study (Oosthuizen, 2022; Chapter 4.1) in January 2021-March 2021.

4.3.4.1 Physico-chemical profiles

It is well known that long-term point and non-point pollution sources can have a detrimental effect on river water quality. Perhaps the best example of this is the physico-chemical profile of the Mosselbank river in Study 1 (Table 4.3.2). The extremely high TDS content previously reported specifically for the Mosselbank site by both Bursey (2021) (TDS range: 540-903 $mg.L^{-1}$)(Chapter 4.1) and Oosthuizen (2022) (TDS range: 696-865 $mg.L^{-1}$)(Chapter 4.1), was confirmed again in this study (701 $mg.L^{-1}$) (Table 4.3.2). As the results indicate, both in this and previous reports, these elevated levels of TDS are always associated with low UVT% values in this river, ranging between 22-49%UVT (Bursey, 2021) and 31-49%UVT (Oosthuizen, 2022) previously, and 40%UVT in this study (Table 4.3.2). As speculated before, this phenomenon might be attributed to the Mosselbank site being very close to a WWTP (Table 4.3.1). Additional insights were, however, obtained in this study by conducting elemental analysis on the river water samples from all three rivers (BIOGRIP results in Table 4.3.3). These indicate very clearly that water from the Mosselbank site has very high concentrations of certain elements, when compared to the other rivers. These elements include, in decreasing order,

chlorine, sodium, sulphate, calcium and magnesium (Table 4.3.3), which could be indicative of chemicals commonly used during WWTP's operations.

According to Venkatesh & Brattebø (2011), typical WWTPs consume a variety of chemicals as part of various processes. Their study, conducted in Norway, indicated the widespread use of chemicals, including coagulants such as iron chloride, iron sulphate, poly-aluminium chloride, and calcium hydroxide. Although it is assumed that these coagulants would be completely assimilated as part of sludge production, excess coagulant could be washed out of the WWTP if added at higher concentrations than what is consumed during the sludge digestion processes (Venkatesh & Brattebø, 2011). Belgiorno *et al*. (2007) have also reported that the advanced oxidation processes used in WWTP's could result in elevated TDS levels of the final effluent and Navamani Kartic *et al*. (2018) specifically reported that sulphate (in the form of sodium sulphate) can lead to elevated TDS levels during wastewater treatment. According to Wilson et al. (2014), water treatment plants do not usually have final stage treatments to remove high TDS levels, resulting in effluents with elevated TDS levels exiting into surface water sources such as river systems. Similar operations might also have contributed to the high concentrations of chlorine, sodium, sulphate and calcium observed in water from the Mosselbank river site.

The higher levels of nitrate observed in the Mosselbank river in relation to the other two rivers (Table 4.3.3) could also have been as a direct result of the presence of the WWTP upstream. Nitrogen present in urban wastewater can be converted to different forms as part of the WWTP operations, some of which leave the plant in the form of nitrates in the WWTP effluent (Venkatesh & Brattebø, 2011). Removing nitrogen (along with phosphorous) from WWTP effluents has been labelled an important priority in order to limit eutrophication and reduce the environmental impact of WWTPs (Venkatesh & Brattebø, 2011).

In Study 2 Farm C had the highest TDS results of the three sites tested (Table 4.3.4), and the second highest TDS over both Studies 1 and 2 with an average TDS of 363.5 $mg.L^{-1}$. Farm C is downstream from the town of Stellenbosch, where the Eerste river forms as a result of the merging of the Plankenburg and Jonkershoek rivers. As reported by Oosthuizen 2021, the Plankenburg river specifically has a history of poor physicochemical and microbial properties, which some researchers have argued is the result of both the informal settlement and the Stellenbosch industrial area upstream of the Plankenburg river sampling site. This is confirmed by the findings in Study 1 for the Plankenburg site, for which an average TDS of 208 $mg.L^{-1}$, and an initial *E. coli* concentration >2 log $CFU.mL^{-1}$ (which is double the guideline limit, as presented in Table 3.2) were recorded. After the town of Stellenbosch, the Eerste river flows past a WWTP effluent stream as well as agricultural land, all of which could have contributed to the high TDS values observed at Farm C. The high TDS value recorded at this site as part of study 2 also appear to be a long-term occurrence, as high TDS values were also reported before at an Eerste river sampling site downstream from Farm C by Bursey (2021) (TDS range: 245-316 $mg.L^{-1}$) and Oosthuizen (2022) (TDS range: 298-370 $mg.L^{-1}$). The TDS levels do not appear to affect the UVT % measured in this study (76%) or the previous studies by Bursey (2021) (UVT% range: 52-

62%) and Oosthuizen (2022) (UVT% range: 63-69%), which all are within the UVT% ranges observed for other river sites which had lower TDS levels.

4.3.4.2 Microbial analyses

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 m^3.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 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 (UVT % of 40.1% and TDS value of 701 mg. L^{-1}). The highest possible UV dose that could be applied in real-time (as automatically measured and displayed on the UV system's display screen) for the Mosselbank river water was 17 mJ.cm^{-2} at 100% system power. As a result, the doses applied to the Mosselbank river water were 1 x 17 mJ.cm^{-2} and 2 x17 mJ.cm^{-2}. With these limitations in mind, 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 rivers tested, and that the actual UV dose that could be applied to a specific river, and measured in real-time (e.g. 17 mJ.cm^{-2}), would be recorded. In addition, it was decided that river water with a UVT % below 40%, would be considered untreatable given the practical limitations of this UV system. The physico-chemical profiles of the Plankenburg and Franschhoek rivers were better (Table 4.3.2), and it was possible to apply UV doses of (1x 20 mJ.cm^{-2}) and (2 x 20 mJ.cm^{-2}) for both these rivers. In Study 2 the UVT% values for all the sites were above 60%, and a minimum UV dose of 20 mJ.cm^{-2} could easily be achieved.

Both the single UV dose (20 mJ.cm^{-2}), double UV dose (2x20 mJ.cm^{-2} = 40 mJ.cm^{-2}) and triple UV dose (3x20 mJ.cm^{-2} = 60 mJ.cm^{-2}) applied to water from the respective river sites in Studies 1 and 2 were effective in reducing the Total Coliforms and *E. coli* concentrations to below detectable limits directly after UV treatment with the colony count method (Figures 4.3.2, 4.3.3, 4.3.4 and 4.3.5). Recovery of Total coliforms was, however observed, especially under light conditions, in both Studies 1 and 2 (Figures 4.3.2, 4.3.3, 4.3.4 and 4.3.6). In Study 2 light recovery was observed only for Farm B after a double UV dose, and for all locations after a triple UV dose. Whether regrowth of the Coliform population after UV treatment holds any risk for consumers of fresh produce would depend on various factors, including whether the specific species of coliforms that survive are pathogenic or harbour antimicrobial resistance genes.

The limitations of the colony counting methods employed in both Studies 1 and 2 have been discussed in the 'Results' section, especially regarding the detection of *E. coli* if the latter is present at very low levels. Membrane filtration was successfully applied to determine the presence of *E. coli* in water from Farm C before UV treatment, but the MF methodology could not be applied as part of the recovery procedures (listed in 'Materials and Methods') due to sample volume limitations. The *E. coli* enrichment strategy employed as part of Study 2 was, however, able to pick up viable *E. coli* before UV treatment and after recovery procedures in the presence of light and darkness were concluded. Based on these findings it

can be concluded that UV treatment can consistently reduce *E. coli* to below guideline limits (<10 CFU.mL^{-1}) (Figures 4.3.2, 4.3.3, 4.3.4 and 4.3.5), but that *E. coli* has the ability recover and persist (Table 4.3.7), albeit at low levels. It can be argued, that given time and optimal growth conditions *E. coli* might recover further after UV treatment (Wang *et al*., 2021; Reddy & Krishnamurthy, 2020; Moreno-Andres *et al*., 2019; Mao *et al*., 2018; Shafaei *et al.,* 2017). The potential risk this has for irrigation water and fresh produce safety will depend on the properties of the *E. coli* that persists.

Quek & Hu (2008) investigated which medium-pressure UV doses are required to reach a 3-log reduction in lab-cultured *E.coli* strains and reported as follows: ATCC 11229 – 4.7 mJ.cm^{-2}; ATCC 11775 – 2.4 mJ.cm^{-2}; ATCC 15597 – 8.3 mJ.cm^{-2}; ATCC 700891 – 8.2 mJ.cm^{-2}. These values are almost half of UV dose used in this study. It is however important to note that the above-mentioned study did not take reactivation into account and lab-cultured strains were used. An increased UV resistance of environmental bacteria, compared to lab-cultured strains, have been reported in several studies (Hijnen *et al*., 2006). Maya *et al*. (2003) reported that a UV dose of 13 mJ.cm^{-2} lead to a 3-log reduction in faecal coliforms. Again, recovery after UV treatment was not considered. From the microbial analysis results obtained in both studies, it can be concluded that coliforms are prone to recovery.

Heterotrophic bacteria's photoreactivation capabilities have been evaluated by Ansa *et al.,* (2017) in bottled water after inactivation with pulsed ultraviolet light. The results showed photoreactivation ranging from 0.4-1.7 log units for the various samples and the authors attributed this variation to differences in ionic composition. This once again demonstrates the impact of that dissolved solids may have on UV efficacy.

In all three rivers from Study 1 (Figures 4.3.2, 4.3.3, 4.3.4) the HPC populations were the most UV resistant and demonstrated 1-2 log CFU.mL^{-1} recovery for light and dark conditions. The same trend was observed In Study 2, the HPC bacteria was still resistant with populations never decreasing to levels below 3 log CFU.mL^{-1}. Recovery under light and dark conditions was, however, restricted following a higher UV dose (3x20 mJ.cm^{-2} = 60 mJ.cm^{-2}) (Fig 4.3.7). HPC bacteria constitute a wide range of Gram-positive and Gram-negative species, including some pathogens, which could potentially be a public health threat depending on the type of species that survive UV irradiation (Camper 2004; Beech & Sunner 2004; Emtiazi *et al*. 2004; Regan *et al.* 2003). Isolation and strain identification of environmental strains after UV treatment were thus included in both Studies 1 and 2 and will be discussed further on.

Based on the results of both Studies 1 and 2 (Tables 4.3.5 & 4.3.7), as well as the results presented in previous chapters (Table 4.1.6 Chapter 4.1 ; Bursey, 2021; Oosthuizen, 2022) it can be argued that the presence of *Salmonella* spp. and *Listeria monocytogenes* in river water can be considered as sporadic. This observed infrequent occurrence can, however, not be ignored, as both these pathogens are frequently linked to fresh produce related food borne outbreaks globally. Bursey's previous results (Table 4.1.6 chapter 4.1 ; Bursey, 2021) indicated that all UV doses (applied at laboratory-scale using an LP lamp system) were effective at reducing *Salmonella* and *L. monocytogenes* levels, but the impact of potential recovery was not considered. As part of Chapter 4.2 of this report, the UV sensitivity and post-UV recovery potential of pure

strains of *Salmonella* and *L. monocytogenes* (at high CFU concentrations) were investigated (at laboratory-scale using an LP lamp system). Results indicated that, of the strains tested, *Salmonella* might have the best ability to recover post-UV.

The results presented as part of this chapter indicated that in the untreated river water *L. monocytogenes* could only be detected twice in Study 1 (Table 4.3.5), while *Salmonella*-like colonies was only detected once in Study 2 (Table 4.3.7). If the potential to recover post-UV is considered, it can be concluded that the UV treatment applied as part of Study 1 (with doses 20 and 40 mJ.cm^{-2}) was effective to prevent recovery of *L. monocytogenes* under both light and dark conditions in river waters with these physico-chemical profiles. Yousef & Marth (1988) reported a UV dose of 9.6 mJ.cm^{-2} was sufficient for 4-log reduction of *L. monocytogenes.*

In contrast, the results of Study 2 (Table 4.3.7) indicate that although *Salmonella*-like colonies were only detected once and could not be detected directly after UV treatment with both the UV doses applied (2 x 20 =40 mJ.cm^{-2} and 3 x 20 = 60 mJ.cm^{-2}), it still had the ability to recover – but only under dark conditions. Literature indicates that *Salmonella* is quite sensitive to UV irradiation, reportedly requiring 7 mJ.cm^{-2} for a 4-log reduction in population (Yaun et al., 2003). Strain identification confirmation of the surviving colonies after recovery did, however, reveal that the population able to recover was of the Citrobacter genus , and not Salmonella (identification results to be discussed in the next sections). The *Salmonella* identification done by Bursey (2021) (Table 4.1.9) did confirm the presence of *Salmonella* in river water samples tested as part of Chapter 4.1. This once again confirms the sporadic nature of this pathogen's occurrence.

It should be noted, however, that microbial recovery observed under light and dark conditions in both Studies 1 and 2 could also have been surviving cells that multiplied or reverted from a viable-but-not-culturable state directly after UV, to a culturable state (following the recovery period) and aren't necessarily only a representation of DNA recovery (also referred to as photoreactivation). The understanding of the photoreactivation phenomenon in particular is mostly based on *in vitro* experiments which do not consider the interaction of microbial populations with real water and wastewater components (Hallmich, 2009). Photoreactivation has not been shown to be impacted by wastewater particle concentration (Whitby & Palmateer 2003; Martin & Gehr 2007), but Curtis et al. (1992) found that factors such as pH, humic substances, and dissolved oxygen were important variables involved in the process by which light damages microorganisms in various environments. Various knowledge gaps regarding the mechanisms involved in microbial recovery after UV treatment still exist, which could be further explored in future research.

Isolate identification in Study 1

As part of Study 1, some HPC colonies that survived the first and second 20 mJ.cm^{-1} UV doses were isolated and further characterised. Following Gram-staining, catalase and oxidase testing, the strains were prepared for MALDI-TOF analysis, using the Biotyper system for species identification of pure strains. The results (Table 4.3.4) indicated that a variety of bacterial species can survive UV treatment. This is in line with previous

findings (Chapter 4.1). Kizhakkekalam & Chakraborty (2019) also reported the presence of a large diversity of HPC bacteria in water, as 148 HPC bacterial strains of seven different species were isolated during their study. Kizhakkekalam & Chakraborty (2019) noted that even more species (than those which were identified) may be present in a HPC population.

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 4.3.4). The A. *hydrophila* and *A. caviae* obtained MALDI-TOF scores of 1.85 and 2.10, respectively (Table 4.3.4). 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 this genus is difficult when using the MALDI-TOF spectra as basis (Table 4.3.4[ab]). The *A. caviae* MALDI-TOF score was between 2.00-2.40, indicating high probability species identification (Table 4.3.4). In previous work done as part of this project A. *hydrophila* was also detected after UV radiation at the Plankenburg river, supporting the findings of this study which could suggest a regular occurrence of UV resistant *Aeromonas* species in this river. Very limited research is available regarding why *Aeromonas* species are UV resistant. 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 4.3.4). *Enterococcus* species are 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*. (2021) 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 4.3.1).

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 isolate identified as an *Enterococcus* species obtained MALDI-TOF scores of 1.61 and 1.48, respectively (Table 4.3.4). These results suggest that no accurate species identifications could be made, as MALDI-TOF scores was below 1.70 (Table 4.3.4). Further analysis of the results indicated that the culture might have been a mixed isolate as identification results indicated both *Enterococcus hirae* and *Staphylococcus epidermidis* in the Biotyper “top ten” spectra matches for this isolate.

The next species to be identified was *Brevundimonas vesicularis* (Table 4.3.4), 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 4.3.4). The MALDI-TOF scores of both isolates were between 2.00-2.30 (Table 4.3.4), 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 4.3.4). Very limited research is available regarding the UV response of these species.

Overall, these results indicate that a variety of heterotrophic bacteria can survive UV disinfection (Table 4.3.4). This is an area that should be investigated further to determine which heterotrophic genera are the most common survivors on fresh produce, and what their potential impact be on consumer safety may be.

Isolate identification in Study 2

The first and most important observation from the strain identification results in Table 4.3.8 is that the presumptive *Salmonella* isolate on XLD and Hektoen agars is *Citrobacter youngae* and therefore no evidence was found that environmental *Salmonella* spp. survives UV or recovers under light or dark conditions. Hoben *et al*. already identified in 1973 that novobiocin addition is necessary to suppress *Citrobacter* spp. that can often be mistaken for *Salmonella* on Hektoen enteric agar. The Hektoen enteric agar (Oxoid) used in this study does not include novobiocin. *Citrobacter* species typically appear as yellow, opaque colonies on XLD agar as per the supplier documents, but a recent study by Pławińska-Czarnak *et al*. (2021) found that false presumptive *Salmonella* identified by selective agar, including XLD, were common to be *Citrobacter* species. Pilar *et al.* (2020) concluded that *Citrobacter* spp. and *Salmonella* spp. can be phylogenetically similar leading to false identification when using current approved *Salmonella* detection methods.

C. youngae and *C. freundii* were both identified as survivors following a total UV dose of 60 mJ.cm^{-2} and light or dark recovery conditions. *Citrobacter* spp. are mostly considered opportunistic pathogens as they naturally reside in human and animal intestines and can be commonly found in the environment including water and soil (Borenshtein & Schauer, 2006). Various infections have been documented to be associated with *Citrobacter* species (Mair *et al*., 2016; Metri *et al*., 2013; Ranjan & Ranjan, 2013; Samonis *et al*., 2009) and antibiotic resistance studies have shown concerning results (Amaretti *et al.*, 2020; Gajdács & Urbán, 2019). These isolates should therefore be subjected to antimicrobial testing to determine the true threat.

Klebsiella was identified in the surviving coliform communities isolated from every location tested. The two species identified was *K. oxytoca* (Eerste river) and *K. pneumoniae* subsp. *pneumonia* (Berg river). Even though it is a common occupant of the human intestinal tract, when exposed to the respiratory system, it can cause severe infections (Brisse *et al*., 2006). Numerous *Klebsiella* spp. isolates have been characterised to produce ESBLs (Gundogan *et al.,* 2011) and reveal carbapenem resistance (Aquino-Andrade *et al*., 2018) which could make it a bigger threat as it can recover in water after UV treatment irrespective of light or dark conditions.

E. cloacae complex (ECC) occurs in a variety of environments including water, sewage, soil, the human skin and intestinal tract (Grimont & Grimont, 2006). It was the most dominant organisms identified and occurred under Coliform and HPC isolates (Table 4.3.8). Although not generally considered pathogenic, ECC's capability to rapidly acquire multiple antimicrobial resistances (Mezzatesta *et al*., 2012) has led to it being one of the leading causes of hospital-acquired infections worldwide (Guérin *et al.,* 2015).

Pseudomonas aeruginosa is considered an opportunistic pathogen that can cause several human, plant and animal diseases and are omnipresent in water and soil ecosystems (Moore *et al*., 2006). The manifestation of antimicrobial resistance combined with quorum-sensing genes and biofilm formation makes it quite dangerous, especially in hospital environments (Brindhadevi *et al.*, 2020; Thi *et al.*, 2020; Azam & Khan, 2019; Yahr & Parsek, 2006). Recovery of *Ps. aeruginosa* was only observed under light conditions (Table 4.3.8), therefore UV could be considered effective when the water sample is stored in the dark post treatment.

Raoultella planticola is a reclassification of *Klebsiella planticola* by Drancourt *et al*. in 2001. It can be found in soil and aquatic environments and human infections caused by *R. planticola* are few and far between (Yilmaz & Kizilates, 2021; Atıcı *et al*., 2018). In a recent study by Li *et al.* (2022) the virulence and multidrug resistance in a *R. planticola* isolated from hospital sewage was revealed. This is concerning as *R. planticola* was isolated following light and dark recovery conditions (Table 4.3.8).

Lastly, *Staphylococcus aureus*, a well-known human pathogen was isolated from the Berg river following 60 $mJ.cm^{-2}$ UV radiation and three hours recovery in the dark (Table 4.3.8). In 2019 a review article by Kozajda *et al*. identified the environment of wastewater treatment plants and livestock husbandry as high risk due to high concentrations of *S. aureus*, including antibiotic-resistant strains, being present. Antibiotic resistant *S. aureus* strains are frequently encountered of which methicillin-resistant strains are the most concerning as it causes higher morbidity, mortality, and longer hospitalisation (Cheung *et al.,* 2021).

In future, further investigation will be needed to determine the UV dose required to eliminate these UV-treatment resistant strains, as many of them are considered opportunistic pathogens. To determine the true risk they hold for consumers of fresh produce, it should also be determined if these strains harbour any antimicrobial resistance determinants that might disseminate throughout the fresh produce supply chain and have a negative effect on consumer health in future.

4.3.5 CONCLUSIONS

Overall, several conclusions could be drawn based on the results obtained in this study:

Firstly, the physico-chemical and microbial analysis prior to UV demonstrated again that for certain rivers (two out of three in study 1) pollution is a long-term problem, that brings with it potential long-term health risks. This confirms the necessity of water disinfection prior to irrigation in order to reduce the consumer's risk of exposure.

Secondly it has been observed that effluents from WWTPs can have a significant impact on the TDS content of a river, which in turn affect the UVT%, and subsequently the susceptibility of microbial populations to UV irradiation. Removing TDS content prior to UV irradiation cannot be done using a standard bag filter (as was demonstrated in the appendix D) and it is an environmental challenge that should ideally be addressed at municipal level as part of the final WWTP effluent polishing, before wastewater is discharged into waterways. This will also limit long-term environmental eutrophication, reduce the carbon footprint of a WWTP, and support agriculture. Communication with stakeholders and policy makers in this regard should be a priority considering the state of WWTP in South Africa in general, as highlighted in the recent Green Drop report.

Thirdly, for *E. coli* specifically, levels were sufficiently reduced in all river water samples during UV treatment, and although recovery occurred (measured both as colony counts in study 1 and as a presence /absence test following selective enrichment in study 2), levels remained within the guideline limits.

Fourthly, the presence of *L. monocytogenes*, which is ubiquitous in nature, could also not be detected after UV and no recovery post-UV was observed for this pathogen. *Salmonella* spp. on the other hand could be a greater concern (also considering the findings reported in Chapter 4.2 related to pure strains' response to UV irradiation and recovery). It's presence in river water is, however, infrequent, as demonstrated in this and previous reports. As *Salmonella* is an important pathogen frequently associated with fresh produce, it is advisable to monitor irrigation water for its presence on a continuous basis, even if UV treatment of irrigation water is implemented. If it is detected prior to UV treatment, it might be advisable to add an additional disinfection treatment such as chlorine, to inhibit recovery of *Salmonella* after UV treatment.

Fifthly, notable recoveries post-UV were also observed for the coliform and HPC populations. The potential risk this could have for consumers of fresh produce depends on various factors (including the presence of antimicrobial resistance phenotypes or other pathogens not monitored as part of this study, as well as microbial survival on fresh produce surfaces). This should be explored further in future research as it is clear from the surviving bacteria identified in this study, that when given time and favourable conditions for recovery, opportunistic pathogens might remain after UV irradiation. The technology does however decrease the populations significantly, which would also limit the risk of transfer during irrigation. UV irradiation could be successfully utilised as part of a hurdle strategy to improve the safety of irrigation water from contaminated river sources and prevent pathogens from entering the fresh produce supply chain.

Lastly, upscaling UV treatment does bring with it certain operational difficulties which needs to be addressed from a design perspective prior to installation. For the UV system used in this study it was, however, clear that for river water with a low UVT%, higher UV-C doses can only be applied cumulatively by water recirculation and repetitive exposure to lower doses (e.g. 2 x 20 = 40 $mJ.cm^{-2}$ or 3 x 20 $mJ.cm^{-2}$ = 60 $mJ.cm^{-2}$). It can, however, be argued that recirculation of water through a UV irradiation device might even be advantageous in the disinfection of water of poor quality with high suspended and dissolved matter contents that might contribute to shielding of microbes against a once-off, in-line UV irradiation application.

4.4 Antibiotic resistance profiling of UV resistant bacterial isolates from river waters

Please note: This chapter is an extract from the MSc thesis of Kuster (Currently in progress)

4.4.1 BACKGROUND & AIM

4.4.1.1 Background

Antibiotics are rapidly becoming the cause of the most important public health concern facing the human population in the 21st century: antibiotic resistance (Sharma *et al.,* 2016). The combination of an ever-increasing population and the evolutionary response by microbes to the excessive use of antimicrobials may have devastating consequences on the human population, with a rise in outbreaks of untreatable disease being predicted (Michael *et al.,* 2014).

High volumes of antibiotics (of the ingested, 30 to 90%) may enter wastewaters through faecal and urinal excretions due to an inability of humans and animals to completely absorb and metabolise these compounds (Du & Liu, 2012). As regulations and guidelines regarding antibiotic concentration levels in wastewater and treated water have yet to be established (Ezeuko *et al.,* 2021), high levels of residual antibiotics may directly enter water sources.

Concern regarding the spread of antibiotic resistant bacteria (ARB) via bodies of water has arisen in South Africa in recent research, with multiple studies finding high levels of ARB in both raw and treated waters. Concern is deepened through the discovery of large percentages of these ARB being resistant to three or more classes of antibiotics, commonly referred to as multidrug resistant bacteria, highlighting the need for further research into the severity of the issue (Biyela and Bezuidenhout, 2004; Lamprecht *et al.,* 2014; Ateba *et al.,* 2020).

An area of particular concern, in antibiotic resistance associated with water, is the presence antibiotic resistant genes (ARGs) encoding for extended-spectrum beta-lactamases (ESBLs). Extended spectrum beta-lactamases are largely responsible for resistance to beta-lactams, which are antibiotics employed in the treatment of infections caused by *Enterobacteriacea*e (Korzeniewska & Harnisz, 2013). Transfer of these genes occurs either horizontally, or vertically. These acquired plasmid-mediated enzymes make use of mobile genetic elements encoding for resistance to antimicrobial agents. ESBL proliferation in microbial populations, much like the spread of other antibiotic resistance determinants, may be attributed to the excessive use of antibiotics, specifically broad-spectrum antibiotics, in the health and agricultural sectors (Korzeniewska & Harnisz, 2013).

ESBLs are posing a great problem globally and are of particular interest as ESBL positive isolates have been identified in rivers in and around the Stellenbosch area (Krom, Mosselbank, Eerste, Plankenburg and Franschhoek rivers) (Sivhute, 2019; Oosthuizen, 2021; Chapter 4.1, Tables 4.1.16 and A1). Thus, while the

presence of ESBL-producing microorganisms in the above-mentioned rivers has been investigated, more in-depth antibiotic susceptibility profiles have not yet been established.

4.4.1.2 Aim

This study therefore aimed to expand on the work performed by Oosthuizen (2022) and Jankowitz (In press), by extensively testing antibiotic resistance of isolates to 19 antibiotics: ampicillin, amoxicillin/clavulanic acid, cefalexin, cefalotin, cefpodoxime, cefovecin, ceftiofur, imipenem, amikacin, gentamicin, neomycin, enrofloxacin, marbofloxacin, pradofloxacin, doxycycline, tetracycline, nitrofurantoin, chloramphenicol, trimethoprim/sulfamethoxazole, using the Vitek® 2 compact system.

4.4.2 MATERIALS & METHODS

Strain revival and purification

Microbial stock isolates (final glycerol concentration of 25% ($v.v^{-1}$)), previously isolated by Oosthuizen (2022) and Jankowitz (In press), were removed from the -80°C freezer and revived in sterile TSB (Oxoid, South Africa) at the appropriate incubation temperatures. To confirm identification, isolates were streaked onto selective media, similar to the agars used to prepare the isolates prior to storage. Selective agars for *E. coli* included Levine's Eosin Methylene-Blue Lactose Sucrose agar (L-EMB) (Oxoid, South Africa) and Brilliance *E. coli* agar (Oxoid, South Africa). Salmonella isolates was streaked onto Xylose Lysine Deoxycholate Agar (XLD) (Oxoid, South Africa), and all HPC isolates were streaked onto Plate Count Agar (PCA) (Oxoid, South Africa). All strains were transferred to Nutrient agar, after which Gram staining, oxidase testing and catalase testing followed to confirm previous findings.

ESBL testing

All Gram-negative isolates were screened for ESBL production by streaking a single colony onto CHROMagar ESBL plates (MediaMage, South Africa), and incubated inversely at 37°C for 24 hours. Presumptive positive strains were streaked from the CHROMagar ESBL onto non-selective Nutrient Agar and inversely incubated for 24 hours at 37°C The ESBL phenotype was confirmed using the EUCAST (2022) disc diffusion testing procedure according to standard methods, in duplicate, using the following discs: Ceftazidime [30 µg], cefotaxime [30 µg] and cefepime [30 µg], each individually and in combination with clavulanic acid [10 µg] (Davies Diagnostics, South Africa).Once discs had been placed, plates were incubated inversely at 37°C for 18 ±2 hours. Plates were analysed following EUCAST (2022) guidelines which indicate 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.

Antibiotic susceptibility testing (AST) and strain identification

Strain identification and AST was done using the VITEK® 2 Compact Automated ID/AST Instrument according to the manufacturer's instructions (BioMérieux, South Africa). For the identification of Gram-negative microorganisms, the VITEK® 2 GN TEST KIT cards were used (BioMèrieux, South Africa). For susceptibility testing, the VITEK® 2 AST-GN97 TEST KIT cards were used, which test for susceptibility to the following antibiotics: ampicillin, amoxicillin/clavulanic acid, cefalexin, cefalotin, cefpodoxime, cefovecin, ceftiofur, imipenem, amikacin, gentamicin, neomycin, enrofloxacin, marbofloxacin, pradofloxacin, doxycycline, tetracycline, nitrofurantoin, chloramphenicol, trimethoprim/sulfamethoxazole, as well as for the production of ESBLs.

4.4.3 RESULTS & DISCUSSION

The VITEK® 2 identification results of isolates from the river sites at Farms A, B, and C, identified by Jankowitz (In press), are presented in Table 4.4.1. These isolates, termed 'UV-resistant' in the scope of this study, were isolated from river water samples following the application of three consecutive doses of MP UV irradiation (3x20 $mJ.cm^{-2}$), followed by a three-hour recovery period (as discussed in Chapter 4.3). The results (Table 4.4.1) highlight the prevalence of *E. coli* (39%) among the isolates from three river sites, as well as the wide range of other Gram-negative microorganisms present. Rivers can act as the main receptors of human and animal faecal contamination (Bessa *et al.,* 2014), which could contribute to *E. coli* being present in surface waters.

Table 4.4.1 depicts the results of isolates isolated from different sites in the same river, (the Berg river), as well as from the Eerste river. Similar genera (*E. coli, Enterobacter cloacae* and *Klebsiella*) were identified at all three sites, despite the fact that the sites are very far apart. Diaz-Garvidia *et al.* (2021) in their study into the identification of antibiotic resistant *Enterobacterales* from river waters used for irrigation and vegetables irrigated with these waters, found a predominance of *E. coli* and *Citrobacter* spp., as well as the presence of *Klebsiella* spp. and *Enterobacter cloacae* complex in their samples. The similarities in the microorganisms found by Diaz-Garvidia *et al.* (2021) and the UV-resistant isolates identified by Jankowitz (In press) highlights the widespread presence of these microorganisms in river waters. It also indicates that these organisms have the ability to survive UV treatment. None of the isolates tested positive for ESBL production using the disc diffusion method (results not included in this chapter). Their non-ESBL status was further confirmed by the VITEK® 2 CAS, as shown in Table 4.4.4.

Table 4.4.1 VITEK® 2 identification results, using VITEK® 2 GN TEST KIT cards, of the Farm A Berg river isolates, Farm B Berg river isolates, and Farm C Eerste river isolates (Jankowitz, *In press*)

Isolate	**VITEK® ID Berg River Farm A**
CE 18	*Escherichia coli*
CE 19	*Enterobacter cloacae* complex
CE 22	*Klebsiella pneumoniae pneumonia*
Isolate	**VITEK® ID Berg river Farm B**
CS1	*Citrobacter youngae*
CS2	*Citrobacter youngae*
CE 23	*Enterobacter cloacae complex*
CE 24	*Enterobacter cloacae complex*
CE 35	*Escherichia coli*
CE 20	*Escherichia coli*
CE 17 B	*Escherichia coli*
CE 17 A	*Citrobacter freundii*
CE 15	*Raoultella planticola*
CE 16 A	*Raoultella planticola*
CE 16 B	*Escherichia coli*
CE 34	*Pseudomonas aeruginosa*
CE 38	*Klebsiella pneumoniae pneumonia*
Isolate	***VITEK® ID Eerste river Farm C***
CE 3	*Escherichia coli*
CE7 B	*Escherichia coli*
CE 10	*Escherichia coli*
CE 9	*Escherichia coli*
CE 5	*Enterobacter cloacae complex*
CE 6	*Enterobacter cloacae complex*
CE 8	*Klebsiella oxytoca*

In Table 4.4.2 below, the identification results of microorganisms isolated by Oosthuizen (2022) from five rivers, are presented. All of these strains were isolated before UV treatment. All isolates, except the *Salmonella* strains, were identified as ESBL producers using disc diffusion methodology (Table 4.4.3). Their ESBL status was further confirmed by the VITEK® 2 CAS, as shown in Table 4.4.5. Of the 13 isolates, eight were identified as *Escherichia coli*, predominantly isolated from the Plankenburg, Eerste and Jonkershoek rivers. Furthermore, the two Franschhoek river isolates were identified as *Klebsiella pneumoniae pneumonia* and *Shigella sonnei*. *Klebsiella pneumoniae pneumonia*, also identified in UV resistant isolates in Table 4.4.1, was also present in the Mosselbank river.

Sivhute (2019) identified 11 ESBL-producing isolates, originating from the Krom and Plankenburg rivers, as *E. coli* and *Klebsiella pneumoniae*. Zaatout *et al.* (2021) in their review on the prevalence of ESBL-producing *Enterobacteriaceae* in wastewater, found *E. coli, Klebsiella*, and *Enterobacter* to be the most common producers of ESBL in wastewaters – thus justifying the high prevalence of *E. coli* (8/12) and presence of *Klebsiella pneumoniae pneumonia* in the ESBL-positive isolates. *Shigella* spp., while not commonly identified as ESBL-producers, have been found to harbour different types of ESBL genes and pose a major global health threat to developing countries (Ranjbar & Farahani, 2019).

ESBL-producing *Salmonella* spp. are not often observed in river waters. Raseala *et al.* (2020) have, however, found evidence of the circulation of ESBL-producing *Salmonella* spp. within the agricultural environment and nearby water sources. As ESBL-positive *Salmonella* spp. have frequently been reported in the food production chain (Monte *et al.,* 2019), the likelihood of this species being present in waters relating to food production is high.

Table 4.4.2 VITEK® 2 identification results of isolates (Oosthuizen, 2021), isolated from various rivers in and around the Stellenbosch area, using VITEK® 2 GN TEST KIT cards

Isolate	VITEK® ID	LOCATION*
ESBL 2	*Klebsiella pneumoniae pneumonia*	MBANK
ESBL 3	*Escherichia coli*	MBANK
ESBL 4	*Klebsiella pneumoniae pneumonia*	FRANS
ESBL 5	Shigella sonnei	FRANS
ESBL 6	*Escherichia coli*	PLANK
ESBL 7	*Escherichia coli*	PLANK
ESBL 9	*Escherichia coli*	EERSTE
ESBL 12	*Escherichia coli*	EERSTE
ESBL 13	*Escherichia coli*	JONKERS
ESBL 14	*Escherichia coli*	JONKERS
SALM 1	*Salmonella* group (98%)	PLANK
SALM 4	*Salmonella* group (98%)	MBANK
HPC 1	*Escherichia coli*	PLANK

MBANK – Mosselbank river; FRANS – Franschhoek river; PLANK – Plankenburg river; EERSTE – Eerste river; JONKERS – Jonkershoek river

Table 4.4.3 Results (obtained for two repetitions) of ESBL testing following EUCAST (2022) disk diffusion procedures

	Mean zone diameter (cm)						ESBL
Isolate code	**CAZ**	**CAZ + CV**	**CPM**	**CPM + CV**	**CTX**	**CTX + CV**	**(Yes/No)**
HPC 1	2.65	2.85	2.10	2.55	1.70	2.65	**Yes**
ESBL 2	1.35	2.70	1.60	2.70	1.00	2.85	**Yes**
ESBL 3	1.35	2.85	1.40	2.40	0.90	2.60	**Yes**
ESBL 4	1.33	2.53	1.50	2.40	0.90	2.53	**Yes**
ESBL 5	2.60	2.80	2.00	2.60	1.40	2.95	**Yes**
ESBL 6	2.25	3.05	2.25	3.05	1.65	3.25	**Yes**
ESBL 7	1.10	2.40	1.20	2.30	0.75	2.55	**Yes**
ESBL 9	1.30	2.55	1.75	2.70	1.05	2.9	**Yes**
ESBL 10	2.33	2.87	2.40	2.93	1.70	2.83	**Yes**
ESBL 11	2.30	3.10	2.40	2.80	1.60	2.90	**Yes**
ESBL 12	2.25	3.25	1.95	2.80	1.25	3.10	**Yes**
ESBL 13	2.45	2.20	1.90	2.50	1.70	2.65	**Yes**
ESBL 14	2.80	3.00	2.10	2.75	1.75	2.90	**Yes**
EC ATCC 25922	2.90	2.70	3.15	3.15	3.15	3.15	**No**
KP ATCC 700603	1.50	2.40	2.60	3.10	2.40	3.20	**Yes**

* CAZ – Ceftazidime; CPM – Cefepime; CTX – Cefotaxime; CV – Clavulanic Acid; EC – *Escherichia coli;* KP – *Klebsiella pneumoniae*

Table 4.4.4 shows the VITEK® 2 AST Minimum Inhibitory Concentration (MIC) results obtained for isolates from the Berg river (Farms A and B), and Eerste river (Farm C) sampling sites. MIC, defined as 'the lowest concentration of an antimicrobial that will inhibit the visible growth of a microorganism after overnight incubation', is used to confirm antibiotic resistance (Andrews, 2001). While the root cause of the global development of resistance is unknown, a speculated cause includes the excessive use of antibiotics in the medical, veterinary, and agricultural sectors, with factors including improper sanitation, easy access to over-the-counter antibiotics, discharge of industrial effluents, and discharge of non-metabolised antibiotics via manure having been identified as factors increasing the severity of the problem (Samreen *et al.,* 2021).

Berg river Farm B isolates showed the greatest levels of antibiotic resistance, with moderate levels of amoxicillin/clavulanic acid (5/12 isolates – CS 1, CS 2, CE 23, CE 24, CE 17A) and ampicillin (4/12 isolates – CE 20, CE 15, CE 16A, CE 38) resistance being detected. Isolates CS 1 and CS 2 (both *Citrobacter youngae*) displayed intermediate resistance to chloramphenicol – a trait also displayed by isolates CE 35, CE 17B and CE 16B (all *E. coli*). Intermediate resistance to nitrofurantoin was seen in CE 23 (*Enterobacter cloacae* complex) and CE 38 (*Klebsiella pneumoniae pneumonia*). CE 20 (*E. coli*), which exhibits resistance to ampicillin, doxycycline, and tetracycline, is the only isolate of those isolated by Jankowitz (*In press*) to show resistance to trimethoprim/sulfamethoxazole, with an MIC of ≥320. CE 17A (*Citrobacter freundii*) is the only

isolate of those isolated at Farm B to exhibit resistance to enrofloxacin, marbofloxacin, pradofloxacin, and chloramphenicol. Of the five isolates tested for ESBL production (CE 35, CE 20, CE 17B, CE 16 B, CE 38), four were confirmed as negative.

The Eerste river isolates CE 3 (*E. coli*) and CE 7B (*E. coli*) produced identical results, being susceptible to all 19 antibiotics. Intermediate resistance against chloramphenicol was once again found to be prevalent, with CE 10, CE 9 (both *E. coli*) and CE 5 (*Enterobacter cloacae* complex) displaying this characteristic. CE 5, CE 6 (*Enterobacter cloacae* complex), and CE 8 (*Klebsiella oxytoca*) showed resistance to amoxicillin/clavulanic acid and cefalotin, as well as intermediate resistance to cefovecin. Of the seven isolates, CE 8 showed the highest overall resistance, being resistant to four of the antibiotics and displaying intermediate resistance to nitrofurantoin. Like the isolates tested for ESBL production in Berg river Farm B, all five of the tested Eerste river isolates were negative for ESBL production.

The Berg river Farm A isolates showed low antibiotic resistance, with CE 18 displaying intermediate chloramphenicol resistance. CE 19 (*Enterobacter cloacae* complex) showed resistance to both amoxicillin/clavulanic acid and cefalotin, and intermediate resistance to nitrofurantoin and marbofloxacin. CE 22 (*Klebsiella pneumoniae pneumonia*) was the only isolate of the three to show resistance to ampicillin. Once again, the two isolates tested for ESBL production, like those tested for Farms B and C, were negative.

When comparing the antibiotic resistance profiles of the three UV-resistant isolates from the Berg river Farm A to those of the thirteen isolates from Berg river Farm B, it is clear not only that more UV-resistant bacteria were present downstream on the Berg river, but also that the UV-resistant Farm B bacteria exhibited greater resistance to antibiotics than the upstream, Farm A bacteria. While Farms A and B are both located in agricultural areas with low population densities, the differences in the resistance profiles at these two sampling sites highlights the influence which municipal, industrial, and agricultural areas have on the river profile as it flows between the sites. As the Berg river flows from Farm A to Farm B, it passes through the densely populated town of Paarl, passing through municipal areas (housing, schools, hospitals), and industrial areas. The river also passes by the Paarl Wastewater Treatment Works, a wastewater treatment plant (WWTP), before reaching Farm B.

In treatment of wastewater, removal of antibiotic resistant bacteria (ARB) and antibiotic resistant genes (ARGs) is largely ignored, with most conventional WWTPs being ineffective and inadequate in the removal of these contaminants (Alam *et al.,* 2021; Xu *et al.,* 2021). WWTPs, through their emission of ARGs, have been found to be responsible for an increased concentration of these genes in receiving waters. As WWTPs are classed as point sources for the release of antibiotics and ARGs into the environment (Grenni, 2022; Brown *et al.,* 2019), bacteria flowing from Farm A to Farm B may therefore acquire antibiotic resistance via resistance genes when passing by the WWTP.

Broad-spectrum penicillins have consistently been found to be the most consumed antibiotic class in retail and hospital sectors, with consumption in low- and middle-income countries increasing 56% between 2000 and 2015 (Klein *et al.,* 2018). Cephalosporin and quinolone consumption in these countries showed

increases of 399 and 125%, respectively (Klein *et al.,* 2018). Resistance to antibiotics of the cephalosporin class was prevalent at all three sites, particularly to cefalotin (9/23). Isolates CS1 and CS2, originating from Farm B, both identified as *Citrobacter youngae*, were also resistant to cefpodoxime and cefovecin, third generation cephalosporins used in both human and veterinary treatments.

The Eerste river Farm C sampling site, is situated in the winelands. The sampling site is at the convergence of the Eerste river, which flows through central Stellenbosch (largely residential regions, but also industrial), and the Blouklip river, which flows through agricultural, less densely populated areas in Stellenbosch. Given the agricultural location of the sampling site, the antibiotic resistance profiles of Farm C isolates were expected to produce similar results to those isolated at Farm A as both sites are downstream and removed from densely populated areas.

All three farms are situated in agricultural areas, and given that tetracyclines, macrolides and penicillins have been found to be the most used antimicrobials of 27 classes commonly implemented in agriculture (Laxminarayan *et al.,* 2015), resistance among the 23 isolates to these classes was expected. The prevalence of amoxicillin/clavulanic acid and ampicillin resistance, both of the penicillin class, in isolates of all three sampling sites is thus likely caused by the extensive use of these antibiotics in agriculture, as well as their high consumption in hospitals and retail. As tetracyclines have been reported to be found in most rivers (Xu *et al.,* 2021), Farm B being the only sampling site at which resistance to antibiotics in the tetracycline class (doxycycline, tetracycline) was found, was surprising. Both tetracycline-resistant isolates were identified as *E. coli*. Intermediate resistance to chloramphenicol and nitrofurantoin was prevalent with 9/23 and 6/23 isolates showing intermediate resistance to these antibiotics, respectively. Chloramphenicol and nitrofurantoin act as broad-spectrum bacterial antibiotics and are commonly used to treat a variety of illnesses in both humans and animals, particularly in developing countries (Nguyen *et al.,* 2022; Munoz-Davila, 2014). Chloramphenicol has been found to accumulate in bodies of water with high frequency (Nguyen *et al.,* 2022), thus explaining the detection of chloramphenicol-resistant bacteria in the river water samples.

In her review looking into the role which old antibiotics play in the era of antibiotic resistance, Munoz-Davila (2014) found resistance levels to nitrofurantoin to have remained largely unchanged since its discovery in 1953 and labelled the role of nitrofurantoin in the treatment of multidrug resistant bacteria as ‘crucial’ as it may be a successful treatment mechanism for infections resistant to newer antibiotics. While this sentiment has been echoed by others (Gardiner *et al.,* 2019), the increased use and prescription of ‘forgotten’ antibiotics like nitrofurantoin, is likely to result in the inevitable emergence of resistance. The presence of bacteria intermediately resistant to the antibiotic in the isolates therefore indicate that the emergence of resistance has already begun.

Suhartono *et al.,* (2016) found that trimethoprim/sulfamethoxazole resistant-*E. coli* were present in large concentrations in river waters, and that half of the plasmids detected in these resistant bacteria were transmissible. Thus, while only one of the isolates (CE 20 – *E. coli*) identified was resistant to

trimethoprim/sulfamethoxazole, the potential for the spread of resistance to this antibiotic combination exists. Similarly, while only one isolate showed resistance to fluoroquinolones (CE 17A – *Citrobacter freundii*), Yim *et al.,* (2013) found evidence for the spread of fluoroquinolone-resistance in bacteria inhabiting WWTPs via plasmid-borne resistance genes from resistant pathogens, further highlighting the threat which even a single resistant isolate may pose in waters in the dissemination of antibiotic resistance.

Table 4.4.5 shows the VITEK® 2 AST MIC results of isolates, isolated by Oosthuizen (2021), found to be ESBL-producers. Compared to the resistance results given in Table 4.4.4, isolates in Table 4.4.5 show far greater levels of antibiotic resistance. High levels of resistance to ampicillin (11/12 isolates), cefalotin (12/12 isolates), cefpodoxime (11/12 isolates), cefovecin (11/12 isolates), ceftiofur (11/12 isolates), trimethoprim/sulfamethoxazole (7/12 isolates), tetracycline (6/12) and doxycycline (6/12) were recorded. Gentamicin resistance was prevalent in four isolates. Intermediate enrofloxacin, pradofloxacin, chloramphenicol, neomycin and amoxicillin/clavulanic acid resistance was also detected in multiple isolates. Of the ten isolates tested for ESBL-production, ten were found to be positive.

When comparing the profiles of the ESBL-producers isolated before UV treatment by Oosthuizen (2022) to the non-ESBL producers isolated after UV treatment by Jankowitz (*In press*), the ESBL-producers exhibit increased resistance across the antibiotic classes. ESBL-producing *Enterobacteriaceae* are commonly reported to be multi-drug resistant, with resistance to fluoroquinolones, aminoglycosides, trimethoprim-sulfamethoxazole and tetracyclines being most prominent (Hassen *et al.,* 2020). Multidrug resistance may be defined as 'co-resistance to three or more classes of antimicrobial drugs' (Doyle *et al.,* 2013), and poses a major threat to human health as research has found that multidrug resistance patterns in both Gram-negative and Gram-positive bacteria have resulted in difficult-to-treat and, occasionally, untreatable infections when using conventional antimicrobial methods (Frieri *et al.,* 2017). Teklu *et al.* (2019) in their study of ESBL-production and MDR in *Enterobacteriaceae,* found 246/426 clinically sourced *Enterobacteriaceae* to be ESBL-producers, of which 96.3% were MDR. Thus, when comparing the 67% MDR-prevalence (ESBL 2, ESBL 3, ESBL 4, ESBL 5, HPC 1, ESBL 9, ESBL 13, ESBL 14) in the isolates isolated by Oosthuizen (2022)(Table 4.4.5) to the 13% MDR-prevalence (CE 20, CE 17A, CE 8) in those identified by Jankowitz (*In press*) Table 4.4.4, the link between ESBL-production and MDR-prevalence is evident.

The high resistance shown by almost all isolates to ampicillin, cefpodoxime, cefovecin, and ceftiofur is unsurprising as ESBLs confer resistance to most beta-lactam antibiotics (including penicillins, and third and fourth generation cephalosporins) (Blaak *et al.,* 2014; Teklu *et al.,* 2019) – with ampicillin being a penicillin, and cefpodoxime, cefovecin, and ceftiofur being third generation cephalosporins. ESBLs have also proven to be successful in the hydrolysis of narrow spectrum cephalosporins (Lim *et al.,*2015), a category into which cefalotin falls. High susceptibility to imipenem was exhibited by 100% of isolates and may be explained by the inability of ESBLs to inhibit carbapenems (Malande *et al.,* 2019). These findings are encouraging, as carbapenems are commonly used as a 'last resort' treatment for Gram-negative bacterial infections, and the incidence of carbapenem resistance is increasingly reported (Kelly *et al.,* 2017). While ESBLs confer resistance

to penicillins, they are inhibited by β-lactamase inhibitors such as clavulanic acid (Malande *et al.,* 2019), thus the use of β-lactams in combination with clavulanic acid, has shown success in treating infections relating to ESBL-producers (Huttner *et al.,* 2020). Therefore, the increased susceptibility of isolates to the amoxicillin/clavulanic acid combination is likely due to the presence of clavulanic acid. As the plasmids encoding for ESBLs often carry genes encoding resistance to trimethoprim/sulfamethoxazole, tetracyclines, and aminoglycosides (Paterson and Bonomo, 2005), resistance to these antibiotics is common in ESBL-producers (Teklu *et al.,* 2019; Schwaber, 2005). Tacão *et al.* (2014) in their study on co-resistance within ESBL-producers from aquatic systems, found resistance to tetracycline, aminoglycosides, and sulfamethoxazole-trimethoprim to be significantly more prevalent in ESBL-producers.

While such extensive profiling of antibiotic resistance has not yet been performed on river water isolates from the Cape Winelands Region, previous studies have investigated the presence of resistance to various antibiotic classes, and subsequent MDR. Lamprecht *et al.* (2014) detected abundant resistance to ampicillin (100%) and trimethoprim (80%), as well as resistances to tetracycline, streptomycin, and chloramphenicol in their study of *E. coli* isolated over three years from the Plankenburg river. Sivhute (2019) found high levels of resistance to ampicillin (100%) and tetracycline (79%) in isolates originating from the Krom and Plankenburg rivers. Bursey (2021), reported high ampicillin, trimethoprim/sulfamethoxazole, and tetracycline resistances in *Enterobacteriaceae* isolated from the Franschhoek, Mosselbank and Plankenburg rivers, as well as high levels of ampicillin, trimethoprim/sulfamethoxazole, penicillin, and erythromycin resistance in *Listeria monocytogenes* isolates from the same rivers. All three studies detected MDR bacteria, emphasising that resistance to antibiotics has consistently been prevalent in waters in the Cape Winelands Region.

Table 4.4.4 VITEK® 2 AST Minimum Inhibitory Concentration (MIC) results of Farm B, Farm C, and Farm A isolates (Jankowitz, 2022), using VITEK® 2 AST-GN97 TEST KIT cards

*Colours indicate resistance brackets: red – resistant; orange – intermediate; green – susceptible

RIVERS:	Berg river (FARM B)													Eerste river (FARM C)							Berg river (FARM A)		
STRAINS:	**CS 1**	**CS 2**	**CE 23**	**CE 24**	**CE 35**	**CE 20**	**CE 17B**	**CE 17A**	**CE 15**	**CE 16A**	**CE 16B**	**CE 34**	**CE 38**	**CE 3**	**CE 7B**	**CE 10**	**CE 9**	**CE 5**	**CE 6**	**CE 8**	**CE 18**	**CE 19**	**CE 22**
ESBL					-	-	-				-		-	-	-	-	-			-	-		-
AMPICILLIN					≤2	≥32	4		16	16	4		≥32	≤2	≤2	8	8			≥32	4		≥32
AMOXICILLIN/CLAVULANIC ACID	4	4	≥32	≥32	≤2	4	≤2	16	≤2	≤2	≤2		≤2	≤2	≤2	4	4	≥32	≥32	≥32	≤2	≥32	≤2
CEFALEXIN																							
CEFALOTIN	32	32	≥64	≥64	4	8	4	16	≤2	≤2	4		≤2	4	4	8	4	≥64	≥64	≥64	4	≥64	≤2
CEFPODOXIME	≥8	≥8	1	0,5	≤0,25	≤0,25	≤0,25	2	≤0,25	≤0,25	≤0,25		≤0,25	≤0,25	≤0,25	≤0,25	0,5	1	0,5	0,5	≤0,25	1	≤0,25
CEFOVECIN	≥8	≥8	2	2	≤0,5	5	≤0,5	2	≤0,5	≤0,5	≤0,5	≤0,5	≤0,5	≤0,5	≤0,5	1	1	4	4	4	≤0,5	2	≤0,5
CEFTIOFUR	2	2	2	≤1	≤1	≤1	≤1	≤1	≤ 1	≤ 1	≤1	≤ 1	≤ 1	≤1	≤1	≤1	≤1	2	≤ 1	≤ 1	≤1	2	≤ 1
IMIPENEM	≤0,25	≤0,25	0,5	1	≤0,25	≤0,25	≤0,25	≤0,25	0,5	0,5	≤0,25	1	≤0,25	≤0,25	≤0,25	≤0,25	≤0,25	0,5	0,5	0,5	≤0,25	0,5	≤0,25
AMIKACIN	≤2	≤2	≤2	≤2	≤2	≤2	≤2	≤2	≤2	≤2	≤2	≤2	≤2	≤2	≤2	≤2	≤2	≤2	≤2	≤2	≤2	≤2	≤2
GENTAMICIN	≤1	≤1	≤1	≤1	≤1	≤1	≤1	≤1	≤1	≤1	≤1	≤1	≤1	≤1	≤1	≤1	≤1	≤1	≤1	≤1	≤1	≤1	≤1
NEOMYCIN	≤2	≤2	≤2	≤2	≤2	≤2	≤2	≤2	≤2	≤2	≤2		≤2	≤2	≤2	≤2	≤2	≤2	≤2	≤2	≤2	≤2	≤2
ENROFLOXACIN	≤0,25	≤0,25	≤0,25	0,5	≤0,25	≤0,25	≤0,25	≥ 4	≤0,25	≤0,25	≤0,25	≤0,25	≤0,25	≤0,25	≤0,25	≤0,25	≤0,25	≤0,25	≤0,25	≤0,25	≤0,25	≤0,1	≤0,12
MARBOFLOXACIN	≤0,25	≤0,25	≤0,25	≤0,25	≤0,25	≤0,25	≤0,25	≥ 4	≤0,25	≤0,25	≤0,25	≤0,25	≤0,25	≤0,25	≤0,25	≤0,25	≤0,25	≤0,25	≤0,25	≤0,25	≤0,25	≤0,5	≤0,5
PRADOFLOXACIN	≤0,25	≤0,25	≤0,25	0,2	≤0,25	≤0,25	≤0,25	≥ 4	≤0,25	≤0,25	≤0,25		≤0,25	≤0,25	≤0,25	≤0,25	≤0,25	≤0,25	≤0,25	≤0,25	≤0,25	≤0,1	≤0,12
DOXYCYCLINE	2	2	8	2	≥16	≥16	2	2	1	1	2		1	≤0,5	≤0,5	2	2	4	4	4	2	8	1
TETRACYCLINE	2	2	2	2	≥16	≥16	≤1	≤1	≤1	≤1	≤1		≤1	≤1	≤1	2	2	2	2	2	≤1	4	≤1
NITROFURANTOIN	≤16	≤16	64	32	≤16	32	≤16	≤16	≤16	≤16	≤16		64	≤16	≤16	≤16	≤16	64	32	64	≤16	64	64
CHLORAMPHENICOL	16	16	8	8	16	8	16	32	≤2	≤2	16		4	4	4	16	16	16	8	16	16	8	≤2
TRIMETHOPRIM/SULFAMETHOXAZOLE	≤20	≤20	≤20	≤20	≤20	≥320	≤20	≤20	≤20	≤20	≤20		≤20	≤20	≤20	≤20	≤20	≤20	≤20	≤20	≤20	≤20	≤20

Table 4.4.5 VITEK® 2 AST Minimum Inhibitory Concentration (MIC) results of isolates (Oosthuizen, 2021), isolated from various rivers in and around the Stellenbosch area, using VITEK® 2 AST-GN97 TEST KIT cards

*Colours indicate resistance brackets: red – resistant; orange – intermediate; green – susceptible

RIVERS:	MBANK		FRANS		PLANK				EERSTE		JONKERS	
STRAINS:	**ESBL 2**	**ESBL 3**	**ESBL 4**	**ESBL 5**	**ESBL 6**	**ESBL 7**	**SALM1**	**HPC 1**	**ESBL 9**	**ESBL 12**	**ESBL 13**	**ESBL 14**
ESBL	+	+	+		+	+		+	+	+	+	+
AMPICILLIN	≥32	≥32	≥32	≥32	≥32	≥32	≤2	≥32	≥32	≥32	≥32	≥32
AMOXICILLIN/CLAVULANIC ACID	16	8	16	≤2	4	4	≤2	4	16	≤2	4	4
CEFALEXIN												
CEFALOTIN	≥64	≥64	≥64	≥64	≥64	≥64	≤2	≥64	≥64	≥64	≥64	≥64
CEFPODOXIME	≥8	≥8	≥8	≥8	≥8	≥8	≤0,25	≥8	≥8	≥8	≥8	≥8
CEFOVECIN	≥8	≥8	≥8	≥8	≥8	≥8	1	≥8	≥8	≥8	≥8	≥8
CEFTIOFUR	≥8	≥8	≥8	≥8	≥8	≥8	≤1	≥8	≥8	≥8	≥8	≥8
IMIPENEM	≤0,25	≤0,25	≤0,25	≤0,25	≤0,25	≤0,25	≤0,25	≤0,25	≤0,25	≤0,25	≤0,25	≤0,25
AMIKACIN	≤2	≤2	≤2	≤2	≤2	≤2	≤2	≤2	≤2	≤2	≤2	≤2
GENTAMICIN	≥16	≤1	≥16	≤1	≤1	≤1	≤1	≤1	≥16	≤1	≤1	≤1
NEOMYCIN	≤2	≤2	16	≤2	≤2	≤2	≤2	16	≤2	≤2	≤2	16
ENROFLOXACIN	≤0,12	1	1	1	1	1	≤0,12	≤0,12	≥4	0,5	≤0,12	≤0,12
MARBOFLOXACIN	≤0,5	≤0,5	≤0,5	1	1	1	≤0,5	≤0,5	≥4	≤0,5	≤0,5	≤0,5
PRADOFLOXACIN	≤0,12	0,25	1	1	0,5	0,5	≤0,12	≤0,12	≥4	0,25	≤0,12	≤0,12
DOXYCYCLINE	≥16	1	≥16	1	1	2	1	≥16	≥16	≤0,5	≥16	≥16
TETRACYCLINE	≥16	≤1	≥16	≤1	2	2	≤1	≥16	≥16	≤1	≥16	≥16
NITROFURANTOIN	32	≤16	64	≤16	≤16	≤16	≤16	≤16	≤16	≤16	≤16	≤16
CHLORAMPHENICOL	≤2	8	4	8	16	16	4	8	16	≤2	8	8
TRIMETHOPRIM/SULFAMETHOXAZOLE	≥ 320	≥ 320	≥ 320	≥ 320	≤20	≤20	≤20	≥ 320	≥ 320	≤20	≤20	≥ 320

In Table 4.4.6 below, the 19 antibiotics tested for in this study (using the VITEK® 2 AST-GN97 TEST KIT cards) are arranged based on the World Health Organisation *Critically Important Antimicrobials for Human Medicine* List *(*WHO CIA List, 2018*)*. The ranking of antibiotics in the list is used as a tool for the risk management of antimicrobial resistance – highlighting which critically important antibiotics should be used sparingly to minimise dissemination of antibiotic resistance.

Table 4.4.6 Importance classification of antibiotics tested for using the VITEK® 2 AST-GN97 TEST KIT card according to the *WHO CIA List* (2018)

Antibiotic	Critically important	Highly important	Important
AMPICILLIN	x		
AMOXICILLIN/CLAVULANIC ACID	x		
CEFALEXIN		x	
CEFALOTIN		x	
CEFPODOXIME	x		
CEFOVECIN	x		
CEFTIOFUR	x		
IMIPENEM	x		
AMIKACIN	x		
GENTAMICIN	x		
NEOMYCIN	x		
ENROFLOXACIN	x		
MARBOFLOXACIN	x		
PRADOFLOXACIN	x		
DOXYCYCLINE		x	
TETRACYCLINE		x	
NITROFURANTOIN			x
CHLORAMPHENICOL		x	
TRIMETHOPRIM/SULFAMETHOXAZOLE		x	

Of the 19 antibiotics tested for, 12 were classed as 'critically important', six as 'highly important', and one as 'important'. Given the resistance profiles for the isolates isolated by both Jankowitz (*In press*) and Oosthuizen (2022), the prevalence of resistance to antibiotics labelled as 'critically important' and 'highly important' is very concerning, as this indicates the potential dissemination of ARG's in surface waters in the Cape Winelands Region.

4.4.4 CONCLUSIONS

While it is encouraging that the UV-resistant isolates identified by Jankowitz (*In press*) were not found to be ESBL-producers, the presence of multidrug resistant isolates and resistance of multiple isolates to 'critically important' and 'highly important' antimicrobials in human medicine is greatly

concerning. A clear link between ESBL-production and the increased prevalence of multidrug resistance in producers could be made. While the limited sample size of isolates examined at each of the locations by Oosthuizen (2021), restricts the ability to draw definitive conclusions on the influence of location on the isolates, the presence of ESBL-producers in all five rivers, and overwhelming resistance of isolates to both 'critically important' and 'highly important' antimicrobials highlights the need for further investigation into the factors influencing these very concerning antibiotic resistant profiles. The findings point to the potential widespread dissemination of ARG's in surface waters in the Cape Winelands Region.

5. GENERAL CONCLUSIONS

Previous and current research (as discussed in Chapters 1 and 2) has highlighted the continuing deterioration of the microbial quality of South African surface waters. The recent Green Drop Report (DWS, 2022) furthermore emphasises the fact that very limited number of WWTP's function properly, which implies that improperly treated wastewater gets released into the environment on a daily basis in the South African context. This is concerning from both a food security and food safety perspective, as most of South Africa's irrigation water is sourced from surface waters. The potential health implications this could have for the consumers of fresh produce urgently warrants some form of water treatment prior to crop irrigation, to prevent pathogens from entering the food distribution chain.

A variety of water treatment methods have been used in the past, of which the most commonly used ones are of a chemical nature. As concerns rise regarding the environmental impact, and detrimental health effects of disinfection byproducts, the advantages of residue-free UV-based disinfection become apparent. It is, however, not without its challenges and it is against this backdrop that the previous scoping study (Sigge et al., 2016), as well as the current project has been undertaken. Investigations (summarised in Chapters 4.1, 4.2 and 4.3) included testing for the presence of *Listeria monocytogenes,* STEC and *Salmonella* spp. before and after UV treatment. UV resistance profiles and recovery potential of isolates obtained from the rivers were also tested (Chapters 4.2 and 4.3), and included antimicrobial resistance testing (Chapters 4.1, 4.2, 4.3 and 4.4). Lastly, by moving from an LP laboratory-scale UV system (Chapters 4.1 and 4.2) to a pilot-scale MP UV system (Chapter 4.3) this study intended to fill knowledge gaps and contribute towards the successful future application of UV radiation in irrigation water treatment at farm-scale.

Findings related to the physico-chemical and microbial profiles of rivers

As the previous scoping study (Sigge et al., 2016) evaluated aspects of UV disinfection while focusing on water from only one site, this project aimed to evaluate the efficacy of UV radiation – both at laboratory-scale and pilot-scale – on a variety of river water sources of varying water qualities. Based on irrigation water guidelines (summarised in Section 3.1, Chapter 3), the previous scoping study and other research (Sigge et al., 2016; Banach et al., 2021) have focused mainly on *E coli* as indicator organism for UV disinfection efficiency. This is in spite of the fact that a number of other pathogens can be associated with contaminated fresh produce and cause disease (as discussed in Chapter 2). The effect of UV on important food pathogens other than *E coli* was thus an important aim of this project. Including this research aim in the project was well justified considering the findings that related to the presence of specific pathogens in river water samples during the course of this study (summarised in Table 5.1).

Table 5.1. A summary of the presence of microbial populations of concern detected at four different river sites sampled at different times during 2019-2021 during the course of this project (Bursey, 2021; Oosthuizen, 2022).

(Where applicable, n=total number of sampling events)

Populations present	Plankenburg	Mosselbank	Franschhoek	Eerste
E. coli (>3 log CFU.mL^{-1})	8 (n=8)	8 (n=8)	1 (n=5)	3 (n=5)
Salmonella spp.	4 (n=6)	5 (n=9)	0 (n=6)	1 (n=5)
Listeria monocytogenes	6 (n=6)	9 (n=9)	3 (n=6)	4 (n=5)
STEC molecular detection	2 (n=3)	2 (n=2)	0 (n=2)	1 (n=1)
ESBL positive strains isolated	Yes	Yes	Yes	No

STEC – Shiga toxin-producing E coli; ESBL – Extended Spectrum Beta-Lactamase

The motivation to include other river water sources in this study was also based on the findings of the previous study which reported fluctuations in the physico-chemical nature of river water quality over time at the same site. This observation was also confirmed by the results of the current project. UVT% is an important parameter to consider in UV-irradiation applications, and if the results of this project over time are considered (as summarised in Figure 5.1), it is apparent that substantial variations occurred in UVT% over time at the different sites included in this project (Figure 5.1).

As highlighted in Figure 5.1 (and in Chapters 4.1-4.3), the Mosselbank river consistently had the poorest UVT%, compared to the Franschhoek river, which had the best UVT% profile. The causes for the poor quality observed at the Mosselbank site – both in terms of physico-chemical profiles and microbial risks (Table 5.1 – have been discussed in detail (Chapters 4.1-4.3, Bursey, 2021; Oosthuizen, 2022) and could be directly related to the WWTP situated upstream of the sampling site, which is not unique in the South African context (Green Drop Report – DWS, 2022). What should be noted, though, are the variations in UVT% observed at the three "better" sites over time. If these variations are compared UVT% values reported in literature for water in other countries (Table 5.2), and how UVT% values should be classified (Table 5.3), it can be concluded that at some sampling occasions during the course of this project, water from all four sites could have been classified as similar to a standard of secondary wastewater effluent (UVT% equal to 60% and lower). This brings with it certain design requirements for wastewater (USEPA, 1999) that should be considered in large-scale UV installations treating river water in the South African setting.

Figure 5.1. UVT% and HPC numbers (in log.CFU.mL^{-1}) measured by Bursey (2021) and Oosthuizen (2022) for four rivers at different times during 2019-2021

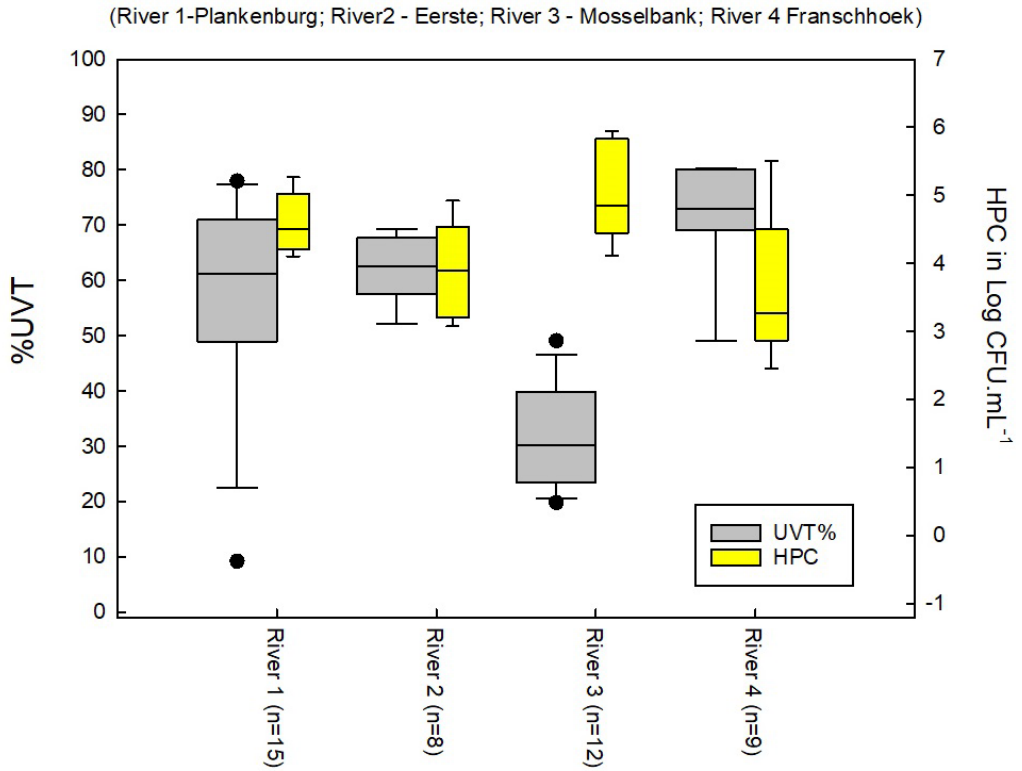


Table 5.2 UVT% values reported in literature for various water sources in other countries

UVT%	Water type	References
>98%	Ultrapure water	Nakova, 2023*
70-96%	Drinking water	Nakova, 2023*
94-95%	River, ground and lake water at camp sites	Younis et al., 2019 (California)
70-90%	Sea water	Nakova, 2023*
88-93%	Sea water (Norway)	Liltved et al., 2011
60-93%	River water (Four rivers)	Cantwell & Hoffman, 2008 (Canada)
60-70%	Tertiary treated wastewater effluent	Nakova, 2023*
55%-65%	"Typical" wastewater at WWTP	Templeton et al., 2006 (Canada)
45-60%	Secondary treated wastewater effluent	Nakova, 2023*
45-70%	Secondary treated wastewater effluent (Israel)	Nasser et al., 2006
30-65%	Storm water flows of secondary treated wastewater effluent (to sea)	Muller & Lem, 2011 (Wales)
20-45%	Primary treated wastewater effluent	Nakova, 2023*

* https://www.weuvcare.com/what-is-uv-transmittance-uvt-and-why-is-it-important-to-know/

Table 5.3 Classification of water quality according to UVT%

UVT%	Comments on water quality/suitability for UV irradiation	References
95%	Excellent	USEPA, 1999b; Chen et al., 2006
85%	Good	USEPA, 1999b; Chen et al., 2006
75%	Fair	USEPA, 1999b; Chen et al., 2006
65%	Pretreatment before UV is advised; OR special UV reactor design requirements need to be considered, which include factors such as more powerful, and closer arranged, lamps, all chosen while considering the cost of design, and cost of operational tradeoffs	Chen et al., 2006; Muller & Lem, 2011
>65%	Typical guideline for effective UV disinfection at WWTPs	Bolyard et al., 2019

Findings related to UV treatment efficacy

Point source pollution from WWTP's are not the only contamination sources to consider, as pollutants in rivers can also include agricultural chemicals and pesticides, sewage, personal care products, and pharmaceutical residues (Spangenberg et al., 2021) that could all impact UV efficacy. Improving UVT% by the addition of pre-treatments is also a possibility, but it does inevitably add to the total cost of treatment and might also have addition environmental impacts. The only pre-treatment included as part of the pilot plant UV treatment done in this study was 5um bag filtration (chapter 4.3, Appendix D). It has however, been demonstrated in other research (Cantwell & Hoffmann, 2008) that UV disinfection of unfiltered surface waters, although partially inhibited, still lead to significant reductions in coliform levels.

In agreement with the findings of Catwell & Hoffmann (2008) significant reductions in microbial indicator levels were observed throughout this project, for UV doses up to 60 mJ.cm^2 in both the LP UV and the MP UV-based studies in spite of the varying UVT% levels observed. In addition, UV treatments could also successfully inactivate *Listeria monocytogenes* and *Salmonella* at the levels that they were present in the river water samples. Molecular detection of STEC also did not show any present after UV treatment. Laboratory studies on the UV susceptibility of pure *Salmonella* (Chapter 4.2) did reveal that it might be more prone to recovery post-UV than *L. monocytogenes*.

It has also been demonstrated that certain bacteria can survive and recover post-UV after the doses applied (20-60 mJ.cm^2) (Chapters 4.1-4.3). Identification and characterisation of the strains have revealed the presence of opportunistic pathogens and strains that carry a wide range of antimicrobial

resistance (AMR) determinants, even to critically important antibiotics (Chapter 4.1-4.4). The latter is a great concern, as this study provides further proof of the rapid spread of AMR within the South African aquatic environment.

The direct threat that surviving strains entering the fresh produce food chain holds for the consumers of fresh products does, however, depend on a variety of factors. These factors do, for instance, include the microbes' ability to attach and form biofilms in irrigation water distribution systems and on plant surfaces. This is an area that warrants further research within the South African setting.

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7. CAPACITY BUILDING AND PRODUCTS

List of students involved in this project.

1. **Caroline Rose Bursey** (MSc in Food Science – graduated March/April 2021)
   Thesis title: CHARACTERISING THE MICROBIAL PROFILES OF VARIOUS RIVER SOURCES AND INVESTIGATING THE EFFICACY OF UV RADIATION TO REDUCE MICROBIAL LOADS FOR IMPROVED CROP SAFETY
   Thesis abstract:
   The rivers used for the irrigation of fresh produce in the Western Cape have been under frequent investigation in recent years. Results have frequently shown that in rivers used for irrigation, the faecal coliform concentrations (*Escherichia coli*) frequently exceed the guideline limit of 1 000 colony forming units per 100 mL. These findings present a health risk for the consumers of fresh produce. Ultraviolet (UV) radiation treatment has been proven to offer some advantages for water disinfection over conventional treatment methods such as filtration and chemical treatments. However, is not a common practice yet in South Africa. Knowledge gaps exist with regard to the efficacy of UV radiation on environmental strains of pathogenic microorganisms such as *Salmonella* species and *Listeria monocytogenes*. The aim of this study was to investigate the effect of low-pressure (LP) UV radiation on water obtained from various river water sources, in order to disinfect water used for irrigation purposes to ultimately reduce the risk of contaminating the consumers of the fresh produce.

   Four rivers in the Western Cape were sampled five times each between the wet winter and dry summer seasons, to establish the microbial and physico-chemical profiles of the rivers. These results were compared to the guideline limits. The samples were exposed to three doses (20, 40 and 60 mJ.cm-2) of LP UV radiation at laboratory-scale. It was established that LP UV radiation was effective at reducing the microbial loads to non-detectable levels. Pathogenic microorganisms were successfully inactivated after a dose of 20 mJ.cm-2. Heterotrophic Plate Count colony numbers were lowered more steadily, and therefore, showed greater resistance to treatment. Thirteen strains were isolated and stored for future experiments. It was suggested that a pre-treatment step be implemented to improve the physical quality of the river water prior to treatment.

   The stored *L. monocytogenes* isolates (n = 8) were subjected to lineage typing experiments, where it was established that all isolates were lineage I. This lineage is most frequently associated with listeriosis. Extended-spectrum beta-lactamase (ESBL) testing indicated that none of the *Enterobacteriaceae* isolates (n=5) were ESBL-producers. All *Enterobacteriaceae* isolates showed resistance to tetracycline, ampicillin and trimethoprim-sulfamethoxazole. Resistance of *L. monocytogenes* isolates (n=5) was observed against trimethoprim-sulfamethoxazole, while four
   L. monocytogenes isolates showed resistance to ampicillin, penicillin and erythromycin. Multi-drug resistance was reported for 90% of river water isolates (n=9).

   Four different bag filter pore sizes (5, 20, 50 and 100 µm) were investigated to determine the most effective pre-treatment step to improve the UV transmission (UVT%) of the water. This experiment was performed on the 'worst case scenario' river, the Mosselbank River. Improvements in the total suspended solids, chemical oxygen demand and turbidity were reported, however, the extremely high total dissolved solids content (728.67 $mg.L^{-1}$) prevented a larger improvement in the UVT %. It was established that the 5 µm bag filter was the most effective pore size.

In the current study, LP UV radiation was successfully able to produce water of an acceptable standard for the irrigation of fresh produce. The physical quality of the water did not prevent a successful disinfection, but rather increased the exposure time required to deliver a specific dose and therefore, decreased efficiency. It was established that LP UV radiation is able to reduce pathogenic microorganisms to non-detectable levels. This method of disinfection, therefore, shows promise for full-scale application of irrigation water treatment.

2. **Marco Oosthuizen** (MSc in Food Science – graduated March/April 2022)
Thesis title: THE QUEST FOR SAFE IRRIGATION WATER: INVESTIGATING UV IRRADIATION TREATMENT OF RIVER WATER TO REDUCE MICROBIAL LOADS
Thesis 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. 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 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 were identified from the surviving populations. It was also observed that UV irradiation eliminated most STEC and ESBL-producing strains.

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 larger volumes 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.

3. **Corani Jankowitz** (MSc in Food Science – In process)
   Thesis title: SURVIVAL POTENTIAL OF FOOD PATHOGENS IN RIVER WATER AFTER UV-C IRRADIATION TREATMENT
   Thesis abstract:
   In process – Estimated graduation date: December 2023

4. **Margot Küster** (MSc in Food Science – In process)
   Preliminary thesis title (still to be finalised): THE IMPACT OF UV ON ANTIBIOTIC RESISTANT BACTERIA FROM RIVER WATER
   Thesis abstract:
   In process – Estimated graduation date: December 2024

8. SUPPLEMENTARY MATERIAL

8.1 APPENDIX A
8.2 APPENDIX B
8.3 APPENDIX C
8.4 APPENDIX D

8.1 APPENDIX A

Table A1 Results obtained with the ESBL testing procedures (EUCAST, 2021) of all *E. coli* isolates pre-UV treatment (Study 2) (Oosthuizen, 2022)

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

8.2 APPENDIX B

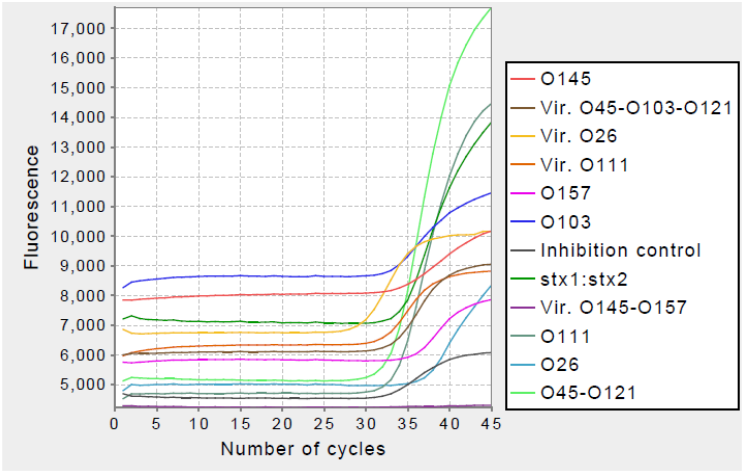


Fig. B1

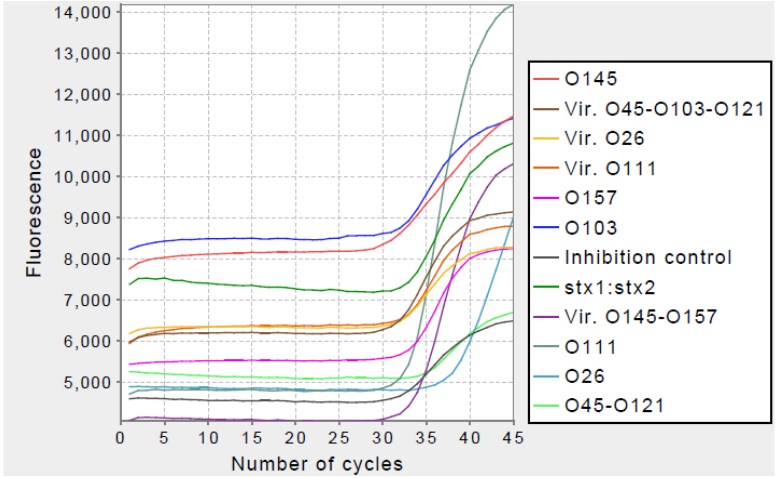


Fig. B2

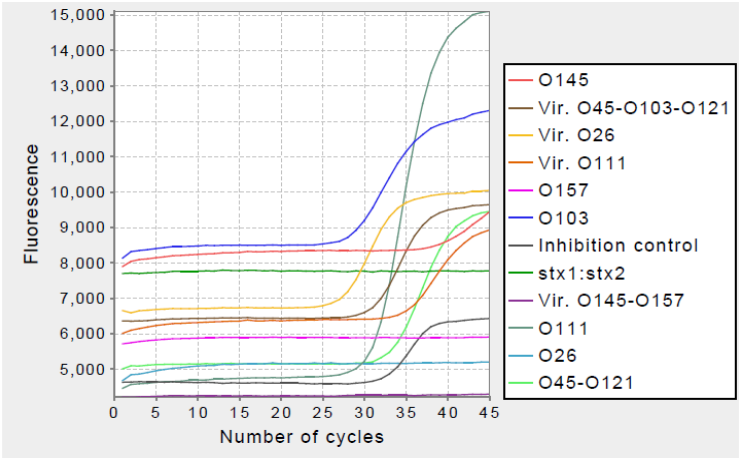


Fig. B3

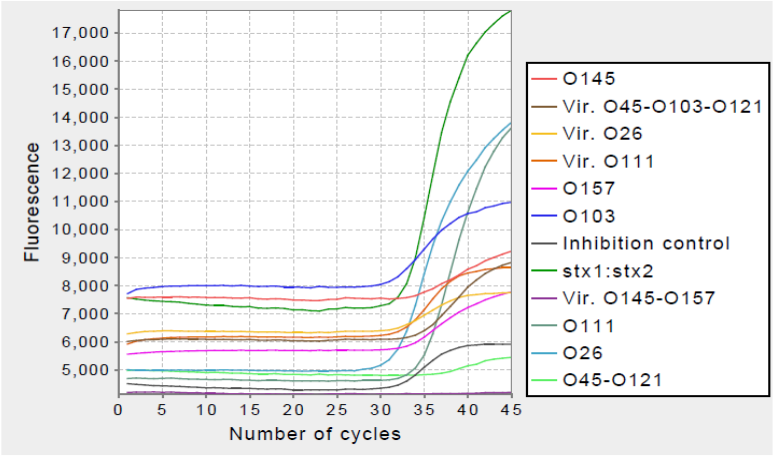


Fig. B4

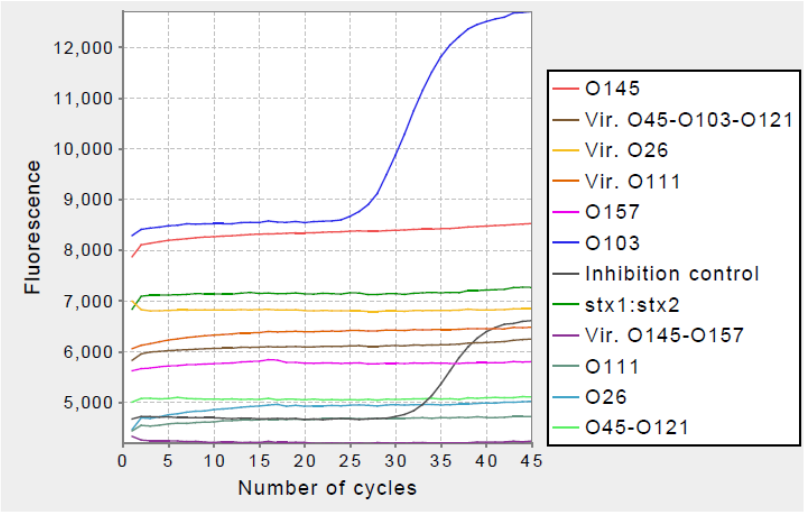
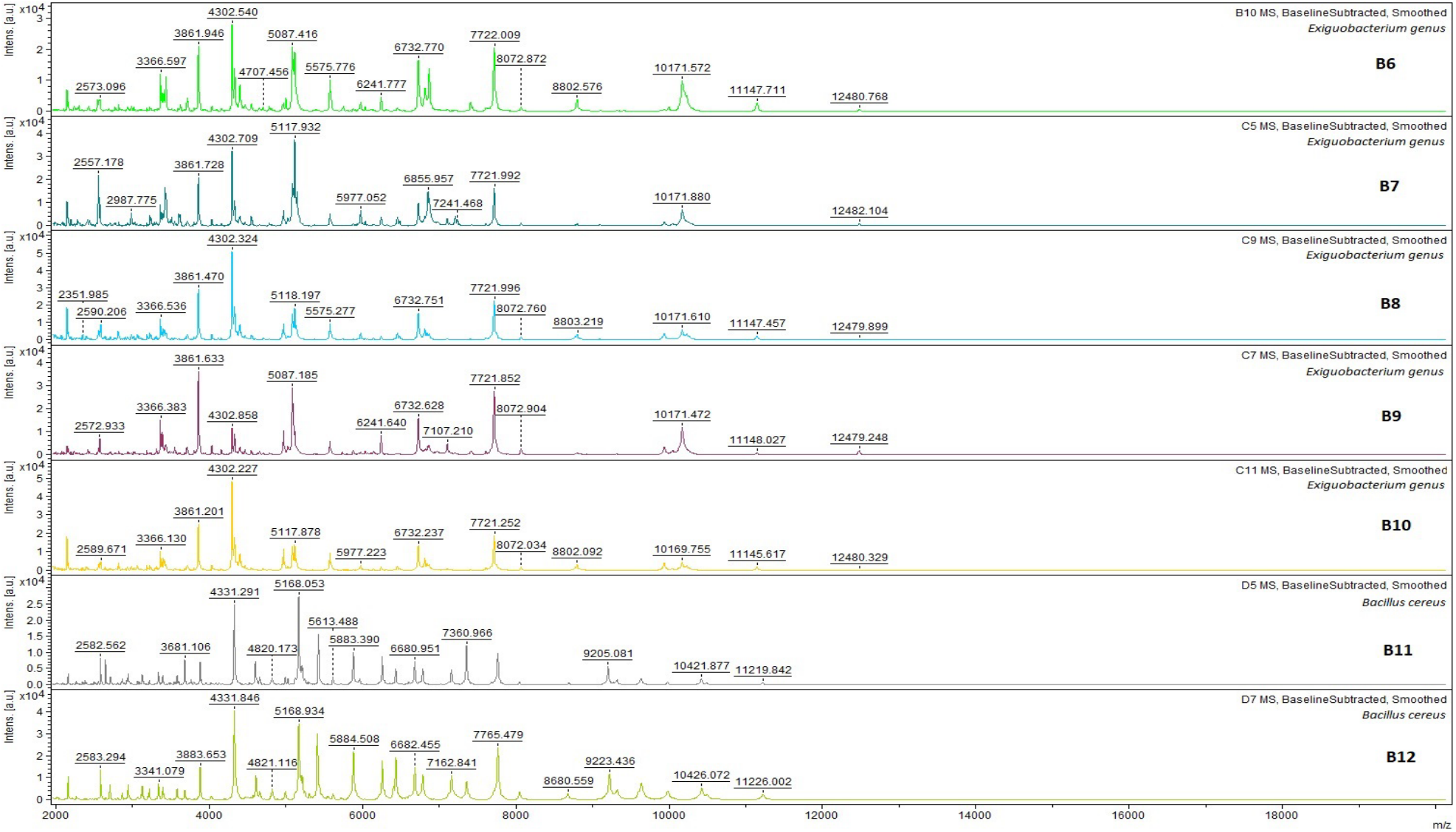


Fig. B5

Figures B1-B5 Representation of the spectra obtained from the Pall GeneDisc STEC Top 7 test results (Study 2) (Oosthuizen, 2022)


B10 MS, BaselineSubtracted, Smoothed
Exiguobacterium genus
B6
4302.540
3861.946
5087.416
6732.770
7722.009
3366.597
4707.456
5575.776
8072.872
2573.096
6241.777
8802.576
10171.572
11147.711
12480.768
C5 MS, BaselineSubtracted, Smoothed
Exiguobacterium genus
B7
5117.932
4302.709
2557.178
3861.728
6855.957
7721.992
2987.775
5977.052
7241.468
10171.880
12482.104
C9 MS, BaselineSubtracted, Smoothed
Exiguobacterium genus
B8
4302.324
3861.470
7721.996
2351.985
5118.197
6732.751
2590.206
3366.536
5575.277
8072.760
8803.219
10171.610
11147.457
12479.899
C7 MS, BaselineSubtracted, Smoothed
Exiguobacterium genus
B9
3861.633
5087.185
7721.852
3366.383
6732.628
8072.904
4302.858
2572.933
6241.640
7107.210
10171.472
11148.027
12479.248
C11 MS, BaselineSubtracted, Smoothed
Exiguobacterium genus
B10
4302.227
3861.201
5117.878
7721.252
3366.130
6732.237
2589.671
5977.223
8072.034
8802.092
10169.755
11145.617
12480.329
D5 MS, BaselineSubtracted, Smoothed
Bacillus cereus
B11
4331.291
5168.053
5613.488
5883.390
7360.966
2582.562
3681.106
4820.173
6680.951
9205.081
10421.877
11219.842
D7 MS, BaselineSubtracted, Smoothed
Bacillus cereus
B12
4331.846
5168.934
5884.508
6682.455
7765.479
2583.294
3883.653
3341.079
4821.116
7162.841
9223.436
8680.559
10426.072
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10000
12000
14000
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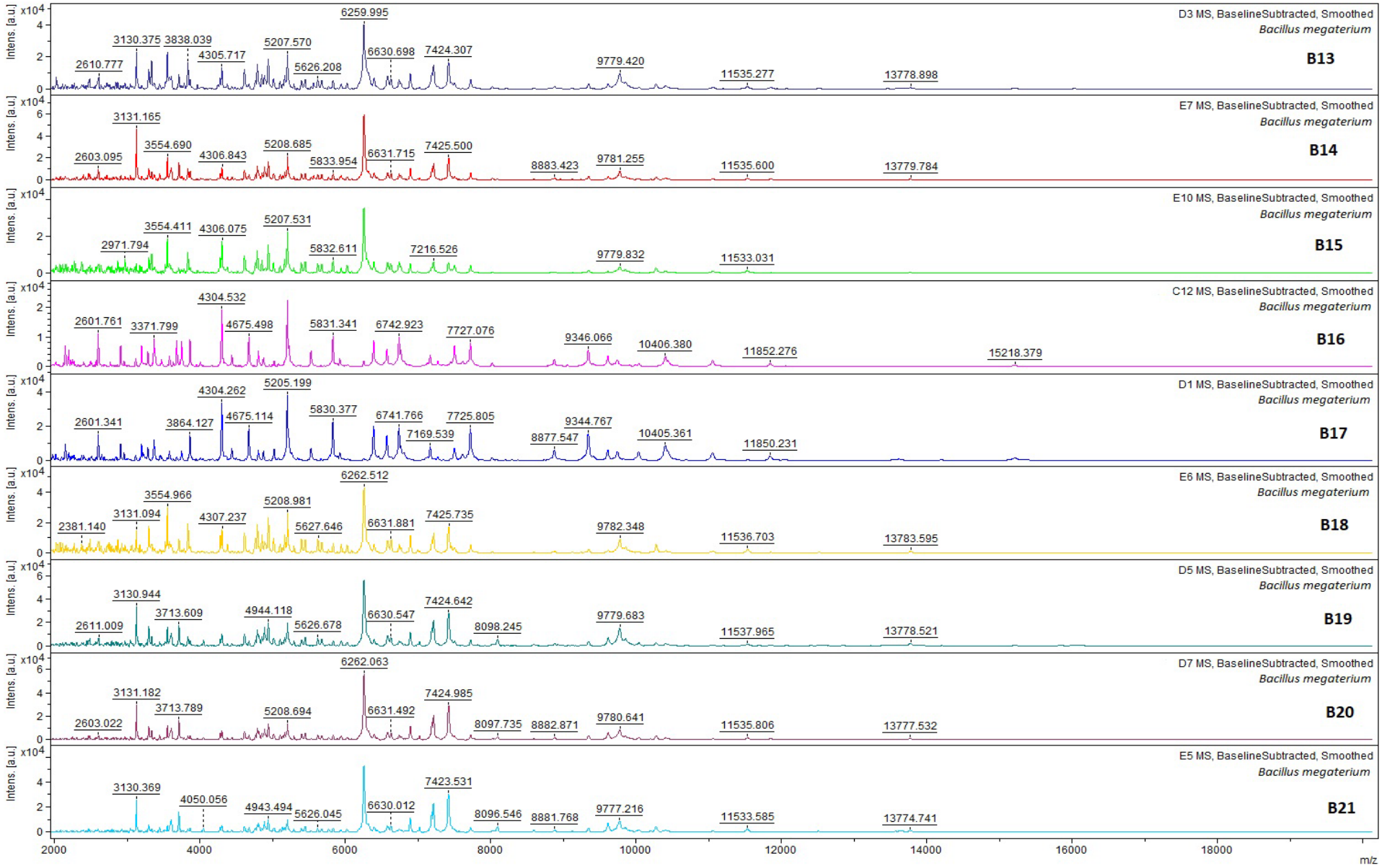
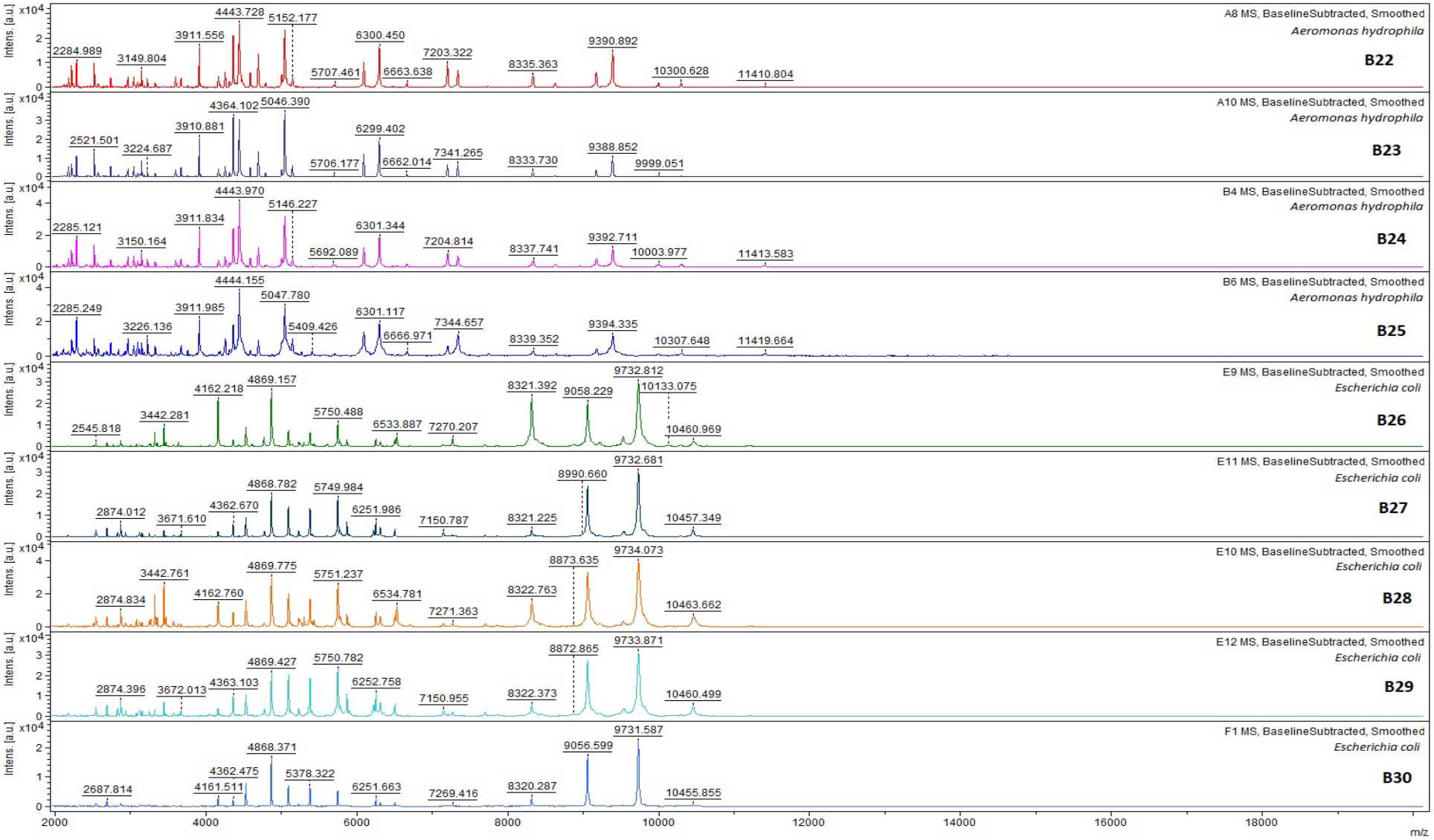
18000
m/z


D3 MS, BaselineSubtracted, Smoothed
Bacillus megaterium
B13
6259.995
3130.375
3838.039
5207.570
2610.777
4305.717
6630.698
7424.307
5626.208
9779.420
11535.277
13778.898
E7 MS, BaselineSubtracted, Smoothed
Bacillus megaterium
B14
3131.165
3554.690
5208.685
2603.095
4306.843
6631.715
7425.500
5833.954
8883.423
9781.255
11535.600
13779.784
E10 MS, BaselineSubtracted, Smoothed
Bacillus megaterium
B15
3554.411
4306.075
5207.531
2971.794
5832.611
7216.526
9779.832
11533.031
C12 MS, BaselineSubtracted, Smoothed
Bacillus megaterium
B16
4304.532
2601.761
3371.799
4675.498
5831.341
6742.923
7727.076
9346.066
10406.380
11852.276
15218.379
D1 MS, BaselineSubtracted, Smoothed
Bacillus megaterium
B17
4304.262
5205.199
2601.341
3864.127
4675.114
5830.377
6741.766
7725.805
9344.767
7169.539
8877.547
10405.361
11850.231
E6 MS, BaselineSubtracted, Smoothed
Bacillus megaterium
B18
6262.512
3554.966
3131.094
5208.981
2381.140
4307.237
5627.646
6631.881
7425.735
9782.348
11536.703
13783.595
D5 MS, BaselineSubtracted, Smoothed
Bacillus megaterium
B19
3130.944
3713.609
4944.118
2611.009
5626.678
6630.547
7424.642
8098.245
9779.683
11537.965
13778.521
D7 MS, BaselineSubtracted, Smoothed
Bacillus megaterium
B20
6262.063
3131.182
3713.789
5208.694
2603.022
6631.492
7424.985
8097.735
8882.871
9780.641
11535.806
13777.532
E5 MS, BaselineSubtracted, Smoothed
Bacillus megaterium
B21
3130.369
7423.531
4050.056
4943.494
5626.045
6630.012
8096.546
8881.768
9777.216
11533.585
13774.741
Intens. [a.u.]
x10^4
2000
4000
6000
8000
10000
12000
14000
16000
18000
m/z



Figures B6-B30 MALDI-TOF spectra of individual organisms detected during Study 2 (Oosthuizen, 2022

8.3 APPENDIX C: MALDI-TOF spectra of isolates **(Oosthuizen, 2021)**

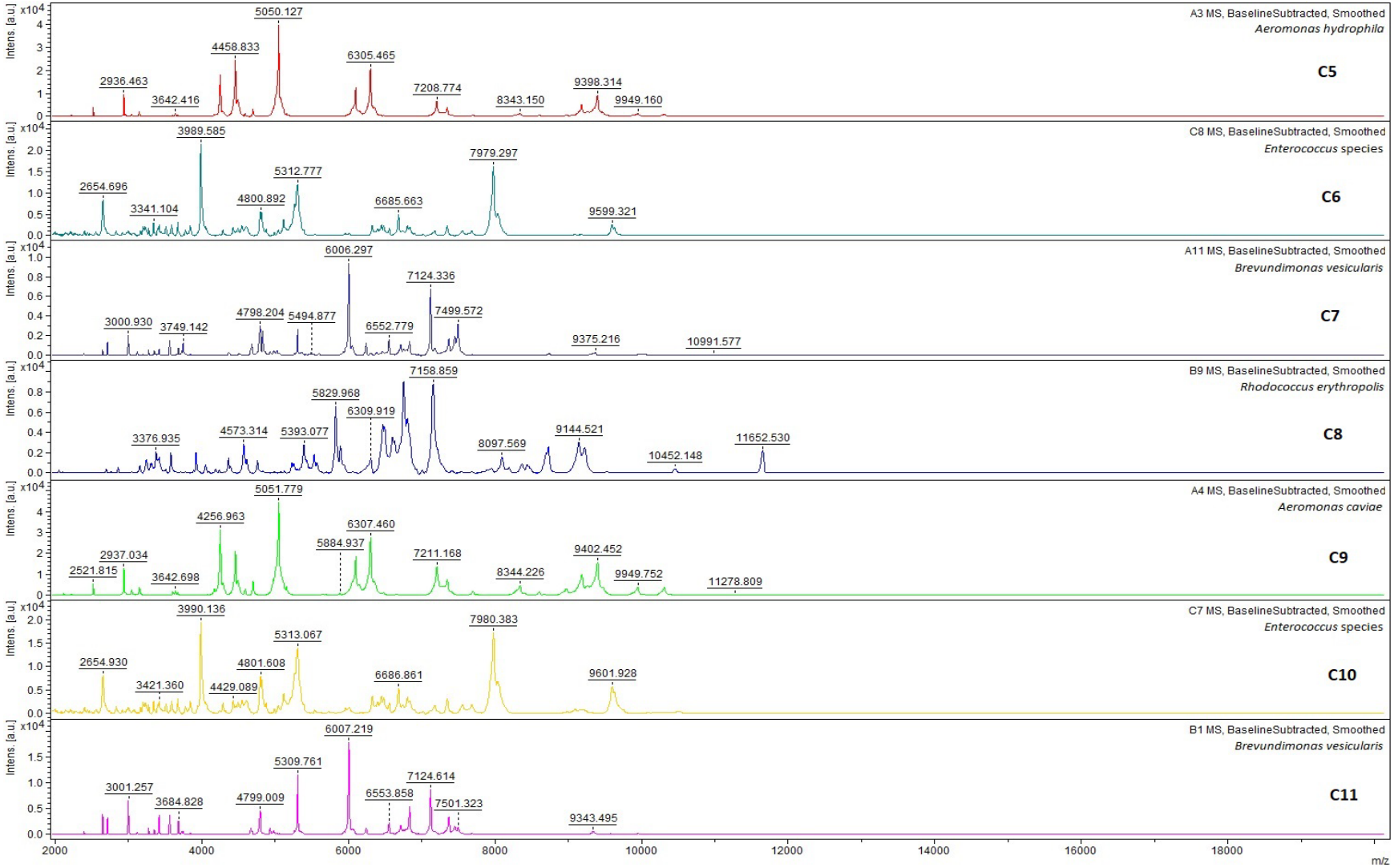


Figure C.1 MALDI-TOF spectra of individual organisms identified as part of Study 1 and listed in Table 4.3.4.

8.4 APPENDIX D : IMPACT OF BAG FILTRATION ON THE RIVER WATER CHARACTERISTICS PRIOR TO UV TREATMENT (Oosthuizen, 2021)

BACKGROUND

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). 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 (Figure D.1). A water sample was taken prior to bag filtration, as demonstrated in Fig D.1, 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 (Fig D.1). 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 1000L batch of water sampled. Three 1000L batches were collected from the Plankenburg river for this optimisation study. Physicochemical and microbial analysis of *E coli*, coliforms and HPC populations were done as described in the main report.

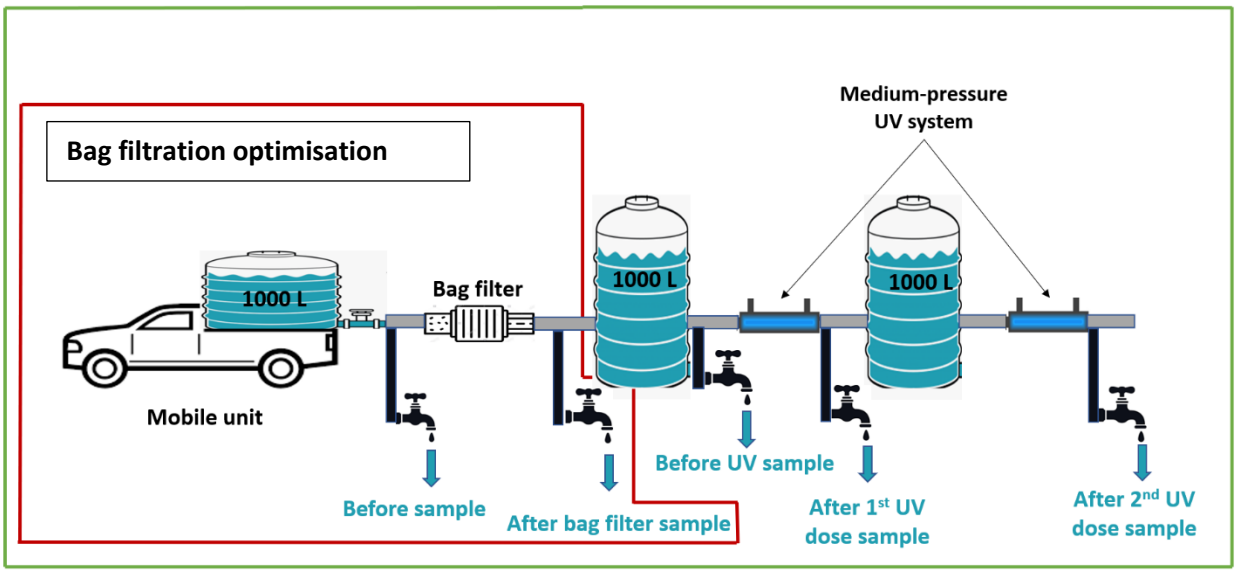


Figure D.1. Visual illustration of the pilot plant system that includes bag filtration and UV disinfection.

RESULTS (Appendix D)

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, as this river site has been indicated more than once in the previous studies as extremely polluted, with high TSS values and microbial loads.

The effects of filtration on the physico-chemical characteristics are presented in Table D.1. Furthermore, Figures D.2, D.3 and D.4 represent the microbial results obtained for the HPC, *E. coli* and coliforms, respectively, before and after filtration.

Table D.1 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 Augustus 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

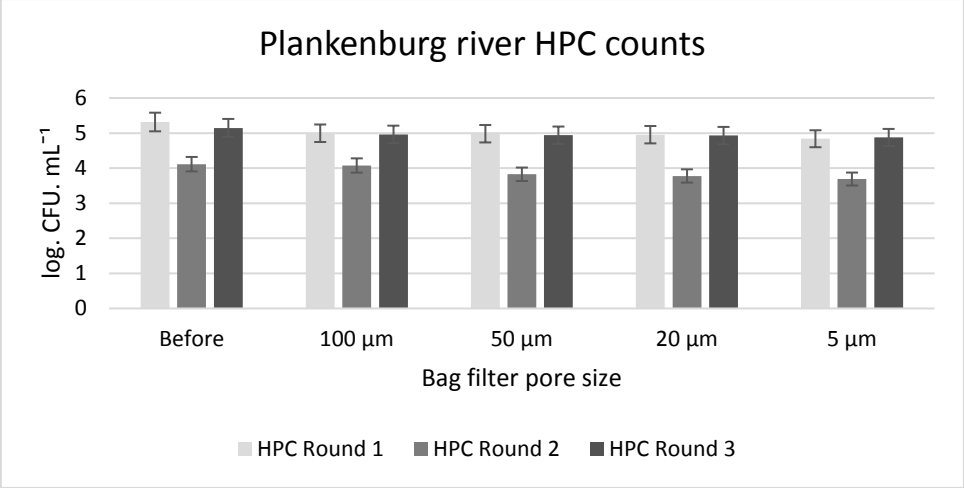


Figure D.2 HPC counts expressed in log CFU. mL before and after various bag filters, with error bars indicating standard deviation across thee sampling occasions.

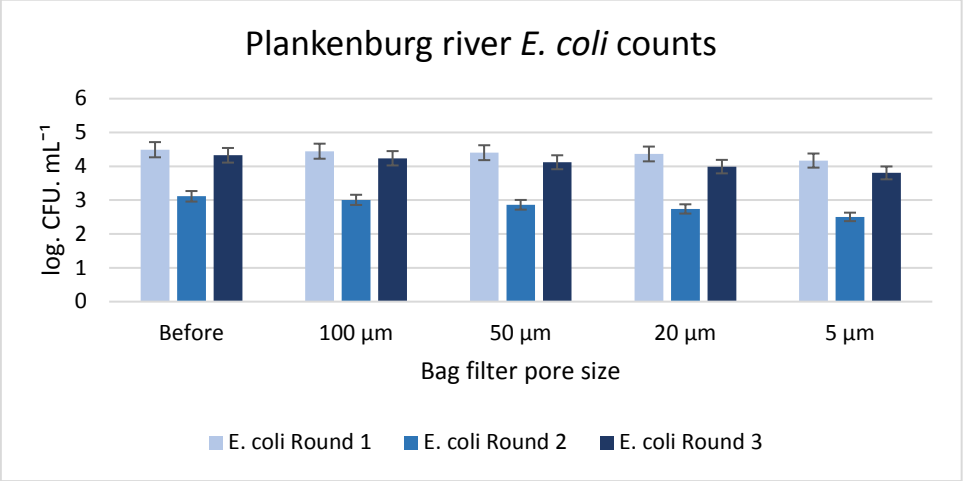


Figure D.3 *E. coli* counts expressed in log CFU. mL before and after various bag filters, with error bars indicating standard deviation across thee sampling occasions.

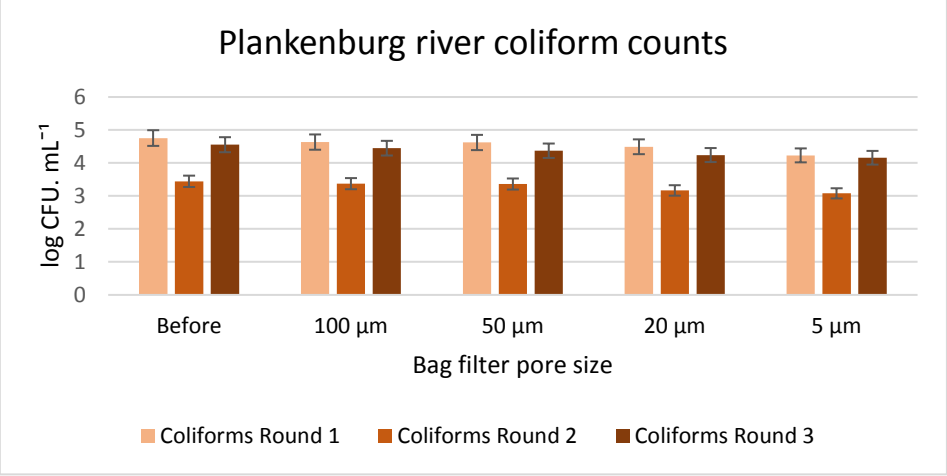


Figure D.4 Coliform counts expressed in log CFU. mL before and after various bag filters, with error bars indicating standard deviation across thee sampling occasions.

DISCUSSION (Appendix D)

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. 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 step before UV irradiation to improve water quality and subsequently, UV 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.

As seen in Table D.1, various physico-chemical characteristics were considered in this study. However, 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 with certain ions (Dong *et al.*, 2020). Gayán et al. (2012) reported that physico-chemical parameters such as UVT%, COD, TSS and TDS determine 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 before UV irradiation treatment is applied, varied significantly ($p<0.05$) between the three sampling rounds (Table D.1). 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 D.1), which could be the reason for the high levels of turbidity (129 NTU) observed (Table D.1). The flooding could also have caused the high TSS and COD values observed here after the first sampling occasion (Table D.1).

Considering the guideline limits, it should be noted that the Irrigation Water Guidelines (DWAF, 1996a) (Table 3.2, Chapter 3) 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 D.1 indicate that, overall, bag filtration does not have a significant effect on UVT%, as the average results for the 5 µm bag filter only indicated improvement of only 1.7%, when compared to the average 'before filtration' sample results (Table D.1).

With regards to the TDS content, the first sampling round showed an average 'before filtration' 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) (Table 3.2, Chapter 3). However, the second and third sampling occasions obtained average 'before' values of 354 and 311 mg. L^{-1} (Table D.1), 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 D.1). Furthermore, the high TDS values are in line with values measured for the Plankenburg river previously (previous progress reports submitted during 2020-2021). 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 4.3.1.

It was expected that the suspended solids content of the river water would be the most affected by bag filtration. The TSS results in Table D.1 indicated that this was indeed the case. 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 D.1). 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 D.1). When considering the 'before' bag filtration values obtained during sampling round one and three (Table D.1) both exceeded the Irrigation Water Guidelines (DWAF, 1996a) (Table 3.2, Chapter 3), which stipulate that only levels 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 filtration' sample and after the 100 µm bag filter (Table D.1). 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 following physico-chemical parameters: pH, EC, and alkalinity (Table D.1). 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 influence these parameters.

With regards to the microbial results (figures D.2, D.3 and D.4), it was expected that the bag filtration will have minimal effect on the initial microbial counts, as Ong et al. (2018) reported that 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 D.3, indicating that *E. coli* loads were not significantly reduced ($p>0.05$) by bag filtration. The slight decrease observed between the 'before filtration' sample and 5 µm bag filter sample (Fig. D3) 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 C.3 indicated an average 'before filtration' 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}.

When considering the initial microbial loads of the three rounds, it can be seen that water from the first sampling round had the highest loads (figures D.2, D.3 and D.4). 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. These results furthermore 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.D.3). 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) (Table 3.1, Chapter 3)

Overall, this study indicated that bag filtration did not improve physico-chemical and microbial characteristics of this river water source significantly (Table D.1). 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 (2020) also studied the same four bag filter options using water from the 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 D.1). During this study UVT% of the Plankenburg river water did not improve significantly after filtration (Table D.1). A similar observation was made by Bursey (2020), 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, 2020). Therefore, the bag filter of 5 µm was chosen as the pre-treatment option for the medium-pressure UV disinfection system used for the main 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 D.1), 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.