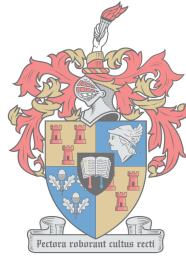


Sustainable Point-of-use Solar Disinfection System for Roof-Harvested Rainwater Treatment

by

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*Thesis presented in partial fulfilment of the requirements for the degree
Master of Science at Stellenbosch University*



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DECLARATION

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SUMMARY

Numerous countries worldwide, particularly in sub-Saharan Africa, are currently experiencing severe water shortages and drought conditions. Domestic rainwater harvesting (DRWH) has thus been earmarked as an alternative water source that could provide water directly to households. However, research has indicated that the microbial quality of rainwater is sub-standard and does not comply with drinking water standards as established by various water associations. It is thus recommended that roof-harvested rainwater should be treated prior to use for potable purposes. While the implementation of a first flush (FF) diverter as part of a DRWH system improves the microbial quality of roof-harvested rainwater, cost-effective primary treatment methods such as solar disinfection (SODIS) still need to be implemented on-site to significantly reduce the microbial load (**Chapter one**).

The primary aim of **Chapter two** was thus to design and construct a pilot-scale SODIS batch system fitted with a compound parabolic collector (CPC) which was (i) constructed from cost-effective materials; (ii) robust in nature in order to withstand adverse environmental conditions and (iii) required minimum maintenance. Two SODIS-CPC systems were constructed and connected to two separate rainwater harvesting tanks. One rainwater harvesting tank was utilised without pre-treatment, while the second tank was connected to a FF diverter. To determine the efficiency of the SODIS-CPC systems, the chemical (anion and cation concentrations as well as turbidity and water hardness) and the microbial quality [indicator organisms including *Escherichia coli* (*E. coli*), heterotrophic plate counts (HPC), enterococci, total and faecal coliforms] of untreated and SODIS treated rainwater samples were assessed during seven sampling events. In addition, the viable *Legionella* and *Pseudomonas* population present in the untreated and SODIS treated rainwater was determined using ethidium monoazide bromide quantitative polymerase chain reaction (EMA-qPCR) assays.

Chemical analysis indicated that both the anion and cation concentrations before [Tank 1 and Tank 2 (FF)] and after SODIS treatment [SODIS-CPC-1 and SODIS-CPC-2 (FF)] were within the drinking water standards as stipulated by various national and international water associations. In addition, the turbidity of all untreated and SODIS treated rainwater samples were within the aesthetic drinking water guideline, while the total water hardness of all samples were classified as soft. Microbial analysis further indicated that the microbiological quality of the untreated rainwater [Tank 1 and Tank 2 (FF)] was compromised as *E. coli*, HPC and total coliforms were detected at concentrations exceeding drinking water guidelines. However, after SODIS treatment, the *E. coli* and HPC were reduced to within the drinking water guidelines. In contrast, while total coliforms were reduced to within the drinking water guidelines during sampling sessions 1 to 4, counts exceeding the guidelines were obtained in the treated samples collected during sampling sessions 5 to 7 for both SODIS-CPC-1 and SODIS-CPC-2 (FF) systems. Moreover, viable *Legionella* spp. and *Pseudomonas* spp. were detected in the Tank 1 and Tank 2 (FF) rainwater samples. The copy numbers of these

organisms then decreased significantly ($p < 0.05$) after SODIS treatment in the SODIS-CPC-1 rainwater samples. However, while both *Legionella* spp. and *Pseudomonas* spp. copy numbers decreased after treatment in the SODIS-CPC-2 (FF) system, the decrease was not significant ($p = 0.195$). As results indicated that opportunistic pathogenic genera (*Legionella* spp. and *Pseudomonas* spp.) were still viable after SODIS treatment, the primary aim of **Chapter three** was to investigate the overall diversity and abundance of the viable bacterial community present in the Tank 1 rainwater and the SODIS-CPC-1 treated rainwater, using Illumina next generation sequencing coupled with EMA. Using this technique, the viable opportunistic pathogenic genera persisting after SODIS treatment in roof-harvested rainwater were also detected and identified.

After taxonomic assignments were performed, various α -diversity indices were utilised to investigate the diversity and abundance of the viable bacterial communities present in the untreated versus SODIS treated rainwater. Results indicated that there was a significant reduction ($p = 0.0033$) in species richness after SODIS treatment, indicating that the number of different species in SODIS-CPC-1 rainwater samples were less than in the Tank 1 rainwater samples. In addition, the Shannon diversity index significantly decreased ($p = 0.0107$) after SODIS treatment, indicating that the species in the SODIS-CPC-1 rainwater samples were less diverse than in the Tank 1 rainwater samples and that the treated rainwater samples were possibly dominated by a smaller group of viable bacteria. The β -diversity was further determined using the Bray-Curtis distance metric and permutational multivariate analysis of variance (PERMANOVA), whereafter results indicated that there was a significant ($p < 0.05$) shift in the viable bacterial community after SODIS treatment. Although the Nocardiaceae family and *Rhodococcus* genus dominated the Tank 1 (16.5 %) and SODIS-CPC-1 rainwater samples (44.0 %), the rest of the viable bacterial community differed. For example, Pseudomonadaceae (8.9 %) was the second most abundant family, followed by Sphingomonadaceae (6.0 %) in the Tank 1 rainwater samples. While in the SODIS-CPC-1 rainwater samples, Micrococcaceae (31.7 %) was the second most abundant family, followed by Oxalobacteraceae (5.0 %). Furthermore, signatures of opportunistic pathogenic genera were detected in both the Tank 1 and SODIS-CPC-1 rainwater samples. In addition, genera such as *Pseudomonas*, *Clostridium sensu stricto*, *Legionella*, *Mycobacterium* and *Yersinia*, amongst others, were detected in rainwater samples after SODIS treatment. It was thus hypothesised that the presence of these potential opportunistic pathogenic genera may be ascribed to debris, leaves, soil, dust and bird faecal matter which contaminated the catchment area either by anthropogenic activity or naturally through wind dispersion, etc.

Based on the results obtained in the current study, it is highly recommended that the catchment area is regularly cleaned, particularly before the rainy season commences and that a FF diverter is routinely installed as part of a RWH system. In addition, it is recommended that the SODIS treated rainwater should primarily be used for domestic purposes such as laundry, irrigation, car washing, etc.

OPSOMMING

Talle lande wêreldwyd, veral in sub-Sahara Afrika, word tans geteister deur ernstige watertekorte en droogtes. Huishoudelike reënwater-oesting (RWO) is dus ten toon gestel as 'n alternatiewe waterbron wat water direk aan huishoudings kan voorsien. Navorsing het egter aangedui dat die mikrobiële kwaliteit van reënwater nie voldoen aan die drinkwaterriglyne soos vasgestel deur verskeie waterverenigings nie. Daar word dus aanbeveel dat dak-opgevangte reënwater vooraf behandel moet word indien dit vir drink doeleindes aangewend word. Terwyl die implementering van 'n eerste spoel (ES) -afleier as deel van 'n RWO-stelsel die mikrobiële kwaliteit van dak-opgevangte reënwater verbeter, moet koste-effektiewe behandelingsmetodes soos sonlig-disinfeksie (SODIS) geïmplementeer word om die mikrobiële lading beduidend te verminder (**Hoofstuk een**).

Die doel van **hoofstuk twee** was dus om 'n kleinskaalse SODIS-stelsel te ontwerp en te bou wat toegerus is met 'n saamgestelde paraboliese versamelaar (SPV) wat (i) uit koste-effektiewe materiale gebou is; (ii) robuus van aard is om uiterse omgewingstoestande te weerstaan en (iii) wat minimum instandhouding vereis. Twee SODIS-SPV stelsels is gebou en gekoppel aan twee afsonderlike reënwater tenke. Een reënwater tenk is gebruik sonder voorafbehandeling, terwyl die tweede tenk aan 'n ES-afleier gekoppel was. Die effektiwiteit van die SODIS-SPV-stelsels was bepaal deur die chemiese (anioon- en kationkonsentrasies sowel as troebelheid en waterhardheid) en die mikrobiële kwaliteit [indikator organismes insluitend *Escherichia coli* (*E. coli*), heterotrofiëse plate tellings (HPT), enterococci, totale en fekale koliforme], van onbehandelde en SODIS-behandelde reënwater-monsters te meet, gedurende sewe monsternemingsessies. Daarbenewens was die aantal lewensvatbare *Legionella*- en *Pseudomonas* spp. wat in die onbehandelde en SODIS behandelde reënwater voorkom, bepaal deur gebruik te maak van etidium monoazied bromied kwantitatiewe polimerase kettingreaksie (EMB-kPKR) analises.

Chemiese analise het aangedui dat beide die anioon- en kationkonsentrasies voor [Tenk 1 en Tenk 2 (ES)] en na SODIS-behandeling [SODIS-SPV-1 en SODIS-SPV-2 (ES)] binne die drinkwaterriglyne was soos gestipuleer deur verskeie waterverenigings. Daaropvolgend het resultate getoon dat die troebelheid van alle onbehandelde en SODIS behandelde reënwater-monsters binne die estetiese drinkwaterriglyn was, terwyl die totale waterhardheid van alle monsters as 'sag' geklassifiseer is. Mikrobiële analises het verder aangedui dat die mikrobiologiese kwaliteit van die onbehandelde reënwater [Tenk 1 en Tenk 2 (ES)] ongeskik is vir drink doeleindes aangesien *E. coli*, HPT en totale koliforme opgespoor is by konsentrasies wat drinkwaterriglyne oorskry. Na die SODIS behandeling is *E. coli* en HPT egter verminder tot binne die drinkwaterstandaarde. In teenstelling, terwyl die totale koliforme verminder is tot binne die drinkwaterriglyne gedurende monsternemingsessies 1 tot 4, het die koliforme telling, van beide SODIS-SPV-1 en SODIS-SPV-2 (ES) sisteme gedurende monsterneming-sessies 5 tot 7, die drinkwaterriglyne oorskry. Verder was lewensvatbare *Legionella* en *Pseudomonas* spp. opgespoor in Tenk 1 en Tenk 2 (ES) reënwater-monsters.

Die kopie-getalle van hierdie organismes het beduidend afgeneem ($p < 0.05$) na SODIS behandeling in die SODIS-SPV-1 reënwater-monsters. Alhoewel beide *Legionella* en *Pseudomonas* kopie getalle afgeneem het na SODIS behandeling in die SODIS-SPV-2 (ES) stelsel, was hierdie afname nie beduidend nie ($p = 0.195$). Aangesien resultate daarop dui dat opportunistiese patogeniese genera (*Legionella* spp. en *Pseudomonas* spp.) nog steeds lewensvatbaar was na SODIS behandeling, was die doel van **Hoofstuk drie** om die algehele diversiteit en oorvloedigheid van die totale lewensvatbare bakteriese gemeenskap wat in beide Tenk 1 en SODIS-SPV-1 voorkom, te bepaal deur gebruik te maak van Illumina volgende generasie volgordebepaling gekoppel aan EMA. Met behulp van hierdie tegniek, is die lewensvatbare patogeniese en opportunistiese patogeniese genera wat voort leef na SODIS-behandeling van dak-opgevangre reënwater, ook opgespoor en geïdentifiseer.

Taksonomiese analyses was uitgevoer deur verskeie α -diversiteitsindekse te gebruik om die diversiteit en oorvloedigheid van die lewensvatbare bakteriese gemeenskap, teenwoordig in die onbehandelde sowel as die SODIS-behandelde reënwater, te ondersoek. Resultate het aangedui dat daar 'n beduidende afname ($p = 0.0033$) was in spesierikheid na SODIS behandeling, wat daarop dui dat die aantal verskillende spesies in SODIS-SPV-1 reënwater-monsters minder was as in die Tenk 1 reënwater-monsters. Daaropvolglik, het die Shannon-diversiteitsindeks beduidend afgeneem ($p = 0.0107$) na SODIS-behandeling wat aandui dat die spesies in die SODIS-SPV-1 reënwater-monsters minder divers was as in die Tenk 1 reënwater-monsters en dat die behandelde reënwater-monsters moontlik gedomineer is deur 'n kleiner groep lewensvatbare bakterieë. Die β -diversiteit is verder bepaal met behulp van die Bray-Curtis-afstandmatriks en permutatiewe multi-variante ontleding van variansie wat aangedui het dat daar 'n beduidende ($p < 0.05$) verandering in die lewensvatbare bakteriese gemeenskap na SODIS-behandeling was. Alhoewel die Nocardiaceae familie en die *Rhodococcus* genus die Tenk 1 (16.5 %) en SODIS-SPV-1 reënwater-monsters (44.0 %) oorheers het, was daar 'n verskil in die res van die lewensvatbare bakteriese gemeenskap. Byvoorbeeld, Pseudomonadaceae (8.9 %) was die tweede oorvloedigste familie, gevolg deur Sphingomonadaceae (6.0 %) in die Tenk 1 reënwater-monsters. In die SODIS-SPV-1 reënwater-monsters was Micrococcaceae (31.7 %) die tweede oorvloedigste familie, gevolg deur Oxalobacteraceae (5.0 %). Verder is opportunistiese patogeniese genera in beide Tenk 1 en SODIS-SPV-1 reënwater-monsters opgespoor. Daarbenewens was genera soos *Pseudomonas*, *Clostridium sensu stricto*, *Legionella*, *Mycobacterium* en *Yersinia*, onder andere, in die reënwater-monsters aangetref na SODIS-behandeling. Die teenwoordigheid van hierdie opportunistiese patogeniese genera kan moontlik toegeskryf word aan blare, stof en voëlfekale afval wat die opvanggebied besoedel, moontlik a.g.v. menslike aktiwiteite of natuurlik deur windverspreiding, ens.

Na afloop van die huidige studie, word dit sterk aanbeveel dat die opvanggebied gereeld skoongemaak word, veral voor die reënseisoen begin, en dat 'n ES-afgeleier geïnstalleer moet word as deel van die RWO-stelsel. Verder word dit aanbeveel dat die SODIS behandelde reënwater hoofsaaklik vir huishoudelike doeleindes gebruik word, soos wasgoed, besproeiing, motorwas, ens.

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LIST OF ABBREVIATIONS AND ACRONYMS

ADWG	Australian Drinking Water Guidelines	PVC	Polyvinyl Chloride
ATP	Adenosine Triphosphate	qPCR	Quantitative or Real-Time Polymerase Chain Reaction
BDL	Below Detection Limit	r²	Correlation Coefficient
BLAST	Basic Local Alignment Search Tool	R²	Regression Coefficient
CAF	Central Analytical Facility	R2A	Reasoner's 2 Agar
CDC	Centres for Disease Control and Prevention	RELMA	Regional Land Management Unit
CFU	Colony Forming Units	RDP	Ribosomal Database Project
CPC	Compound Parabolic Collector	RNA	Ribonucleic Acid
DNA	Deoxyribonucleic Acids	ROS	Reactive Oxygen Species
dNTP's	Deoxyribonucleotide Triphosphate	rRNA	Ribosomal Ribonucleic Acid
DNI	Direct Normal Irradiance	RWH	Rainwater Harvesting
DRWH	Domestic Rainwater Harvesting	SABS	South African Bureau of Standards
DWA	Department of Water Affairs	SANS	South African National Standards
DWAF	Department of Water Affairs and Forestry	SDG	Sustainable Development Goals
ELISA	Enzyme-linked Immunosorbent Assays	SFIEST	Swiss Federal Institute for Environmental Science and Technology
EMA	Ethidium Monoazide Bromide	SIDA	Swedish International Development Cooperation Agency
EMA-qPCR	Ethidium Monoazide Bromide Quantitative Polymerase Chain Reaction	SODIS	Solar Disinfection
FF	First Flush	SOPAS	Solar Pasteurization
HPC	Heterotrophic Plate Count	UK	United Kingdom
LLOD	Lower Limit of Detection	UN	United Nations
MDG	Millennium Development Goals	UNEP	United Nations Environment Programme
NCBI	National Centre for Biotechnology Information	UNICEF	United Nations International Children's Emergency Fund
NHMRC	National Health and Medical Research Council	US	United States
NRMMC	Natural Resource Management Ministerial Council	USA	United States of America
NTU	Nephelometric Turbidity Unit	USEPA	United States Environmental Protection Agency
PCR	Polymerase Chain Reaction	UV	Ultra-violet
PET	Polyethylene Terephthalate	WHO	World Health Organisation
PMA	Propidium Monoazide		

Chapter 1: Literature Review

(UK spelling is employed)

1.1. Introduction

In 2015 it was estimated that globally, 663 million people lacked access to a safe drinking water source and 1.8 billion people used drinking water contaminated by faecal matter [United Nations (UN), 2015a]. As a result, it is postulated that approximately 800 children die each day due to water- and sanitation-related diarrhoeal diseases, most of which are preventable. The Sustainable Development Goals (SDG) were thus assembled in 2015, to succeed the Millennium Development Goals (MDG). The SDG specified 17 goals of which the sixth was to ensure global access to clean water and sanitation services by 2030. Eight primary targets intrinsic to the sixth goal were identified. These included, amongst others: (i) achieving access to safe and affordable drinking water, sanitation and hygiene; (ii) improving water quality by reducing pollution, eliminating dumping and minimising the release of hazardous chemicals and materials into water sources; (iii) halving the proportion of untreated wastewater, substantially increasing water recycling and safe reuse globally and, (iv) increasing water-use efficiency across all sectors. If achieved, all these targets should reduce substantially the number of people adversely affected by water scarcity and inferior water quality (UN, 2015b).

To attain the targets of the sixth SDG goal, many countries are adopting strategies to achieve equitable access to safe drinking water sources. Domestic rainwater harvesting (DRWH) has previously been described as an alternative and sustainable water source that could provide water directly to households (Amin & Han, 2009; De Kwaadsteniet et al. 2013). This method of harvesting refers to the catchment and storage of rainwater from rooftops and diverse surfaces during a rain event (De Kwaadsteniet et al. 2013). Furthermore, South Africa, especially the Western Cape region, is currently experiencing a severe drought and on 6 Desember 2017, catchment dams in the latter province were estimated to have a combined residual water capacity of only 35.1 % (City of Cape Town, 2017). As a result of the ongoing and future droughts, the Department of Water and Sanitation has earmarked rainwater harvesting (RWH) as a sustainable means of providing households with a direct alternative water source (Mwenge Kahinda & Taigbenu, 2011).

Although rainwater is considered a pure water source, undesirable microbial and chemical contamination often occurs during the harvesting process (Abbasi & Abbasi, 2011). For example, rainwater can become contaminated when the rain traverses the air. This is due to the presence of airborne microorganisms and particles (heavy metals and dust). Further contamination can occur when rain flows over a catchment area where faecal matter (which may contain chemicals such as phosphorous, nitrogen and trace elements) and/or organic debris have accumulated (Helmreich & Horn, 2009; Abbasi & Abbasi, 2011; De Kwaadsteniet et al. 2013). Numerous studies have thus reported that various microorganisms including bacteria [virulent *Escherichia coli* (*E. coli*) and *Legionella* spp.], viruses (adenovirus) and protozoa (*Cryptosporidium* spp.) are major contaminants of harvested rainwater systems (Helmreich & Horn, 2009; Dobrowsky et al. 2014a; 2014b).

Pseudomonas is one of the primary bacteria identified in harvested rainwater and various species are associated with diseases such as bacteraemia, endocarditis, osteomyelitis, gastrointestinal infections, urinary tract infections and septicaemia (Lyczak et al. 2000; Giamarellou, 2002; Mena & Gerba, 2009). Nosocomial infections in individuals with vulnerable immune systems have also been ascribed to *Pseudomonas* (Giamarellou, 2002). *Legionella* is a well-known waterborne opportunistic pathogen and has also been detected in various water sources, including harvested rainwater (Simmons et al. 2001; Dobrowsky et al. 2014b; 2015; Reyneke et al. 2016). *Legionella* causes Legionellosis and once contaminated water droplets are inhaled by humans, Legionnaires' disease (an acute type of pneumonia) or Pontiac fever (mild non-pneumonic illness) can occur [World Health Organisation (WHO), 2007].

Accordingly, the microbial quality of harvested rainwater does not adhere to the minimum requirements stipulated by the Department of Water Affairs and Forestry (DWAF) (DWAF, 1996) and the WHO (WHO, 2011) and it is therefore not suitable for potable purposes. It is thus essential that roof-harvested rainwater should be treated to render it microbiologically safe as a primary source of drinking water (Ahmed et al. 2012; Huston et al. 2012; Adler et al. 2014; Dobrowsky et al. 2014a). Solar disinfection (SODIS) is recognised as an efficient, cost-effective method to reduce microbial loading in contaminated water sources. This treatment method inactivates microorganisms through the synergistic effect of ultra-violet (UV) radiation and solar mild-heat (Amin & Han, 2009; McGuigan et al. 2012; Amin et al. 2014).

An example of a simple SODIS system is the use of a 2 to 5 L transparent container [polyethylene terephthalate (PET) bottle] filled with contaminated water, which is continuously exposed to direct sunlight for at least six to eight hours (Safapour & Metcalf, 1999). While research has indicated that the microbial quality of the water source is substantially improved after exposure to SODIS, a disadvantage of the method is that only limited volumes of water can be treated at a given time. The UN recommends a volume of 25 L water per person per day (UN, 2010). Thus the volume of water produced by a simple SODIS system may not be sufficient to meet the daily potable water demands of a household. Hence, while contemporary small-volume SODIS systems yield good microbial inactivation efficiencies, optimisation of this treatment method is required in order to generate larger volumes of water of acceptable microbial standard.

The primary aim of the current study was therefore to design a SODIS system, which was robust, cost-effective, of low maintenance and easy to implement, with a high efficiency to treat sufficient quantities of rainwater for potable purposes. This aim was achieved by designing a SODIS system connected to a compound parabolic collector (CPC) which functioned to concentrate solar irradiation onto the primary reactor. After designing and constructing the system, a pilot scale study was carried out to monitor and compare the overall quality of treated water. Two SODIS systems were installed at the Welgevallen Experimental Farm (Stellenbosch University). The first system was connected to a previously installed rainwater harvesting tank, while the second system was attached to a rainwater

harvesting tank coupled to a first flush (FF) diverter. The microbial and chemical quality of the water was routinely monitored before and after treatment of up to a maximum of eight hours of continuous sunlight exposure.

Moreover, a recent study conducted by Strauss et al. (2016) demonstrated the presence of viable *Pseudomonas* spp. and *Legionella* spp. in rainwater after solar pasteurization (SOPAS) (Phungamanzi™ system) and SODIS (2 L PET bottles placed in a solar cooker) treatment, respectively. Ethidium monoazide bromide quantitative polymerase chain reaction (EMA-qPCR) analysis was thus performed to analyse the efficiency of the SODIS-CPC treatment systems designed and used in the current study to reduce the level of viable *Pseudomonas* and *Legionella* spp. present in harvested rainwater. Ethidium monoazide bromide (EMA) is a nucleic acid binding dye that can be used to bind to the deoxyribonucleic acid (DNA) of microbial cells (after photoactivation) with damaged and/or permeable membranes (non-viable cells). The binding of the dye to DNA prevents PCR amplification of the DNA and thereby leads to a strong signal reduction during qPCR as only the DNA from intact (viable) cells will be amplified. Additionally, EMA was used in combination with the Illumina next generation sequencing platform to identify the viable bacterial population (culturable and non-culturable) present in the SODIS treated and untreated harvested rainwater.

1.2. Domestic rainwater harvesting

One millimetre of rain falling onto a surface of one square metre yields one litre of harvested rainwater (Helmreich & Horn, 2009), thus offering a sustainable source of fresh water to a community. A minimal capital investment is also required for the installation of a rainwater harvesting system in comparison to the municipal pipeline systems, which are conventionally used to supply water for agricultural, industrial and domestic purposes. Rainwater harvesting systems have been implemented and are functioning all over the world, including in Asia (e.g. Japan and Philippines), Australia (e.g. Australia and New Zealand), Europe (e.g. Denmark and Germany), United Kingdom (e.g. Ireland), North America (e.g. Canada), South America (e.g. Brazil) and Africa (e.g. Kenya and South Africa) (Uba & Aghogho, 2000; Albrechtsen, 2002; Li et al. 2010; Ahmed et al. 2011; Mwenge Kahinda & Taigbenu, 2011; Global Development Research Centre, 2017). In developed countries such as Australia, France, New Zealand and the United Kingdom, rainwater harvesting is promoted by the government by the use of subsidies and tax incentives to encourage households to use rainwater as an alternative water source. For example, in Australia, the Queensland government offered “WaterWise Rebates” to households across the state for a number of water saving devices including rainwater harvesting systems. An estimated 260 000 households enrolled in the scheme within the first two years of implementation.

On the African continent, numerous countries including Botswana, Kenya, Ghana, Ethiopia, Namibia, Malawi and South Africa have rainwater harvesting projects operating to supply

households with water (Mwenge Kahinda & Taigbenu, 2011). For example, a combined study conducted by the Swedish International Development Cooperation agency (SIDA) and Regional Land Management Unit (RELMA) assisted individuals in Kenya and Ethiopia with the development of methods to enable storage of rainwater for domestic purposes (Nega & Kimeu, 2002). In Namibia, Baker et al. (2007) initiated a project which focused on the implementation of cost-effective rainwater harvesting systems constructed from materials such as plastic sheeting and steel drums for the provision of a supplementary water source.

As rainwater harvesting requires minimal infrastructural changes in a community, it has also been earmarked by the South African government as an effective strategy, particularly for rural and urban informal settlements, for obtaining water for daily household usage (Mwenge Kahinda & Taigbenu, 2011). Currently domestic rainwater harvesting systems are distributed across all nine provinces of South Africa, especially in the Eastern Cape and KwaZulu-Natal, where households use roof-harvested rainwater as a primary water source. It was estimated that in 2011, 26 500 households in South Africa used domestic rainwater harvesting as their primary water source. By 2016, this number had increased to approximately 69 746 (Malema et al. 2016) (**Figure 1.1**).

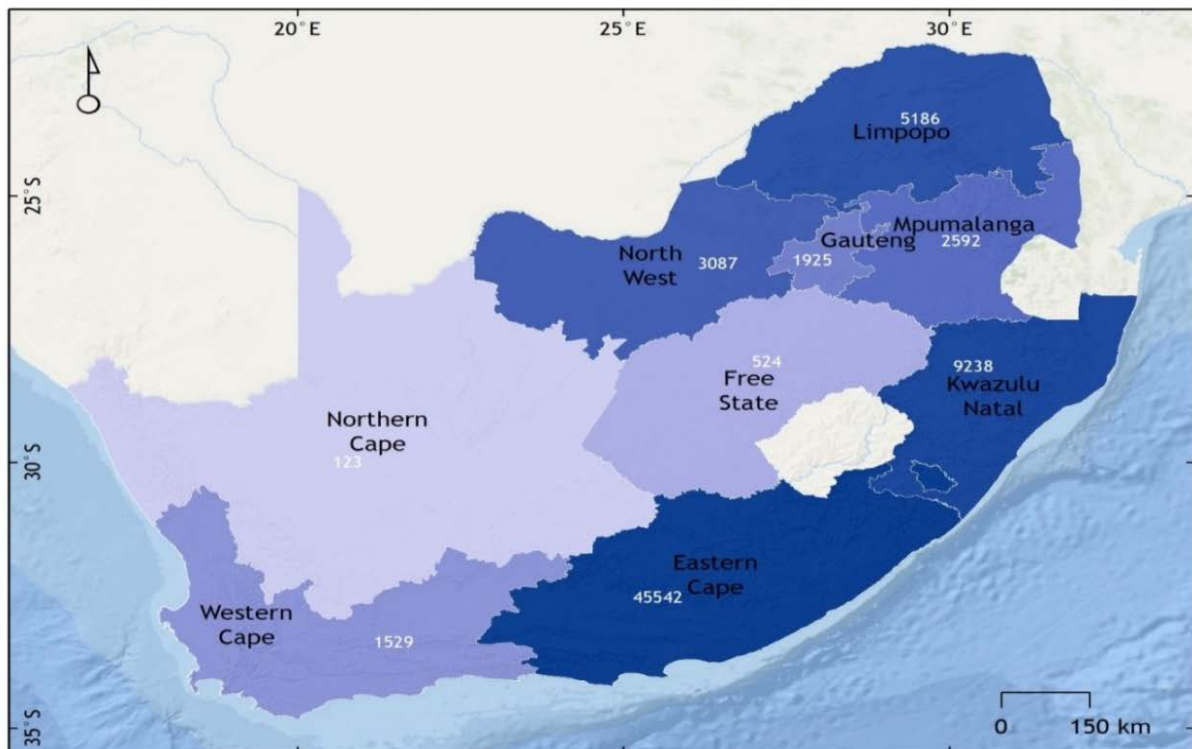


Figure 1.1: Distribution of the number of households using domestic rainwater harvesting systems as their primary water source in the nine provinces of South Africa (adopted from Malema et al. 2016).

Most of the rainwater harvesting projects initiated in South Africa form part of green initiatives or community development projects, all aiming to provide a sustainable quality of living for disadvantaged people. For example, a rainwater harvesting project was launched in Botlhabelo village development, where 520 housing units were constructed with a rainwater harvesting system

installed adjacent to each unit (Social Housing Foundation, 2010). Similarly, in the Cato Manor Development project, 27 rainwater harvesting tanks were installed near domestic residences (Naidoo, 2011; Botes, 2012). The Breaking New Ground project was also launched in the peri-urban coastal town of Kleinmond, South Africa, where 411 houses were constructed. Amongst other sustainable initiatives, each house was provided with a 2 000 L rainwater harvesting tank.

1.2.1. General characteristics of domestic rainwater harvesting systems

Three major categories of rainwater harvesting systems exist viz. in situ, external and domestic rainwater harvesting. In situ rainwater harvesting refers to practices where the catchment and storage areas are situated in low topographic depressions and the rainwater is used on site primarily for irrigation purposes (Ibraimo & Munguambe, 2007). External rainwater harvesting refers to the collection and storage of surface runoff rainwater off-site in a constructed tank, while domestic rainwater harvesting generally refers to the collection of rainwater from diverse surfaces during a rain event and the subsequent storage of the captured rain in an underground or above ground harvesting tank (Helmreich & Horn, 2009). The rainwater collected during in situ and external rainwater harvesting is generally suitable for agricultural purposes, while rainwater captured during the domestic rainwater harvesting process can be used for daily household activities such as cooking, sanitation and laundry (Helmreich & Horn, 2009). A simple domestic rainwater harvesting system consists of a catchment area, a conveyance system (gutter pipes) and a storage tank (Amin & Han, 2009; Helmreich & Horn, 2009; Amin et al. 2014).

1.2.1.1. Catchment area

The catchment area is the largest component of a rainwater harvesting system and usually refers to the rooftops of houses. For an optimal catchment area, a roof must be designed with a steep slope to ensure a high runoff coefficient, which is required for rainwater harvesting (Li et al. 2010). The runoff coefficient refers to the ratio of the volume of water that flows over an area versus the volume of water that falls onto that specific area. It is known that a well-designed roof with a runoff coefficient of 0.7 to 0.9 is required for optimal rainwater harvesting (Gould & Nissen-Petersen, 1999). In order to harvest the maximum rainwater volume, the catchment surface area should also be expansive (Abdulla & Al-Shareef, 2009). However, catchment systems with larger surface areas may contribute to an increased contamination load in the rainwater entering a storage tank. Hence, when constructing the catchment area, the material used must be taken into consideration, as the surface and type of roofing material can reduce contamination [United Nations Environment Programme (UNEP), 2016]. Smooth, impermeable and superior materials, such as corrugated plastic, galvanised iron sheets and tiles are preferred as roof catchment surfaces (Li et al. 2010). Flat cement or felt-covered roofs can also be used, provided they are regularly cleaned (Gould & Nissen-Petersen, 1999).

The materials commonly used for rooftop catchment construction include clay tiles, concrete, aluminium and galvanised metal sheets, polycarbonate plastic, flat gravel roofs, thatch (raffia palm, leaves or grass), polyethylene plastics and asbestos (Uba & Aghogho, 2000; Handia et al. 2003; Gwenzi et al. 2015). In South Africa, tiles, corrugated plastic, galvanised and corrugated iron sheets are frequently used by households to construct the roofing system as these materials are readily available, show suitable durability and are of moderate cost (Enniful, 2013). An important consideration for metal roofing systems is that chemicals may leach from the material and wash into the rainwater harvesting tank during a rain event (UNEP, 2016). While unpainted and uncoated roof surfaces are the preferred option (Li et al. 2010), non-toxic painted or coated roofs can also be used for rainwater harvesting. However, caution must be exercised when utilising painted or coated roofing systems, as flakes of paint or coating can wash into the tank through the conveyance system.

1.2.1.2. Conveyance system

The conveyance system connects the catchment area to the storage tank and consists of gutters and downpipes. A well-designed and maintained conveyance system is capable of diverting over 90 % of the rainwater runoff into the storage tank. However, the realistic collection efficiency is usually between 80 % and 90 % (Li et al. 2010). Fibreglass, stainless steel, galvanised steel and polyethylene are the most common materials used for the construction of gutters, however polyethylene is considered the most effective as rainwater can have a low pH (acidic), thus causing corrosion and mobilisation of metals when metal pipes are used (UNEP, 2016). In South Africa, conveyance systems constructed from plastic (polyethylene) gutters are used extensively as this material is considered the most cost-effective option and exhibits good durability (Marley Pipesystems, 2016). Gutters are generally suspended from the eaves and slope towards the downpipes. Semi-circular gutters have been recognised as the most efficient for conveying water from the catchment area to the storage tank (Li et al. 2010). In a study conducted by Gould and Nissen-Petersen (1999), it was demonstrated that a gutter cross-sectional area of 1 cm² was required for each 1 m² of roof area. In addition, splash-guards can be used to prevent water overflowing from the gutter. However, it is important to design a gutter of appropriate size in order to discharge water into the storage tank and prevent the wastage of water caused by overflow.

Maintenance and cleaning of the conveyance system is essential in order to prevent unnecessary contamination of collected water and to ensure that the system functions effectively. As the catchment area is vast and normally contributes to organic contamination, a cleaning device is often required to reduce contamination of the rainwater inside the storage tank. For example, the Superhead Rainwater Tank Filter[®] is a South African product which combines a first flush diverter with a leaf catcher and automatically filters and discards the first batch of rainwater during a rain event (Water Conservation Systems, 2016). In this manner the filter prevents bird and animal droppings, insects, leaves, dirt and dust from entering the storage tank. Moreover, the filter is

constructed from polyethylene and does not contain any mechanical parts making it cost-effective, neat and easy to install (Water Conservation Systems, 2016).

1.2.1.3. Storage tanks

After rainwater has been captured and passed through the gutters, it is collected in a storage tank. Precautions are required to minimise contamination of tank contents. Preventative measures include the construction of a protective enclosure around the tank as well as the use of a tight cover to seal the tank. Thus, contamination by animal, human and other environmental pollutants is minimised. In addition, mosquito breeding and algal proliferation in the tank are prevented (Helmreich & Horn, 2009). Furthermore, the type of material from which the tank is constructed will determine whether the tank will be used for above ground or underground storage (Helmreich & Horn, 2009).

Storage tanks can thus be separated into two categories viz. above ground or underground (Helmreich & Horn, 2009; Sturm et al. 2009). The implementation of underground tanks is considered labour intensive as a ground surface area to accommodate the dimensions of the tank must be excavated. In the past, cement and bricks were predominantly used to construct underground tanks. However, today polyethylene or metal tanks are more frequently used because of their increased availability and durability. The size of the polyethylene and metal tanks is often limited as large-scale tanks constructed from these materials are costly. Corrosion of metals used to construct an underground tank can also be problematic as metals leach into the stored rainwater, which adversely affects water quality.

Figure 1.2 depicts underground storage tanks constructed from polyethylene (**Figure 1.2A**) and cement (**Figure 1.2B**). Underground tanks are often used if above ground space is limited (Helmreich & Horn, 2009). The principal concern when using this type of storage tank is the difficulty associated with extracting the stored water to ground level. This often necessitates the use of a pump which increases costs as electricity will be needed (Helmreich & Horn, 2009). A further disadvantage is that it is difficult to detect cracks and leakages. Should a leak arise, runoff water and groundwater can enter the tank, contributing to the contamination of stored rainwater. Underground tanks are used predominantly in the rural areas of South Africa.

In urban areas of South Africa, above ground tanks are used more often than underground tanks. Above ground tanks are less expensive and the installation of these tanks is less labour intensive as no excavation is required. Cracks and leakages are easier to detect and the tanks can be readily drained for cleaning. Furthermore, a major advantage of installing rainwater tanks above ground is that water can be extracted passively from the tank by means of gravity (**Figure 1.3**). Cement-brick, metal, plain-cement, concrete and polyethylene are the materials most frequently used for the construction of an above ground tank. A tank constructed from these materials is generally

watertight, durable and affordable and the stored water is exposed to minimal contamination (Sturm et al. 2009; Li et al. 2010).

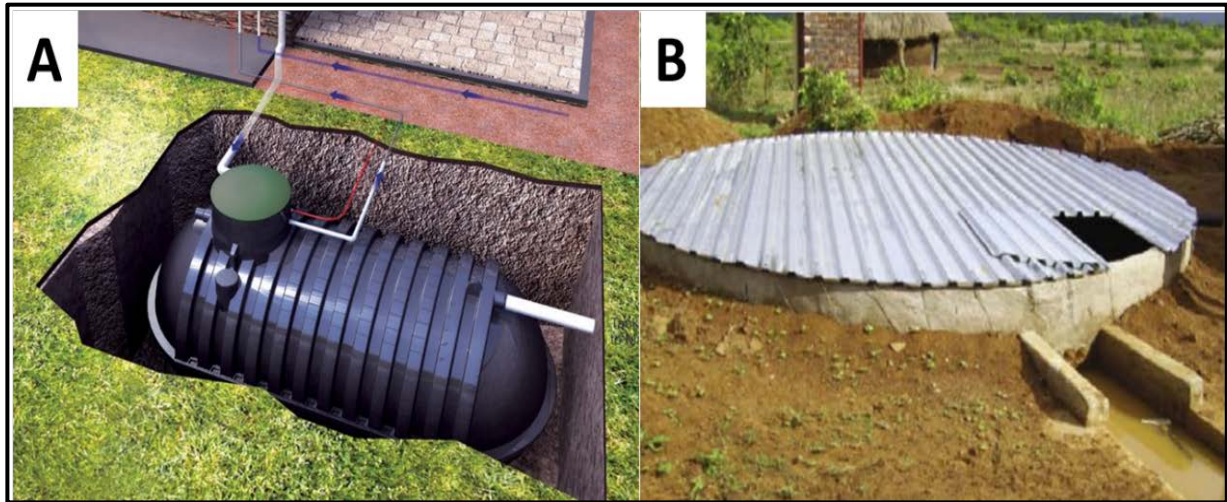


Figure 1.2: (A) An underground rainwater storage tank constructed from high grade polyethylene. (B) A cement rainwater storage tank covered with zinc sheets to prevent pollutants and sunlight from entering the tank (adapted from Helmreich & Horn, 2009).

Selection of a suitable storage tank shape depends on whether the tank is located above ground or underground. A rectangular or square tank is most often used when the tank is located above ground as these are easier to construct (Li et al. 2010). Li et al. (2010) stated that tanks located underground must be cylindrical or hemispherical in shape, as these shapes have the advantage of resisting the substantial pressure exerted on the tank wall by soil, particularly when the tank is empty.



Figure 1.3: Above ground high grade polyethylene storage tank installed on a metal stand to enhance passive flow of the water from the tank.

1.3. Quality of roof-harvested rainwater

Rainwater may become polluted with various microorganisms and organic and inorganic matter during the harvesting process (Helmreich & Horn, 2009; Abbasi & Abbasi, 2011; De Kwaadsteniet et al. 2013). Research has indicated that the quality of harvested rainwater is markedly influenced by atmospheric conditions which in turn can be influenced by the anthropogenic activity in the surrounding environment. The topography and weather conditions at the catchment site also determine the quality of the harvested water (Evans et al. 2006). Previous studies have indicated that urban areas exhibit high levels of airborne pollutants originating from industrial activities and motor vehicle emissions (Helmreich & Horn, 2009; Huston et al. 2009; 2012). In rural areas, however, the overall quality of the air is less polluted than that of urban areas, although factors such as gravel roads, gases and particles originating from organic matter (cattle manure), may exert considerable influence on the air quality (Waweru, 2014). Williams et al. (2015) investigated the relationship between the quality of fresh rainwater¹ and the surrounding air on a farm situated on the periphery of a town in the Western Cape (South Africa). Phylogenetic analysis of samples collected showed that similar bacterial genera were present in the fresh rainwater and the surrounding air. In addition, results obtained from the study indicated that as the wind speed increased, correspondingly, the microbial contamination in the air and rainwater samples increased (Williams et al. 2015).

A general perception however, is that poor roof and gutter maintenance primarily influences the microbial and chemical contamination of rainwater. Any rooftop catchment may be contaminated with dust, leaves, debris and bird and animal faecal matter (Simmons et al. 2001; Lee et al. 2010). When rain flows over a catchment area, chemical (such as heavy metals) and particularly microbial contaminants, may wash into the rainwater tank (Evans et al. 2006; Abbasi & Abbasi, 2011). Similarly, organic (decaying animals and plants) and inorganic (dust and debris) materials contaminate the rainwater when it enters the gutter system (Evans et al. 2006). Moreover, rainwater harvesting tanks may serve as breeding sites for various insect and parasite vectors, such as mosquitoes (Mandel et al. 2011; WHO, 2011), which are responsible for the transmission of diseases such as malaria (Mwenge Kahinda & Taigbenu, 2007; Chidamba, 2015). It is thus essential that any harvesting tank is adequately sealed with a well-fitted cover to limit the entry of external contaminants (bioaerosols and insects). Research has however, indicated that all of the above factors, including roof geometry and roof and gutter materials, influence both the chemical and microbial quality of harvested rainwater (Uygur et al. 2010; Abbasi & Abbasi, 2011).

¹ Rainwater collected directly from the open air in a sampling container.

1.3.1. Chemical quality of roof-harvested rainwater

Depending on the surrounding environment, the atmosphere can be polluted with exhaust fumes from motor vehicles, industrial pollutants, industrial burning of coal and the spraying of pesticides. Most of these pollutants originate from rapid industrialisation, unplanned urbanisation and increased agricultural activities (Abbasi & Abbasi, 2011; Mishra et al. 2012). Varying concentrations of heavy metals and organic pollutants in urban, industrial or rural areas accumulate in the atmosphere and negatively impact rainwater quality (Helmreich & Horn, 2009; Huston et al. 2009). Mishra et al. (2012) reported that rainwater traversing the atmosphere is an efficient means of removing gases and other pollutants from the air, however in the process the rainwater becomes contaminated. In a study conducted in Australia, atmospheric deposition was characterised as a source of contamination in urban rainwater tanks (Huston et al. 2009). Huston et al. (2009) reported that 17.7 % ($n = 31$), 10.3 % ($n = 18$) and 1.7 % ($n = 3$) of all atmospheric deposition samples had concentrations of iron, lead and cadmium, respectively, in excess of the stipulated Australian Drinking Water Guidelines (ADWG) [National Health and Medical Research Council & National Resource Management Ministerial Council (NHMRC & NRMCC), 2004]. Furthermore, the study highlighted that increased chemical contamination levels were recorded in industrial and traffic dense areas. It was thus concluded that atmospheric deposition directly contributed to the contamination of harvested rainwater in an urban environment and that the impact of chemical pollution on harvested rainwater must be determined, especially in areas where air pollution is severe (Huston et al. 2009).

A study conducted by Spinks et al. (2006) in Australia investigated the chemical quality of rainwater in harvesting tanks after a bushfire event. A major concern was that smoke and ash would contaminate the catchment area and subsequently wash into the harvesting tank after a rain event. Contaminants of particular concern were the polycyclic aromatic hydrocarbons (originating from the incomplete combustion of organic matter), which are classified as possible human carcinogens (International Agency for Research on Cancer, 1973). In addition, it was hypothesised that copper chrome arsenate, used to treat wooden products (including furniture, fencing and outdoor structures such as Wendy houses and huts), may have contaminated the rainwater. Such contamination was undesirable as copper chrome arsenate, when ingested in its organic and inorganic forms, is responsible for a wide range of deleterious systemic health effects including cancer (Agency for Toxic Substances and Disease Registry, 2000). However, results obtained from the study showed that the concentration of these compounds were well within the ADWG specifications (NHMRC & NRMCC, 2004) for all 49 harvesting tanks sampled.

The roof catchment area is generally constructed from materials such as zinc sheets, concrete tiles, Chromadek[®], asbestos and aluminium (Lye, 2009; Dobrowsky et al. 2017a). Lead nails and screws are also often used on roofs for construction purposes and paint or zinc galvanisation is used as a coating material aiding in the prevention of corrosion (Sullivan & Worsley, 2002). These materials could be major contributors to metal contamination of water as the acidity of rainwater (pH 5.0 – 5.6)

together with the exposure of the roof surface to the sun, facilitate possible leaching of metals from the roofing material (Lye, 2009). A study conducted by Yaziz et al. (1989) compared the quality of rainwater collected from two different roof catchment areas viz. a concrete tile roof and a galvanised-iron sheet roof. Results showed that rainwater collected from the galvanised-iron sheets had a significantly higher ($p < 0.05$) zinc concentration (423.4 $\mu\text{g/L}$) when compared with rainwater collected from a concrete tile roof (78 $\mu\text{g/L}$). However, the increased zinc concentration reported complied with levels specified by drinking water guidelines stipulated by the WHO (1984). The authors attributed this increased zinc concentration to the leaching of the metal from the galvanised-iron sheets into the water, as the rainwater had an average pH of 5.9, which could have enhanced the leaching process (Yaziz et al. 1989).

In a recent study conducted by Dobrowsky et al. (2017a), the incidence of *Acanthamoeba* spp. and *Legionella* spp. was correlated with the chemical and microbial quality of rainwater harvested from catchment systems constructed from Chromadek®, galvanized zinc and asbestos roofing materials. Dobrowsky et al. (2017a) reported that the concentrations of anions did not differ significantly ($p > 0.05$) among the different tank samples. In contrast, various cation concentrations (including zinc and iron), differed significantly ($p < 0.05$) among the tank water samples collected from the different roofing catchment materials. For example, the zinc concentration recorded in the rainwater collected from the galvanised zinc roof was significantly higher ($p < 0.05$) than the zinc concentrations recorded in rainwater samples collected from the Chromadek® and asbestos roofing materials. This was attributed to zinc leaching directly from the galvanised zinc roofing material.

The chemical quality of harvested rainwater has also been extensively studied in countries where rainwater is utilised as a primary or supplementary water source (Sazakli et al. 2007; Peters et al. 2008; Huston et al. 2009; Lee et al. 2010; Gikas & Tsihrintzis, 2012; Huston et al. 2012; Strauss et al. 2016) (**Table 1.1**). As there are no guidelines stipulating the concentrations of chemical compounds in rainwater, the majority of these studies compared their results with drinking water standards such as those promulgated by the WHO (2011).

A study conducted by Lee et al. (2010) investigated the chemical quality of harvested rainwater in the City of Gangneung, South Korea. Cation (including aluminium, copper, arsenic, lead and zinc) and anion (including chloride, nitrites, nitrates and sulphates) analyses were conducted. Most concentrations occurred within the range acceptable for drinking water (Lee et al. 2010), with the sole exception of aluminium, which exceeded the statutory standard (not specified in the study) of 200 $\mu\text{g/L}$. (**Table 1.1**). The authors attributed the high aluminium concentration recorded (230 $\mu\text{g/L}$) to the leaching of this compound from the aluminium gutter system (Lee et al. 2010). Huston et al. (2012) also detected the presence of several metals such as aluminium, copper, iron, magnesium, manganese and zinc in harvested rainwater in Australia (**Table 1.1**). However, the concentrations recorded for all the metals detected were compliant with the drinking water guidelines stipulated by both the ADWG (NHMRC & NRMCC, 2011) and the WHO (2011).

Table 1.1: Chemical concentrations ($\mu\text{g/L}$) present in harvested rainwater as analysed by various studies, compared to guidelines stipulated by the WHO (2011).

Anion/Cation	Sazakli et al. 2007	Peters et al. 2008	Huston et al. 2009	Lee et al. 2010	Gikas & Tsihrintzis, 2012	Huston et al. 2012	Strauss et al. 2016	WHO, 2011
Aluminium	-	130	310.59	225	-	314	1130	-
Ammonia	-	-	-	90	-	-	-	-
Ammonium	10	-	-	-	1835	-	-	-
Antimony	-	0.86	0.5	-	-	0.15	-	-
Arsenic	-	-	0.97	3	-	0.25	0.37	10
Barium	-	5	7.27	-	-	12	-	700
Boron	-	BDL	156.69	-	-	-	BDL	2.4
Cadmium	0.05	-	0.32	1.5	-	-	BDL	3
Calcium	15200	15	1397.73	6.4	11990	2.4	4220	-
Chloride	7000	3000	6507.67	7500	4522	3900	4553	-
Chromium	< 1.3	0.98	1.81	4.5	-	0.53	-	50
Cobalt	-	-	0.72	-	-	0.17	0.05	-
Copper	< 2.5	1.9	5.52	85	-	21	3.18	2000
Fluoride	< 10	-	-	-	-	-	63	-
Iron	11	17	275.27	-	-	68	101.13	-
Lead	< 2.0	0.47	5.92	27	-	5.4	0.59	10
Lithium	-	-	0.37	-	-	0.55	-	-
Magnesium	600	1500	847.34	1200	1573	500	400	-
Manganese	1	BDL	16.06	115	-	8.7	2.7	-
Mercury	-	BDL	-	-	-	-	-	6
Molybdenum	-	0.2	0.48	-	-	-	0.2	-
Nickel	<10	BDL	1.03	-	-	1.3	2.45	70
Nitrate	7040	5000	2740.85	6800	700	1600	37	50000
Nitrite	13	-	423.43	-	43	600	17	3000
Phosphate	90	-	527.14	20	-	100	153	-
Phosphorus	-	-	-	-	-	-	0.04	-
Potassium	2400	1200	910.95	3100	3357	900	490	-
Selenium	-	0.62	1.76	-	-	-	-	40
Sodium	6000	1300	5880.36	3200	4612	2800	1923	-
Strontium	-	160	8.55	-	-	30	-	-
Sulphate	8000	9700	2547.51	4100	11213	1600	2310	-
Tin	-	BDL	0.35	-	-	0.51	-	-
Vanadium	-	2.6	0.9	-	-	0.32	0.053	-
Zinc	10	23	45.53	160	-	770	16.84	3000

BDL = below detection limit

- = not reported

Research conducted in South Africa by Reyneke et al. (2016) and Strauss et al. (2016) then indicated that anion (sulphate, fluoride, chloride, amongst others) and cation (aluminium, calcium, copper, amongst others) concentrations of harvested rainwater were generally within the national drinking water guidelines stipulated by the South African government [DWAF, 1996; South African Bureau of Standards (SABS), 2005]. However, the iron concentrations in three rainwater samples analysed by Strauss et al. (2016) exceeded the DWAF guideline of $100 \mu\text{g/L}$ (DWAF, 1996). The increased iron concentrations were attributed to the materials (iron nails and screws) used in the catchment system.

Thus, while the chemical quality of harvested rainwater may be affected by the surrounding environment (air quality) or the catchment system itself (type of roofing material, cleanliness and maintenance), numerous studies have indicated that the chemical quality of this water source is of minor concern and levels measured are generally compliant with stipulated drinking water guidelines. In contrast, research has indicated that the microbial quality of roof-harvested rainwater is seriously compromised and often exceeds drinking water guidelines (Ahmed et al. 2010; 2012; De Kwaadsteniet et al. 2013; Dobrowksy et al. 2014a).

1.3.2. Microbial quality of roof-harvested rainwater

Abbasi and Abbasi (2011) indicated that the microbial contamination of roof-harvested rainwater primarily occurs through: (i) leaf and dust accumulation on the catchment surface area; (ii) faecal matter originating from birds and animals that have access to the catchment surface area; (iii) dead animals on the catchment area or in the storage tank; (iv) airborne microbial communities that arise from various sources and are carried by the wind to the catchment surface, and (v) fungal/bacterial growth on the catchment area/roof, especially in areas which experience a moist climate. Roof-harvested rainwater systems may thus be susceptible to major contamination by pathogenic species of bacteria (e.g. *Pseudomonas*, *Legionella* and *Yersinia*), fungi (e.g. *Aspergillus* and *Cladosporium*), protozoa (e.g. *Cryptosporidium* and *Giardia*) and viruses (e.g. adenovirus) (Ahmed et al. 2010; De Kwaadsteniet et al. 2013; Dobrowsky et al. 2014b; Waso et al. 2016).

While the ultimate aim of harvesting rainwater is to supplement potable resources, no guidelines have been formulated by the various statutory bodies to regulate the microbial quality of this water source. Therefore, it is common practice to use drinking water guidelines as a reference to monitor the quality of harvested rainwater (Rompré et al. 2002; Noble et al. 2003; Pitkänen et al. 2007; De Kwaadsteniet et al. 2013). It is however, impractical to screen for all known water-associated pathogens and studies which assess the quality of a water source generally screen for indicator organisms such as *E. coli*, faecal and total coliforms and enterococci (DWAF, 1996; SABS, 2005; NHMRC & NRMCC, 2011; WHO, 2011).

1.3.2.1. Indicator organisms

Indicator organisms are used to assess the microbial quality of a water source and should essentially: (i) be easy to culture and detect; (ii) indicate the presence of pathogens and (iii) be present in higher numbers than pathogens in the analysed water sample (Noble et al. 2003). These microorganisms are generally non-pathogenic and occur in the intestinal microflora of humans and warm-blooded animals. They thus serve as a good indication of the faecal contamination of a water source and correspondingly indicate the presence of potential pathogens (Noble et al. 2003). A combination of indicators established by several water institutions, including coliforms (faecal and total coliforms), *E. coli*, enterococci and heterotrophic bacteria (HPC), are generally used to

determine the microbial quality of water (Ahmed et al. 2008; De Kwaadsteniet et al. 2013).

Coliforms are comprised of a large group of Gram-negative, non-sporulating aerobic and facultative anaerobic bacteria, subdivided into total coliforms and faecal coliforms. Total coliforms do not serve as a direct indication of faecal contamination, but are used to assess the general hygienic quality of water and determine whether water disinfection strategies are functioning optimally (Water Stewardship Information Series, 2007; WHO, 2011). These microbes are more commonly found in soil environments where they can survive at temperatures of up to 37 °C. In contrast, faecal coliforms are thermo-tolerant microorganisms which can survive at temperatures up to 44.5 °C and primarily originate from the intestines of humans and warm-blooded animals. These bacteria belong to the Enterobacteriaceae family and are directly associated with faecal contamination of a water source. *Escherichia coli* is the most characterised species of the Enterobacteriaceae family and commonly occurs in large numbers in faecal matter and thus the presence of this bacterium in a water body specifically indicates faecal contamination. Consequently, the presence of *E. coli* in a water source indicates an imminent health risk and guidelines generally specify that < 1 colony forming unit (CFU) of *E. coli* should be present if a water source is utilised for potable purposes (SABS, 2005; WHO, 2011).

Enterococci are Gram-positive, facultative anaerobic bacteria, able to survive for extended time periods in aquatic habitats (WHO, 2003). They also occur in the colon of mammals and are thus generally associated with direct faecal contamination of a water source (Edberg et al. 2000). Heterotrophic bacteria are ubiquitous in the environment including in water sources and are detected by performing a heterotrophic plate count (HPC) on non-selective culture media. A high HPC count signifies that conditions within the sampled waterbody may be favourable for the growth of many bacterial genera, including pathogens (NHMRC & NRMCC, 2011). Thus, while the HPC test itself does not identify the microbes in a sample, it provides an indication of the number of microorganisms present in a water source and therefore serves as an indirect indicator of the microbial quality of that source (WHO, 2003; Allen et al. 2004).

Numerous studies have detected the presence of indicator organisms in roof-harvested rainwater. (**Table 1.2**). For example, Simmons et al. (2001) detected the presence of total coliforms, faecal coliforms, enterococci and HPC in 125 rainwater tank samples collected from four different districts in Auckland, New Zealand. The indicator organism analysis of 56 % of the samples exceeded the guidelines stipulated by the New Zealand Drinking Water Standards (Ministry of Health, 1995). Similarly, in a study conducted by Sazakli et al. (2007), enterococci, *E. coli* and total coliforms were detected in 29 %, 40.9 % and 80 % of harvested rainwater samples collected in Greece, respectively.

Table 1.2: Studies that have detected the presence of indicator microorganisms in harvested rainwater samples (adapted from Ahmed et al. 2011).

Country	Percentages of samples tested positive (>1 CFU/100 mL) for various indicators (number of samples)					Reference
	HPC	Total coliforms	Faecal coliforms	<i>E. coli</i>	Enterococci	
Australia	NR	52 (100)	38 (100)	NR	NR	Verrinder & Keleher (2001)
Australia	NR	90 (49)	NR	33 (49)	73 (49)	Spinks et al. (2006)
Australia	NR	NR	NR	63 (27)	78 (27)	Ahmed et al. (2008)
Australia	NR	NR	NR	58 (100)	83 (100)	Ahmed et al. (2010)
Australia	NR	NR	NR	33 (49)	73 (49)	Ahmed et al. (2011)
Australia	NR	NR	NR	63	29	Ahmed et al. (2012)
Australia	100 (67)	91 (46)	78 (41)	57 (67)	82 (67)	Chapman et al. (2008)
Australia	NR	NR	83 (6)	NR	NR	Thomas & Green (1993)
Australia	100 (77)	63 (81)	63 (81)	NR	NR	Evans et al. (2006)
Bermuda	NR	90 (102)	NR	66 (102)	NR	Lévesque et al. (2008)
Canada	NR	31 (360)	14 (360)	NR	NR	Despins et al. (2009)
Denmark	100 (14)	NR	NR	NR	NR	Albrechtsen (2002)
Greece	NR	80 (156)	NR	29 (156)	29 (156)	Sazakli et al. (2007)
Hawaii, USA	NR	NR	89(9)	NR	NR	Fujioka et al. (1991)
Micronesia	NR	43 (155)	70 (176)	NR	NR	Dillaha & Zolan (1985)
New Zealand	NR	NR	56 (125)	NR	NR	Simmons et al. (2001)
Nigeria	100 (6)	100 (6)	ND	NR	ND	Uba & Aghogho (2000)
Palestine	NR	95 (100)	57 (100)	NR	NR	Al-Salaymeh et al. (2011)
Palestine	NR	49 (255)	NR	17 (255)	NR	Abo-Shehada et al. (2004)
South Africa	100 (11)	NR	BDL	100 (11)	BDL	Strauss et al. (2016)
South Africa	100 (21)	100 (21)	NR	100(21)	NR	Dobrowsky et al. (2017a)
South Korea	NR	NR	NR	72 (90)	NR	Lee et al. (2010)
Thailand	NR	NR	NR	40 (86)	NR	Pinfold et al. (1993)
U.S. Virgin Islands	86 (45)	57 (45)	36 (45)	NR	NR	Crabtree et al. (1996)
USA	100 (30)	93 (30)	NR	3 (30)	NR	Lye (1987)
Zambia	NR	100 (5)	100 (5)	NR	NR	Handia (2005)

NR - not reported

ND - not detected

BDL - below detection limit

Ahmed et al. (2011) analysed 49 rainwater tanks for the presence of *E. coli* and enterococci. Results indicated that 33 % and 73 % of the samples tested positive for these organisms, respectively. A further study conducted by the same research group detected *E. coli* in 63 % of rainwater samples analysed, while enterococci were present in 92 % of the rainwater samples (Ahmed et al. 2012). In South Africa, Dobrowsky et al. (2017a) confirmed the presence of total coliforms, *E. coli* and HPC in rainwater samples collected from Chromadek®, galvanized zinc and asbestos roofing materials, respectively. However, no significant difference was observed among the mean concentrations of these indicator organisms in rainwater harvested from the three different roofing materials (Dobrowsky et al. 2017a).

Strauss et al. (2016) then assessed the quality of roof-harvested rainwater in South Africa before and after treatment by SODIS (solar cooker) and SOPAS, respectively. The authors reported that while the *E. coli* and HPC counts recorded in the untreated rainwater exceeded drinking water guidelines stipulated by the DWAF (DWAF, 1996), ADWG (NHMRC & NRMCC, 2011) and WHO (WHO, 2011), the numbers of enterococci and faecal coliforms were below the detection limit (< 1 CFU/mL). However, *E. coli* and HPC counts in the water were effectively reduced to below the detection limit (< 1 CFU/mL) after both SODIS and SOPAS treatment.

Thus, based on indicator organism analysis, untreated roof-harvested rainwater frequently does not comply with the microbial standards prescribed for drinking water. Accordingly, this water source is unsuitable for potable purposes as it poses potential health risks to the consumer. Of concern is the frequent detection of indicator organisms in harvested rainwater, which implies that various pathogenic bacteria may also be present in this water source earmarked for human consumption.

1.3.2.2. Pathogens and opportunistic pathogens associated with roof-harvested rainwater

Despite the benefits of harvesting rainwater, studies have detected numerous pathogens and opportunistic pathogens such as *Mycobacterium* (Albrechtsen, 2002), *Klebsiella* (Dobrowsky et al. 2014b), *Legionella* (Ahmed et al. 2010), *Pseudomonas* (Strauss et al. 2016), amongst others, in stored rainwater. Possible sources of these microorganisms include bioaerosol particles which contaminate rain droplets, fungal and algal growth, decaying matter, leaves and debris on catchment systems and faecal material originating from birds and other animals on the roof surfaces (Ahmed et al. 2012; 2014; Sánchez et al. 2015; Waso et al. 2016). Following a rain event, all these contaminants can be washed into the harvesting tank which subsequently compromises the microbial quality of rainwater. As a result, waterborne disease outbreaks have been associated with the consumption of untreated harvested rainwater (Schlech et al. 1985; Merritt et al. 1999; Simmons et al. 2001; Lye 2002; Ahmed et al. 2008; Simmons et al. 2008; Franklin et al. 2009).

The bacterial genus *Campylobacter* is often associated with faecal matter and has frequently been detected in harvested rainwater (Savill et al. 2001; Albrechtsen, 2002; Ahmed et al. 2012). This

organism causes campylobacteriosis in humans, which is characterised by symptoms such as stomach cramps, fever, pain and diarrhoea (Centers for Disease Control and Prevention, 2016). Avian species are the major vectors of this pathogen and in an epidemiological study conducted by Eberhart-Philips et al. (1997), it was hypothesised that nesting of birds near and on the catchment area of a rainwater harvesting system may have caused an outbreak of campylobacteriosis after consumption of contaminated rainwater. Similarly, Merritt et al. (1999) reported on an outbreak of campylobacteriosis after the consumption of contaminated rainwater. Although *Campylobacter* spp. were not found in the rainwater tank samples when culture based methods were used, faecal contamination was detected. The authors concluded that additional techniques should be used for *Campylobacter* detection as the microbe was identified in stool samples from seven patients associated with the outbreak.

Klebsiella spp. occur ubiquitously in nature and have been isolated from plants (Grimont et al. 2003; Grimont & Grimont, 2005), animals (present mainly in the gastrointestinal tract) (Gordon & FitzGibbon, 1999, Davidson et al. 2015) and soil and water (Cabral, 2010). In a study conducted in Singapore, 50 rainwater tanks were screened to assess the microbial quality of the water (Kaushik et al. 2012). Among other genera, *Klebsiella* spp. were found to be present in 12 % ($n = 6$) of the rainwater samples analysed. Kaushik et al. (2012) then suggested that *Klebsiella* contamination occurred predominantly by means of bioaerosols as *Klebsiella* spp. were previously found to be prevalent in air samples (Gauthier & Archibald, 2001). In a study conducted in South Africa, Dobrowsky et al. (2015) investigated the efficiency of a closed-coupled SOPAS system in treating roof-harvested rainwater. Using PCR analysis, *Klebsiella* spp. were also found to persist in 47 % ($n = 15$) of the roof-harvested rainwater samples and were detected at a maximum temperature of 74 °C after SOPAS treatment (Dobrowsky et al. 2015).

Salmonella is most frequently transmitted through contaminated food sources and has previously been detected in various water bodies (natural waters, stormwater runoff, sewage) (Arvanitidou et al. 2005) as a result of animal faecal contamination (Cabral, 2010). *Salmonella* spp. have also been detected in rainwater (Simmons et al. 2001; Ahmed et al. 2008; 2010; Chapman et al. 2008; Dobrowsky et al. 2014b). Ahmed et al. (2008) investigated the microbiological quality of roof-harvested rainwater in Australia by screening for virulence genes specific to certain pathogenic bacteria. Genes screened for included the *Aeromonas hydrophila lip* gene, the *Campylobacter coli ceuE* gene, the *Legionella pneumophila mip* gene and the *Salmonella invA* gene. The *Salmonella invA* gene was detected in 11 % ($n = 3$), the *Legionella pneumophila mip* gene in 26 % ($n = 7$), the *Campylobacter coli ceuE* gene in 41 % ($n = 11$) and the *Aeromonas hydrophila lip* gene in 15 % ($n = 4$) of the samples ($n = 27$) by means of qPCR analyses. A study conducted by Dobrowsky et al. (2014b) in South Africa investigated the presence and frequency distributions of pathogenic bacteria considered indigenous to harvested rainwater by using genus-specific PCR analysis. Amongst other bacterial genera, *Salmonella* was found to be present in 6 % ($n = 7$), *Pseudomonas* spp. in 13 %

($n = 15$) and *Legionella* in 73 % ($n = 85$) of the samples analysed (Dobrowsky et al. 2014b). Furthermore, research conducted by Strauss et al. (2016) showed that viable *Legionella* and *Pseudomonas* spp. persisted in SOPAS and SODIS (solar cooker) treated harvested rainwater. As research has indicated that these two opportunistic pathogens are ubiquitous in harvested rainwater, they are primarily investigated in the research chapters of the current study and literature specific to *Legionella* and *Pseudomonas* spp. is summarised.

1.3.2.2.1. *Legionella* spp.

Legionella are Gram-negative rods and possess lateral or polar flagella (Benson & Fields, 1998). The genus is comprised of more than 50 species, with 70 distinct serotypes of which 39 are associated with human disease (Stout et al. 2003). Most species are considered opportunistic pathogens (Fields, 1996). Accordingly, *Legionella* spp. are able to proliferate in mammalian cells and cause disease in immunocompromised individuals which can result in disorders associated with the respiratory and gastrointestinal tracts and the nervous system. *Legionella* is however, primarily associated with Legionellosis, which is a standard term describing the pneumonic and non-pneumonic types of infection (WHO, 2007). When *Legionella* is inhaled, the bacterium can replicate and infect the alveoli, resulting in Legionnaires' disease or a mild flu-like disease called Pontiac fever (Fields et al. 2002; WHO, 2007). Legionnaires' disease is a multisystem disease which involves pneumonia. Risk factors for Legionnaires' disease include smoking, diabetes, chronic lung disease and lung cancer (Marston et al. 1994).

More than 90 % of all Legionellosis cases worldwide are attributed to infection caused by *L. pneumophila*. However, approximately 24 species, including *L. bozemanii*, *L. longbeachae* and *L. micdadei*, are associated with human illness (Fields et al. 2002; Yu et al. 2002; Mercante & Winchell, 2015) as they are intracellular parasites and are able to penetrate mammalian and protozoan cells. There are two antibiotic classes used to successfully treat *Legionella* spp. infections, viz. macrolides and quinolones (Edelstein & Cianciotto, 2010). Macrolides (azithromycin) act by inhibiting bacterial protein synthesis as they interfere with the peptidyltransferase enzyme thereby preventing ribosomal translation. The quinolones (ciprofloxacin, levofloxacin, gemifloxacin, moxifloxacin and trovofloxacin) inhibit the topoisomerase ligase domain and this causes fragmentation of DNA and inhibits the synthesis of mitochondrial DNA (Suto et al. 1992). A third category of antibiotics viz. tetracyclines, may also be effective against *Legionella* by inhibiting protein synthesis, as the antibiotic prevents the attachment of a transfer ribonucleic acid (RNA) molecule to the prokaryotic ribosome. In contrast, research has indicated that *Legionella* spp. are resistant to both aminoglycosides (inhibits protein synthesis by interfering with peptide elongation on the 30S ribosomal unit) and lincosamides (inhibits protein synthesis by dissociating the peptidyl-transfer RNA molecules on the 50S ribosomal unit) (Mingeot-Leclercq et al. 1999; Tenson et al. 2003; Garau et al. 2010; Bruin et al. 2012).

Legionella spp. are commonly isolated from soil and freshwater environments and may survive and proliferate for extended time periods under low nutrient conditions (Dusserre et al. 2008). In addition, *Legionella* is ubiquitous in water environments and has previously been detected in swimming pools (Coetzee et al. 2012), ice-making machines (Graman et al. 1997), fountains (Palmore et al. 2009), hot springs (Kurosawa et al. 2010), whirlpool spas (Campese et al. 2010) and cooling towers (Osawa et al. 2014). *Legionella* spp. have also been widely detected in harvested rainwater in many countries including Denmark (Albrechtsen, 2002), Australia (Ahmed et al. 2008; 2010), South Africa (Dobrowsky et al. 2015; Reyneke et al. 2016; Strauss et al. 2016) and the US Virgin Islands (Broadhead et al. 1988). A study conducted by Simmons et al. (2001) in New Zealand reported on an outbreak of Legionnaires' disease where individuals were exposed to *Legionella* spp. through contaminated showers connected to rainwater tanks. This was the first Legionnaires' disease outbreak in New Zealand linked to roof-harvested rainwater (Simmons et al. 2001).

Reyneke et al. (2016) conducted a study in South Africa to investigate the efficiency of a closed coupled SOPAS system to reduce *Legionella* spp. copy numbers in harvested rainwater. The EMA-qPCR technique was used to detect and quantify this microorganism whereafter results indicated that *Legionella* spp. copy numbers were significantly reduced ($p < 0.05$) following SOPAS treatment. However, *Legionella* spp. were still detected in harvested rainwater after treatment at 95 °C, suggesting that *Legionella* spp. were still viable (Reyneke et al. 2016). The ability of *Legionella* to survive at high temperatures can be attributed to: (i) the presence of heat shock proteins which confer thermo-tolerance at increased temperatures (> 50 °C) (Fields et al. 2002); (ii) their association with biofilms (Murga et al. 2001; Borella et al. 2005) and (iii) their ability to survive as intracellular parasites in protozoan species (Fields et al. 2002). Dobrowsky et al. (2017b) recently investigated the resistance of five *Legionella* spp. and the protozoan, *Acanthamoeba mauritaniensis* (*A. mauritaniensis*), to heat treatment. Results from the study indicated that while heat treatment (above 50 °C) significantly reduced ($p < 0.05$) the number of viable *Legionella* spp., the association between *A. mauritaniensis* and *L. pneumophila* significantly increased the virulence of this opportunistic pathogen during heat treatment (Dobrowsky et al. 2017b).

1.3.2.2.2. *Pseudomonas* spp.

Pseudomonas are Gram-negative, non-sporulating rod-shaped bacteria which naturally inhabit a wide range of environmental niches, particularly water and soil. They are known to display remarkable metabolic versatility. This enables the bacterium to adapt to various conditions and survive in diverse environments where they exist as planktonic cells or congregate together in a biofilm community (Suzuki et al. 2013). Although this genus is predominantly aerobic, they are able to utilise nitrate (NO_3^-) as an electron acceptor when oxygen (O_2) is limited. *Pseudomonas* consists of 202 described species with numerous pathogenic species included. Many of the pathogenic species are associated with plants, including the well-characterised pathogen *P. syringae* (Río-

Álvarez et al. 2014; Rous et al. 2016). Several human and animal pathogenic species have also been identified.

The pathogenic species of *Pseudomonas* include *P. aeruginosa*, *P. putida* and *P. fluorescens*, amongst others (Mazurier et al. 2015; Kittinger et al. 2016). They are known to cause severe infections such as endocarditis (inflammation of the inner layers of the heart), pneumonia (inflammation of the alveoli in the lungs), malignant otitis externa (infection of the inner and outer ear), bacteraemia/septicaemia (blood infection), keratitis (eye infection), gastro-intestinal, skeletal, skin and soft tissue infections (Noble & Overman, 1994; Mena & Gerba, 2009). These infections may lead to life threatening complications in patients diagnosed with cystic fibrosis, diabetes, tuberculosis and human immunodeficiency virus (HIV) if adequate health care is not provided.

Pseudomonas aeruginosa is the best studied species of this genus and is known to produce various virulence factors including alkaline protease, elastase, exotoxin A, flagella, lipopolysaccharides, phospholipase, pyochelin, pyoverdine and type IV pili, which are regulated by signal transduction systems in response to environmental changes (Llamas et al. 2006; Özen & Ussery, 2012; Vaz-Moreira et al. 2012; Lee et al. 2016). In addition, *P. aeruginosa* is the pathogen most frequently isolated from patients who suffer from hospital-acquired infections (Coutinho et al. 2008; Silby et al. 2011). Several epidemiological studies have tracked the occurrence of this microorganism and have shown that clinical isolates of *P. aeruginosa* exhibit increasing resistance against a wide range of antibiotics such as penicillin G; aminopenicillin, meropenem, piperacillin, tazobactam and ceftazidime (Yayan et al. 2015; Kittinger et al. 2016).

Numerous studies have detected *Pseudomonas* spp. in diverse water environments including lakes and rivers (Knezevic et al. 2009; Malik & Aleem, 2011; Vaz-Moreira et al. 2012), hydro-systems including heat exchangers and whirlpools, hot tubs and swimming pools, which are not adequately chlorinated (Ratnam et al. 1986; Huhulescu et al. 2011; Lutz & Lee, 2011). Ribeiro et al. (2014) investigated the role of aquatic environments in the spread of antibiotic resistance genes after a rain event. The authors isolated 580 *Pseudomonas* isolates from a karst spring in Le Havre, France and showed that all 580 isolates were resistant to at least one antibiotic. They further detected eight *Pseudomonas* isolates, including *P. putida* and *P. fluorescens* in potable tap water (originating from a local spring) and concluded that potable water sources can contribute to the transmission of multi-antibiotic resistant *Pseudomonas* spp.

Pseudomonas spp. have also been detected by numerous research groups in harvested rainwater (Uba & Aghogho, 2000; Albrechtsen, 2002; Kaushik et al. 2012; Amin et al. 2014; Dobrowsky et al. 2014b; Nawaz et al. 2014; Dobrowsky et al. 2015; Strauss et al. 2016). Using culture based assays, Nawaz et al. (2014) investigated the effect of different catchment and storage conditions of harvested rainwater in South Korea on the occurrence of *P. aeruginosa* during both a dry and wet season. It was reported that *P. aeruginosa* counts varied between 30 and 400 CFU/100 mL and 200 to

1800 CFU/mL during the dry and wet seasons, respectively. Although increased CFU counts of *P. aeruginosa* were observed during the wet season, it was suggested that adequate maintenance of the catchment area, suitable storage conditions and efficient harvesting tank designs could lead to an improved harvested rainwater quality (Nawaz et al. 2014).

In a study conducted by Dobrowsky et al. (2014b), the distribution of indigenous bacterial pathogens present in roof-harvested rainwater was investigated in Kleinmond, South Africa. Using molecular based techniques (16S rRNA PCR), *Pseudomonas* spp. were identified in 13 % ($n = 15$) of rainwater samples analysed. In addition, Strauss et al. (2016) detected the presence of viable *Pseudomonas* spp. in roof-harvested rainwater before and after both SOPAS and SODIS (solar cooker) treatment. Strauss et al. (2016) used EMA-qPCR to quantify the viable population present in the untreated and treated rainwater. Although *Pseudomonas* spp. remained present after treatment, a 99.61 % reduction in viable *Pseudomonas* copy numbers was observed after SOPAS treatment, while SODIS treatments of six and eight hours yielded a 47.27 % and 58.31 % decrease in *Pseudomonas* copy numbers, respectively.

1.4. Molecular methods used for the detection of pathogens and opportunistic pathogens in harvested rainwater

Several assays which include culture- and molecular-based techniques are used to screen for microbial pathogens and opportunistic pathogens in harvested rainwater. At the basic microbiological level, general nutrient media are used to screen for a range of microbial species and the use of differential and selective culture media enables the detection of specific genera. However, culture based methods are labour intensive and time-consuming as media preparation is required and results are usually generated only after 24 to 36 hours incubation (Lungu et al. 2012; Law et al. 2014). A further disadvantage of culture based methods is that they merely account for viable and culturable microorganisms present in a water source, while viable and non-culturable species are not detected. To overcome this, molecular based analyses such as the conventional PCR technique are currently employed to detect specific pathogens and opportunistic pathogens in water samples. While results are frequently generated within eight hours, the PCR functions only as a presence/absence indicator and provides no information on the viability and concentration of a specific microorganism in a sample.

Quantitative PCR provides a more sensitive screening method as the number of gene copies of a particular organism can be determined. This method provides essential information as quantitative data are required for accurate risk assessment studies of pathogenic microbial contaminants. In this regard, it is important to note that some pathogens are naturally present in environments and their infectious dose may vary (Haas et al. 1999). Nucleic acid binding dyes such as EMA and propidium monoazide (PMA) are used in combination with qPCR to detect the viable population of microbial species in a water sample (Fittipaldi et al. 2012; Agustí et al. 2013). The nucleic acid binding dye

complexes with the DNA of cells (after photoactivation) with damaged and/or permeable membranes (non-viable cells) (Fittipaldi et al. 2012; Reneyke et al. 2016). The binding effectively prevents the PCR amplification of DNA in non-viable (dead or damaged) cells. This causes a marked signal reduction during qPCR as only the DNA from intact (viable) cells is amplified.

A major disadvantage of EMA-qPCR and PMA-qPCR is that only one genus can be detected per analysis as a normal qPCR assay is limited to only one primer set per specific organism. Several molecular tools have thus been developed for the simultaneous detection of multiple species of pathogens in a single reaction. These techniques include multiplex PCR (Guion et al. 2008; Ramalingam et al. 2010), microarray hybridization (Kostić et al. 2007; Donhauser et al. 2011) and other hybridization-based detection methods. Although these methods greatly reduce the detection time for a contaminant when compared with traditional culture based methods, quantitative information on the target pathogens present in the sample is often not achieved (Ishii et al. 2013). Other disadvantages include: (i) the high cost of a single experiment; (ii) information generated for non-expressed genes is limited in the control sample and (iii) in the case of microarrays, hybridization is dependent upon the length of the DNA sequences (Jaluria et al. 2007; Jaksik et al. 2015).

Next-generation sequencing such as pyrosequencing and Illumina sequencing are high-throughput sequencing techniques which provide detailed information on microbial community structures in combination with excellent taxonomic resolution (Meyer & Kircher, 2010). Briefly, this technique requires the separation of the sample DNA into small fragments which are subsequently molecularly modified at the 5' and 3' ends by using adapter sequences. The modified DNA fragments are then introduced onto a microchip which has oligonucleotides complementary to those of the adapter sequences, thereby permitting DNA hybridization to the chip. Once the DNA fragment is anchored, cluster generation commences and this results in multiple copies of each DNA fragment being produced. Each fragment is amplified during a step known as bridge amplification by using deoxynucleotide triphosphates (dNTP's) and primers which permit amplification of specific variable regions in the 16S rRNA gene. Fluorescently tagged dNTP's² are used, and these have reversible terminators to force the primers to attach to only one nucleotide on the template strand per round of dNTP addition (Meyer & Kircher, 2010). After each round, a fluorescent reading is recorded which allows monitoring of the specific nucleotide incorporated. This process is better known as sequencing by synthesis and the process continues until an entire DNA fragment is sequenced (Meyer & Kircher, 2010).

Ishii et al. (2013) proposed the use of next generation sequencing to determine the abundance and diversity of pathogenic bacteria in a water sample, whereafter qPCR analysis could be performed in order to calculate the numbers of the specific dominant pathogens. A study conducted by Chidamba

² Each type of dNTP has a different fluorescent colour.

and Korsten (2015) employed pyrosequencing, targeting the V1 – V3 hypervariable regions of the prokaryotic 16S rRNA gene, in order to assess microbial communities present in rainwater harvesting systems and river water samples in South Africa. From 10 956 identified unique sequences, 95.6 % ($n \approx 10\,474$) were classified into ten respective phyla of the bacterial kingdom. However, 4.2 % ($n \approx 460$) of the sequences could not be assigned to any known phyla. The genera *Acinetobacter*, *Clostridium*, *Chromobacterium*, *Pseudomonas*, *Legionella*, *Serratia* and *Yersinia*, amongst others, were then identified (Chidamba & Korsten, 2015).

In a recent study conducted by Ahmed et al. (2017), amplicon-based Illumina next-generation sequencing was carried out by targeting the V5 – V6 hypervariable regions of the 16S rRNA gene to determine the abundance and diversity of bacterial communities in rainwater samples collected in Brisbane ($n = 44$) and Currumbin ($n = 44$) in South East Queensland, Australia. From the DNA sequences identified from the Brisbane and Currumbin rainwater samples, 86 % and 89 % respectively were classified into 345 bacterial families. Furthermore, 83 % and 88 % of the sequences identified from the Brisbane and Currumbin rainwater samples respectively, were comprised of 1363 genera (Ahmed et al. 2017). Results then indicated that *Burkholderia*, *Chromobacterium*, *Clostridium*, *Legionella*, *Mycobacterium*, *Nocardia* and *Pseudomonas* were the most commonly occurring opportunistic pathogens present in the rainwater samples at both sites.

1.5. Treatment of harvested rainwater

To improve the quality of contaminated water sources, researchers favour the use of cost- and time-effective treatment methods of low maintenance that use no or limited electricity and are easy to implement (Amin et al. 2014). Chlorination, filtration, SODIS and SOPAS have all been considered and implemented as possible treatment strategies for the removal of microbial contaminants in harvested rainwater (Venkobachar et al. 1977; Burch & Thomas, 1998; Edberg et al. 2000; Helmreich & Horn, 2009; McGuigan et al. 2012; De Kwaadsteniet et al. 2013; Amin et al. 2014). However, the most suitable treatment strategy may depend on the physical location of the rainwater harvesting system (i.e. surrounding environmental pollutants and climate) as well as the water quality prior to treatment (Mwabi et al. 2011). For example, the efficiency of a chlorination and filtration system may depend on the turbidity (concentration of suspended solids) and the bacterial load (CFU, size of the microbes as well as their survival strategy) in the stored rainwater. In contrast, the efficiency of SODIS and SOPAS treatment systems are dependent on high solar irradiance levels and the location (north facing, no obstruction to the passage of sunlight) of the treatment site is thus crucial. In addition, the turbidity of the water will greatly influence the efficiency of SODIS as suspended particles (such as organic matter) may inhibit transmittance of UV rays through the water, hindering disinfection efficiency. As turbidity may affect the treatment efficiency of various systems, current research focuses on the pre-treatment of rainwater e.g. implementation of a first flush diverter, in an attempt to minimise the total suspended solid concentration before it enters the

harvesting tank.

1.5.1. First flush diverter

The first consortium of run-off water, also termed the first flush, is considered to have the highest concentration of pollutants (Sánchez et al. 2015). Research has shown that the installation of a downpipe first flush diverter into the gutter system (**Figure 1.4A**) should improve the quality of roof-harvested rainwater as the diverter acts as a filter for the removal of debris and solid material (Lee et al. 2010). During a rain event, the rainwater initially enters the first flush diverter where large debris are trapped by a leaf screen. Smaller pollutants are then diverted into the flush pipe which slowly becomes saturated. When the flush pipe is at maximum capacity, a floating device (usually a plastic ball) acts as a valve and blocks further entry of rainwater into the first flush system. The volume of the flush pipe is generally proportional to the area of the catchment surface (Sánchez et al. 2015) and as the initial highly contaminated water is diverted into the flush pipe, in principle most of the contaminants should be removed (**Figure 1.4B**). In addition, the flush pipe has a small valve situated at the base which allows this pipe to empty after a rain event, minimising maintenance of the first flush system (Texas Totes & Barrels, 2016). The system is generally constructed from readily available materials [e.g. polyvinyl chloride (PVC) or PET] and is regarded as an inexpensive add-on to a rainwater harvesting system. In addition, it is robust and is easy to install (Helmreich & Horn, 2009).

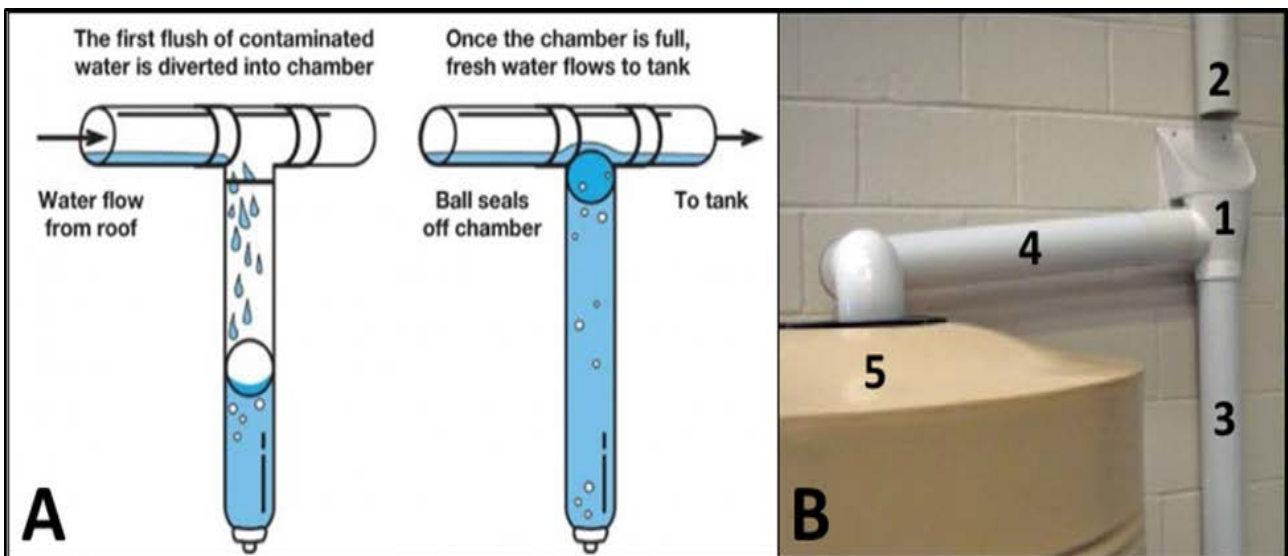


Figure 1.4: A basic first flush diverter. **A)** The initial rainwater is diverted into the flush pipe. The flush pipe slowly fills up and is blocked by a floating ball which acts as a valve. The remaining rainwater (considered cleaner than the first flow of rainwater) is now diverted into the storage tank (adopted from Texas Totes & Barrels, 2016). **B)** Leaf screen (1) which is connected to a vertical gutter (2) from the roof, the flush pipe (3) and diverting pipe (4) leading into the storage tank (5).

For a first flush diverter to function optimally, it is important that the correct volume of the first flush, which should be diverted or discarded, is calculated. This volume depends principally on the intensity

of the rainfall event as well as the number of dry days preceding the event (Sánchez et al. 2015). For example, for a low intensity rainfall event there may be an incomplete wash-off of the catchment surface after the initial rainfall. A study conducted by Egodawatta et al. (2009) showed that only 75 % of all particles present on a catchment surface were removed after 20 mm/h (low intensity) rainfall, while all (100 %) the particles were removed from the catchment surface after a rainfall event of 115 mm/h (high intensity). In addition, Lee et al. (2004) reported that the concentration of pollutants may be up to 20 times higher at the commencement of a wet season when compared to the end of the wet season. This is due to the accumulation of pollutants on the catchment surface during an extended dry period.

While it is generally accepted that first flush diverter systems reduce the initial contamination load in the storage tanks, there are conflicting reports on their efficacy. A study conducted by Gikas and Tsihrintzis (2012) demonstrated that the use of a first flush diverter in a roof rainwater harvesting system did not significantly reduce the microbial contamination in the rainwater. However, an improvement in the physicochemical quality of the water was observed. Studies have also indicated that downstream treatment of harvested rainwater is required even when a first flush diverter is incorporated into the harvesting system, as the microbial quality in particular does not comply with drinking water standards (Doyle, 2008; Mendez et al. 2011). These downstream treatments include SOPAS and SODIS, as well as other methods, as they are considered to be cost-effective antimicrobial strategies (Amin & Han, 2009; Amin et al. 2014).

1.5.2. Solar pasteurization

A SOPAS system relies solely on the thermal effect (minimum 70 °C) originating from solar energy to inactivate microorganisms (Sommer et al. 1997). There are three categories of water heating (SOPAS) systems used for domestic purposes. The first system is a split system consisting of two components, namely a storage tank, where water is heated and a collector system. The collector system is normally placed on top of a roof while the storage tank is installed inside the roof. This allows for a thermo-siphoning effect whereby water is able to circulate through the collector due to differences in temperature (Nieuwoudt & Mathews, 2005). The second system is a closed coupled system which consists of a flat plate collector that heats the water, with an elevated storage tank attached to the end of the collector (Nieuwoudt & Mathews, 2005). The third type is also a closed coupled system which consists of an integrated collector and storage system, or only a collector, used to heat and store the water. The two closed coupled types are passive systems where the water circulates by means of a thermo-siphoning effect (Nieuwoudt & Mathews, 2005).

An example of a simple SOPAS system is the contemporary solar geyser (**Figure 1.5A**), where water fills borosilicate glass tubes that are exposed to solar radiation. The energy obtained from solar radiation is transferred to the water and the temperature increases (Raveendhra et al. 2014). As the water in the tubes heat up, it becomes less dense and moves up into the storage tank by means of

the thermo-siphoning effect. Cold water from the storage tank then moves into the tubes for SOPAS treatment (**Figure 1.5B**). Research has indicated that the time needed to treat water decreases as temperature increases. Thus, the treatment time decreases by a factor of 10 for every 10 °C increase in temperature above 50 °C (Feachem et al. 1983). Furthermore, this system is a cost-effective method that is not influenced by the turbidity or pH of the water (Burch & Thomas, 1998; Dobrowsky et al. 2015). It should however be noted, that while SOPAS treatment systems generally improve the microbial quality of water, it does not improve the chemical quality of treated water (Islam & Johnston, 2006).

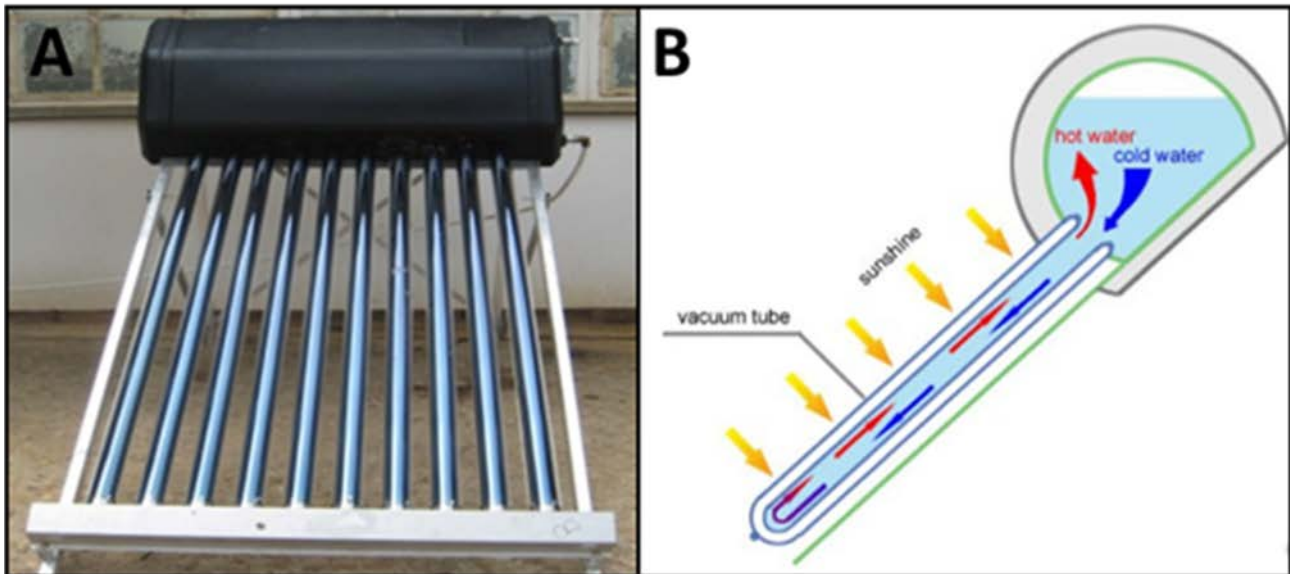


Figure 1.5: **A)** A solar geyser which acts as a SOPAS system (Strauss et al. 2016). **B)** Diagram of the thermo-siphoning effect illustrating the movement of water in a SOPAS system (adapted from Sunflower Solar, 2017).

In a study conducted by Dobrowsky et al. (2015), the efficiency of a closed coupled SOPAS system (Apollo™ system manufactured in China) for the treatment of roof-harvested rainwater was investigated. When the temperature was 72 °C or more, the authors showed that all microbial indicator counts were reduced to below the detection limit. However, opportunistic pathogens including *Klebsiella* spp., *Legionella* spp., *Yersinia* spp. and *Pseudomonas* spp. were still detected by means of the PCR. As referred to in the foregoing section (1.4.), it is recognised that the PCR screens only for the presence/absence of microorganisms. Thus, the viability of the opportunistic pathogens needs to be determined. Furthermore, the authors demonstrated that there was a significant ($p < 0.05$) increase in the concentrations of aluminium, iron, lead and nickel after pasteurization and it was hypothesised that these metals could have leached from the storage tank of the SOPAS system which was constructed from stainless steel (Dobrowsky et al. 2015).

In a follow-up study conducted by the same research group, the authors assessed the viability of *Legionella* spp. by means of EMA-qPCR (Reyneke et al. 2016). When compared with untreated rainwater samples, there was a significant ($p < 0.05$) reduction in viable *Legionella* cells recorded

after SOPAS. However, of concern was the finding that *Legionella* cells remained viable even after pasteurization at a temperature of 95 °C (Reyneke et al. 2016). In addition, the authors showed that after pasteurization, most anions and cations present in the samples occurred at levels compliant with the drinking water guidelines. However, exceptions were aluminium and iron which were present at levels exceeding drinking water guidelines as specified by the DWAF (1996), after treatment (Reyneke et al. 2016).

1.5.3. Solar disinfection

A SODIS system relies on the synergistic effect of direct UV radiation and solar mild-heat to inactivate microorganisms (McGuigan et al. 2012; Amin et al. 2014). This treatment method dates back to the late 1870's when Downes and Blunt (1877) studied the bactericidal effect of sunlight. However, the first successful application of SODIS was recorded by Acra et al. (1980). Thereafter, laboratory and field tests were conducted in 1991 by the Swiss Federal Institute for Environmental Science and Technology (SFIEST) (SFIEST, 2002). Based on this research, it was concluded that SODIS significantly improved the microbial quality of water as it inactivated pathogenic microorganisms that included both protozoa and bacteria. Conversely, anion and cation concentrations may fluctuate after SODIS treatment as mineralisation of organic matter may occur (Gligorovski et al. 2015). However, research has indicated that after SODIS treatment the chemical quality of water sources generally still adheres to drinking water guidelines. Solar disinfection is thus currently recognised and promoted by the WHO as an effective antimicrobial water treatment method and there are an estimated 4.5 million people living predominantly in Africa, Latin America and Asia who use SODIS to treat water contaminated by microorganisms (Alrousan et al. 2012; SFIEST, 2002).

The principle of SODIS is based on solar energy. The sun offers a free sustainable energy source in the form of solar rays (UV-A, UV-B and UV-C) of which UV-C rays (wavelength: 200 – 280 nm) exhibit the most energy and are harmful to all life forms, however it is filtered out by the ozone layer (in the stratosphere). In contrast, UV-B (wavelength: 281 – 320 nm) and UV-A (wavelength: 321 – 400 nm) are characterised as possessing substantial amounts of energy and penetrate the atmosphere. This energy can be harnessed either directly (UV-B) or indirectly (UV-A) by SODIS and is used to treat water contaminated with microbes (Reed, 2004). As nucleic acids (DNA and RNA) and proteins absorb light at wavelengths of 260 nm and 280 nm (Oxford Gene Technology, 2014) respectively, UV-B radiation interferes directly with the genetic material of a cell by causing single nucleotide strand breaks and other nucleic acid modifications both of which may be mutagenic and often lethal (Reed, 2004).

It is well known that DNA repair mechanisms exist within bacterial species and these serve to maintain the integrity of the genome (Žgur-Bertok, 2013). Deoxyribonucleic acid repair mechanisms

in bacteria include photoreactivation³ by the photolyase enzyme, the Adaptive Response (DNA and AidB protein protection in starved cells), the ClpR regulation in *Mycobacteria* and the Damage Response in *Deinococcus radiourans*. The SOS Response is the best elucidated DNA repair mechanism in bacteria and serves multiple purposes among various bacterial species (Kreuzer, 2013). Two key proteins are involved in the SOS Response, viz. LexA (repressor protein) and RecA (inducer protein). These bind to the SOS box which is a consensus palindromic DNA sequence (Butala et al. 2009). When an exogenous trigger (such as UV irradiation, chemicals or antibiotics) or an endogenous trigger (such as metabolic by-products) damage the DNA (Aertsen & Michiels, 2005), RecA is transcribed and binds to the damaged single stranded DNA forming a nucleoprotein complex. This nucleoprotein initiates the cleavage of the repressor protein (LexA) resulting in the transcription of more than 50 genes of the SOS regulon, aiding in bacterial DNA repair (Butala et al. 2009). It should be noted that often the exposure of a bacterium to a continuous high dose of solar radiation will cause irreversible DNA damage and ultimately cell death (Zimmer & Slawson, 2002).

It is however, more common for solar UV to cause indirect cellular damage through UV-A radiation. Photosensitizer molecules occurring in microbial cells include flavins, photosynthetic pigments and porphyrins and these are raised to an excited state once irradiated by UV-A rays (Curtis et al. 1992). The excited molecules directly interact with cellular molecules, known as type I reactions, or with oxygen, resulting in type II reactions. When an excited photosensitizer molecule reacts with oxygen, free radicals [alkoxyl (RO•), hydroperoxyl (HO₂•), hydroxyl (HO•), peroxy (ROO•), superoxide (O₂•⁻)] and non-radicals [hydrogen peroxide (H₂O₂), hypochlorous acid (HOCl) and singlet oxygen (¹O₂)] are formed (Castro-Alferez et al. 2016). These radicals are better known as reactive oxygen species (ROS) and react with cellular constituents such as proteins, DNA, RNA and the cell membrane (especially membrane lipids by lipid peroxidation) (Gourmelon et al. 1994; Castro-Alferez et al. 2016). This can cause an increased cell membrane permeability, followed by disruption of membrane potential gradients thus depleting cellular ATP concentrations. This is generally lethal to the cell. Cells are able to counter the production of ROS and inherently protect against solar radiation damage by the production of antioxidants such as the enzymes catalase and superoxide dismutase. However, it has been shown that these antioxidants are also UV sensitive and are permanently inactivated after being subjected to lethal amounts of UV-A radiation (Kapuscinski & Mitchell, 1981).

Various SODIS systems, ranging from a simple solar cooker to a compound parabolic collector have thus been designed to enhance the SODIS effect. In addition, various additive or photocatalyst supplements are often used with SODIS to increase the bactericidal effect, while simultaneously reducing treatment time.

³ Type of DNA repair mechanism exhibited by bacteria after ultra-violet irradiated damage occurs.

1.5.3.1. Traditional SODIS systems

Traditional SODIS systems are typically used in rural informal settlements where 2 L transparent (PET) bottles are filled with contaminated water and placed in direct sunlight for six to eight hours (up to 48 hours may be required in cloudy weather conditions) (**Figure 1.6**) (Harding & Schwab, 2012). Research groups have demonstrated that such a simple SODIS system improves the microbial quality of harvested rainwater (McGuigan et al. 2006; Amin & Han, 2009; McGuigan et al. 2012; Amin et al. 2014). Furthermore, health impact studies reported a 26 – 37 % decrease in diarrhoeal disease when people used traditional SODIS systems to treat water (Conroy et al. 1996; 1999; 2001; Rose et al. 2006).



Figure 1.6: Transparent (PET) bottles are filled with contaminated water and placed onto rooftops where they are exposed to direct sunlight (adopted from SFIEST, 2002).

Advantages of this system are that a free renewable energy source, the sun, is used to treat water and due to its simplicity, the method can be implemented throughout the world (Safapour & Metcalf, 1999; De Kwaadsteniet et al. 2013). In addition, it is cost-effective as PET bottles, which are readily available and inexpensive, act as “reactors”. However, concern regarding the use of PET bottles as reactors in SODIS has been raised due to the possible leaching of photoproducts from the polymer material after prolonged solar exposure, which could generate genotoxic compounds. Wegelin et al. (2001) however, concluded that there was no migration of photoproducts from the PET material into the water after solar exposure of up to 126 days. In addition, the authors reported that photoproducts were generated on the outer surface of the PET bottles. Similarly, Ubomba-Jaswa et al. (2010a) tested for genotoxic compounds in water stored in PET bottles after solar exposure over a period of six months. Results indicated that in samples where the PET bottles were refilled daily, even after six months of treatment, genotoxicity was absent. In contrast, in samples where water was not replaced on a daily basis, genotoxic compounds were observed after two months exposure. Genotoxic compounds were also detected after two months in untreated control samples where bottles were kept in the dark and the water was not replaced. Thus, it is unlikely that the formation

of genotoxic compounds was related to solar exposure (Ubomba-Jaswa et al. 2010a). As a precaution the authors suggested that PET bottles used during SODIS treatment should be replaced every six months (Ubomba-Jaswa et al. 2010a).

A further disadvantage associated with the use of SODIS treatment is that the efficiency of the traditional SODIS system decreases as the turbidity of the water increases. Water with high Nephelometric Turbidity Units (NTU) usually contains more suspended solids which may prevent solar rays from penetrating the water. Under such conditions, low SODIS efficiency is recorded. Moreover, low solar intensities experienced in poor weather conditions also decrease treatment efficiency (Amin & Han, 2009; McGuigan et al. 2012; Amin et al. 2014).

1.5.3.2. Solar collector disinfection (SOCO-DIS) system/solar cooker

The traditional SODIS system (placing a PET bottle in direct sunlight) concept was improved by the construction of a solar collector that functions to concentrate solar rays onto a water-filled bottle, particularly when solar intensity is low (Amin & Han, 2009). The solar collector consists of four open vertical reflective wings which were constructed at a 30° angle with respect to the horizontal position of the solar collector thus concentrating UV radiation (**Figure 1.7A**). Based on a measured reduction of the indicator organisms *E. coli*, HPC, total and faecal coliforms, Amin and Han (2009) showed that the new enhanced SODIS system, called SOCO-DIS (solar collector disinfection) improved water disinfection efficiency by 20 - 30 % when compared with a traditional SODIS system. It was further demonstrated that as a result of the concentrated sunlight effect, increased disinfection efficiencies were obtained even when sunlight intensity was moderate (Amin & Han, 2009).

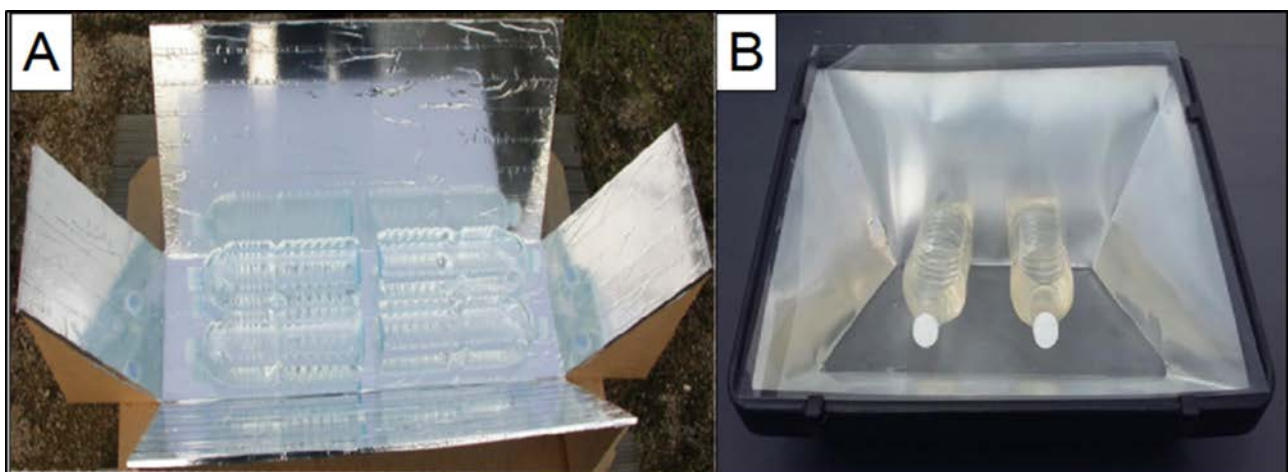


Figure 1.7: **A)** SOCO-DIS system with four open vertical reflective wings constructed at a 30° angle, containing PET bottles filled with harvested rainwater (adopted from Amin & Han, 2009). **B)** A solar cooker, containing 2 L PET bottles, which serves as a SODIS system (Strauss et al. 2016).

A study conducted by Strauss et al. (2016) investigated the microbial inactivation efficiency of a solar cooker SODIS system, which was based on the principles described for the SOCO-DIS system by Amin and Han (2009). **Figure 1.7B** demonstrates the simple design of the solar cooker consisting

of a horizontal rectangular base (painted black to absorb heat and thus increase the temperature of the water) with the inside of the system constructed from a reflective aluminium plate. A black polyethylene material enclosed the vertical panels of the system. In addition, in order to trap heat from solar radiation, the inner section of the system was covered with a transparent Perspex lid. Results obtained from the solar cooker SODIS system used in this study demonstrated that indicator counts (*E. coli*, HPC, total and faecal coliforms) were reduced to below the detection limit of < 1 CFU/mL after six to eight hours of direct sunlight exposure.

Although the systems used by Amin and Han (2009) (SOCO-DIS) and Strauss et al. (2016) (solar cooker) yielded improved SODIS efficiencies when compared with the traditional SODIS system, a few disadvantages hinder the overall efficacy of these systems. A study conducted by Ubomba-Jaswa et al. (2010b) suggested that the nature of material from which the reactor bottle is constructed should be carefully considered as PET has an optical lower limit at 330 nm and only transmits a portion of the UV-A spectra. In addition, a major concern when treating rainwater in PET bottles is that only limited volumes (~5 L) can be treated at one time. This yields insufficient water for a typical household (3-5 people) where the requirement is 25 L per person per day (UN, 2010). Thus, research currently focuses on up-scaling current SODIS technologies in order to treat larger volumes of water and simultaneously improve microbial inactivation efficiencies.

1.5.3.3. Solar disinfection compound parabolic collector (SODIS-CPC) systems

Compound parabolic collectors (CPC) use non-imaging optics to concentrate direct radiation and diffuse radiation into an absorbing reactor without the need to reorient or reposition the CPC system as it tracks the sun throughout the day (Rodriguez et al. 2004; Navntoft et al. 2008). Ubomba-Jaswa et al. (2010b) designed a SODIS-CPC batch reactor system to increase the volume of water that can be treated, together with an improvement in the treatment efficiency (**Figure 1.8A**). The authors used a 25 L methacrylate tube as the reactor (a cylindrical tube filled with water), rather than the smaller volume PET bottles. If PET bottles are used to provide the volume required, a constant supply of these bottles would be needed for the reactor to function effectively at increased volume (**Figure 1.8A**). Although methacrylate is less effective in transmitting UV-A light, when compared with PET and borosilicate glass, the authors justified the choice of reactor material on the premise that methacrylate is a robust material and is cost-effective. Furthermore, the CPC (**Figure 1.8B**) was constructed from a highly reflective anodized aluminium sheet to concentrate available sunlight onto the reactor. This enhanced the solar effect, reducing the time required for disinfection (Ubomba-Jaswa et al. 2009).

Studies have also reported on the use of continuous re-circulating systems to increase the volume of water to be treated by means of SODIS (Ubomba-Jaswa et al. 2010b). However, the flow of water exerted a negative effect on microbial inactivation (Ubomba-Jaswa et al. 2009). Inactivation of microorganisms is more effective when they are exposed to high intensity UV radiation (a lethal

dosage) over a shorter time period in comparison to exposure to repeated sub-lethal doses carried out over longer time intervals (Ubomba-Jaswa et al. 2010b). Furthermore, re-circulating systems require additional maintenance and construction materials as well as an electrical pump to circulate the water, which increases treatment costs.

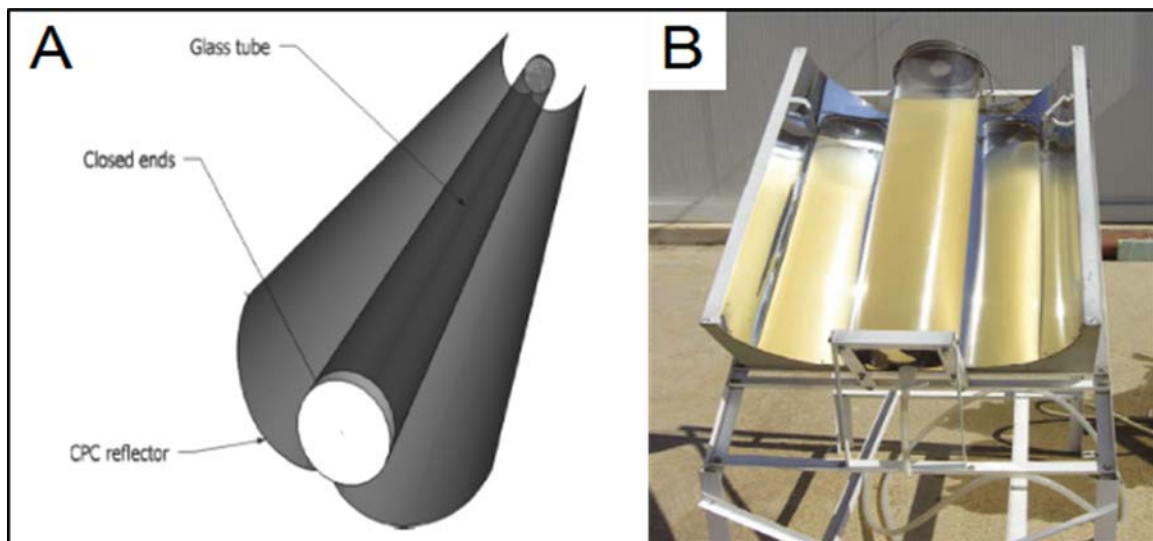


Figure 1.8: **A)** Diagram of a reactor tube placed along the linear focus of a CPC. **B)** A SODIS system fitted with a CPC which can treat up to 25 L of water (adopted from Ubomba-Jaswa et al. 2010b).

1.5.3.4. SODIS supplementation

Numerous studies have reported on the enhancement or acceleration of the SODIS process by using supplementation with readily available and inexpensive additives. These additives include lemon and lime juice, hydrogen peroxide (H_2O_2), vinegar and copper plus ascorbate (Fisher, 2004; Fisher et al. 2008; Harding & Schwab, 2012; McLaughlin et al. 2016). Treatment involves manual dosing of the water with the additive before SODIS treatment. The principle of accelerating SODIS differs depending on which additive is used (Harding & Schwab, 2012). For example, when H_2O_2 is irradiated with UV light, the molecule splits into hydroxyl radicals ($HO\bullet$) and ROS are formed which damage cell membranes (Mamane et al. 2007). In contrast, citrus fruits such as lemons and limes (particularly the peel) contain psoralens, a class of photoactive cyclic biomolecules known to intercalate into DNA double helices (Harding & Schwab, 2012). When psoralens are in the presence of UV-A light, cross-links are formed between parallel DNA strands and this inhibits replication of the microbial genetic material (Cole, 1970; Dall'Acqua et al. 1974; Scott et al. 1976). However, supplementation with certain additives may pose potential health risks when added at high concentrations. For example, hydrogen peroxide can cause systemic toxicity when ingested and leads to asphyxiation. Therefore, low concentrations of additives are generally used and at these concentrations, research has indicated that the effect of the supplement on human health is negligible (Fisher et al. 2008).

Laboratory and field experiments then indicated that the use of additives significantly decreases disinfection time, in both sunny and cloudy weather conditions. However different additives exhibit

varied inactivation efficiencies on microorganisms (Harding & Schwab, 2012). For example, Harding and Schwab (2012) investigated the inactivation effect of lime slurry, lime juice and 5-methoxypsoralen (synthetic psoralens) on *E. coli*, MS2 bacteriophages and murine norovirus under natural sunlight conditions. Results indicated that *E. coli* numbers were significantly reduced (> 6.1 log) by SODIS in combination with lime slurry after 30 minutes of treatment. In contrast, MS2 bacteriophages were only reduced by 3.9 logs after 30 minutes of SODIS and lime slurry treatment, while a 1.8 log reduction in murine norovirus was recorded (Harding & Schwab, 2012). Overall however, SODIS used in combination with lime slurry yielded the best inactivation efficiencies when compared with results obtained for SODIS in combination with lime juice and 5-methoxypsoralen.

The major disadvantage of the supplementation of SODIS with additives is that the contaminated water should be consistently dosed with the additive. Therefore, current research focuses on the addition of a photocatalyst, such as titanium dioxide, which is fixed into the reactor and can be used repeatedly. The ability of titanium dioxide (TiO₂) to act as a photocatalyst is well documented in literature and was studied from the early 1920's (Foster et al. 2011). The compound is widely used as a disinfectant in dental implants, paint, on catheters to prevent urinary tract infections, in cosmetics and as a chalking agent (Mo et al. 2007; Allen et al. 2008; Yao et al. 2008). Titanium dioxide is also extensively applied as an agent on self-cleaning surfaces, such as windows, as it has super-hydrophilic properties allowing for the easy removal of dust and dirt particles (Chen & Poon, 2009). In addition, it is applied as a disinfectant in water, air and on surfaces (Foster et al. 2011) as numerous studies have shown that TiO₂ has the ability to catalyse the oxidation of pollutants (such as organic matter) and inactivate microorganisms (Sichel et al. 2007; Chen et al. 2008; Kozlova et al. 2010). The effectiveness of TiO₂ in the inactivation of a wide range of prokaryotic as well as eukaryotic microorganisms including Gram-positive (Kim et al. 2003) and Gram-negative bacteria (Luo et al. 2008), algae (Peller et al. 2007), fungi (Kühn et al. 2003) and protozoa (Sökmen et al. 2008), has been demonstrated. It has also been reported to effectively inactivate mammalian viruses (Lin et al. 2006; Kozlova et al. 2010) and bacteriophages (Guillard et al. 2008). However, TiO₂ is less effective in inactivating the dormant stages of microorganisms such as endospores, fungal spores and protozoan cysts. This is due to the resilient cell wall of these structures (Foster et al. 2011).

1.6. Project aims

Water scarcity is one of the major problems faced by the world today and approximately 319 million people in sub-Saharan Africa use inadequately treated and contaminated water sources. Domestic rainwater harvesting has thus been earmarked by numerous countries as a supplementary water supply and has been identified by the South African government as an alternative and sustainable water source that could provide water directly to households (Amin & Han, 2009; De Kwaadsteniet et al. 2013). Rainwater is considered a pure water source, however, during the harvesting process, it can become polluted with microorganisms and atmospheric particles such as organic and inorganic

matter (e.g. heavy metals and dust) (Helmreich & Horn, 2009; Abbasi & Abbasi, 2011; De Kwaadsteniet et al. 2013). Numerous studies have thus shown that the chemical and microbial quality of harvested rainwater is compromised and does not adhere to recommended potable water guidelines (Ahmed et al. 2008; Huston et al. 2012; Dobrowsky et al. 2014a; Strauss et al. 2016).

Preliminary treatment of rainwater can be achieved by diverting the first flush of harvested rain, which is thought to contain higher levels of microbial and chemical pollutants (Yaziz et al. 1989). However, primary treatment methods, such as SOPAS and SODIS are required to effectively inactivate specifically pathogenic microorganisms. Solar disinfection is an efficient and cost-effective treatment method and is widely used in northern and sub-Saharan Africa for the treatment of harvested rainwater (Amin & Han, 2009; Amin et al. 2014). While numerous SODIS reactor types have been developed and optimised, current studies focus on the production of SODIS batch reactor systems constructed from inexpensive materials to effectively treat large volumes of water. It is however, essential to accurately monitor the disinfection efficiency of these batch reactor SODIS systems in order to determine whether the water quality is acceptable and fit for human consumption. Whole microbial community profile analysis in combination with viability assays is thus required.

The primary aim of the current study was to design and construct a pilot-scale SODIS batch system fitted with a compound parabolic collector (CPC). Requirements for the enhanced SODIS batch system designed and used for the study were that it was: (i) constructed from cost-effective materials; (ii) robust in nature in order to withstand adverse environmental conditions and (iii) required minimum maintenance.

Objective 1 (**Chapter 2**): Compound parabolic collector solar disinfection system for the treatment of harvested rainwater.

- A pilot scale experiment was conducted using a UV-vis spectrophotometer which measured UV transmittance through borosilicate glass and acrylic plastic. This was done in order to determine which of the two locally produced materials was more suitable for the construction of the reactor.
- A CPC frame was designed. This consisted of two mirrored arches with variable radii, lined with a reflective stainless steel sheet. On the central linear plane of the CPC, a 10.6 L volume borosilicate glass tube was fitted to the system. This functioned as the treatment reactor.
- After completion of the design and construction of the system, a pilot scale study was performed to monitor and compare overall water quality. This incorporated the use of two SODIS systems exposed to eight hours of solar radiation. The first system was connected to a rainwater harvesting tank only, while the second system was connected to a rainwater harvesting tank which was in turn connected to a first flush (FF) diverter.

- The chemical and microbial quality of water samples collected from the two tanks [without FF – Tank 1, and with FF – Tank 2 (FF)] and reactors [SODIS treated rainwater – SODIS-CPC-1, and FF SODIS treated rainwater –SODIS-CPC-2 (FF)] were determined.
- The chemical quality of untreated (collected directly from the rainwater tanks) and SODIS treated rainwater samples was determined by monitoring cation and anion concentrations. The turbidity was also measured.
- For microbial analysis before and after SODIS treatment of the rainwater, the enumeration of traditional indicator bacteria such as *E. coli*, total and faecal coliforms, enterococci and heterotrophic bacteria was determined by using selective growth media for each indicator microorganism.
- Ethidium monoazide bromide quantitative polymerase chain reaction analysis was performed to quantify the number of viable *Pseudomonas* and *Legionella* copy numbers present in both treated and untreated rainwater samples.

Objective 2 (**Chapter 3**): EMA-Amplicon-based taxonomic characterisation of the viable bacterial community present in untreated and SODIS treated roof-harvested rainwater:

*Only samples collected from Tank 1 and SODIS-CPC-1 during sampling sessions 1, 2, 3, 4 and 7 were used in Chapter 3 to investigate the principle of EMA combined with Illumina.

- Prior to DNA extractions, 1 L of concentrated rainwater samples (Tank 1 and SODIS-CPC-1) were pre-treated with the nucleic acid binding dye EMA (6 μ M) for the detection of intact (viable) cells.
- After DNA extraction, the hypervariable region three to hypervariable region four (V3 –V4) of the bacterial 16S rRNA gene originating from all intact (viable) bacterial species, was amplified. Library construction and sequencing, using the 16S MiSeq Illumina platform was then performed to determine the abundance and diversity of the viable bacterial population (both culturable and non-culturable) present in the samples. In addition, four control samples were included as follows, samples from Tank 1 and SODIS-CPC-1 respectively, which were not EMA treated, and samples from Tank 1 and SODIS-CPC-1 respectively, which were EMA treated.
- The relative abundance and diversity of the viable bacterial community present in the untreated (Tank1) and SODIS treated (SODIS-CPC-1) rainwater samples was determined, using alpha-diversity indices [such as species richness, Shannon diversity index and abundance-based coverage estimator (ACE)], to investigate the effect of SODIS on the viable bacterial community.

- Differences in bacterial community composition (beta-diversity) were evaluated through permutational multivariate analysis of variance (PERMANOVA) in order to investigate whether the viable bacterial community differs after SODIS treatment.
- Taxonomic assignments were further used to detect and identify potential pathogenic bacterial genera which were still viable after SODIS treatment.

1.7. References

Abbasi, T. & Abbasi, S.A. 2011. Sources of pollution in rooftop rainwater harvesting systems and their control. *Critical Reviews in Environmental Science and Technology*. 41(23):2097–2167.

Abdulla, F.A. & Al-Shareef, A.W. 2009. Roof rainwater harvesting systems for household water supply in Jordan. *Desalination*. 243(1–3):195–207.

Abo-Shehada, M.N., Hindyia, M. & Saiah, A. 2004. Prevalence of *Cryptosporidium parvum* in private drinking water cisterns in Bani-Kenanah district, northern Jordan. *International Journal of Environmental Health Research*. 14(5):351–358.

Acra, A., Karahagopian, Y., Raffoul, Z. & Dajani, R. 1980. Disinfection of oral rehydration solutions by sunlight. *The Lancet*. 316(8206):1257-1258.

Adler, I., Campos, L. & Bell, S. 2014. In: *Community participation in decentralised rainwater systems: A Mexican case study. Alternative water supply systems*. F.A. Memon, S. Ward, Eds. London, England: IWA Publishing.117-130.

Aertsen, A. & Michiels, C.W. 2005. Mrr instigates the SOS response after high pressure stress in *Escherichia coli*. *Molecular Microbiology*. 58(5):1381-1391.

Agency for Toxic Substances and Disease Registry. 2000. *Toxicological profile for arsenic*. Available: <https://www.atsdr.cdc.gov/> [2017, February 9].

Agustí, G., Fittipaldi, M., Morató, J. & Codony, F. 2013. Viable quantitative PCR for assessing the response of *Candida albicans* to antifungal treatment. *Applied Microbiology and Biotechnology*. 97(1):341-349.

Ahmed, W., Huygens, F., Goonetilleke, A. & Gardner, T. 2008. Real-time PCR detection of pathogenic microorganisms in roof-harvested rainwater in Southeast Queensland, Australia. *Applied and Environmental Microbiology*. 74(17):5490–5496.

Ahmed, W., Goonetilleke, A. & Gardner, T. 2010. Implications of faecal indicator bacteria for the microbiological assessment of roof-harvested rainwater quality in Southeast Queensland, Australia. *Canadian Journal of Microbiology*. 56(6):471–479.

- Ahmed, W., Gardner, T. & Toze, S. 2011. Microbiological quality of roof-harvested rainwater and health risks: A review. *Journal of Environment Quality*. 40(1):13–21.
- Ahmed, W., Hodgers, L., Sidhu, J.P.S. & Toze, S. 2012. Fecal indicators and zoonotic pathogens in household drinking water taps fed from rainwater tanks in Southeast Queensland, Australia. *Applied and Environmental Microbiology*. 78(1):219–226.
- Ahmed, W., Brandes, H., Gyawali, P., Sidhu, J.P.S. & Toze, S. 2014. Opportunistic pathogens in roof-captured rainwater samples, determined using quantitative PCR. *Water Research*. 53:361–369.
- Ahmed, W., Staley, C., Hamilton, K.A., Beale, D.J., Sadowsky, M.J., Toze, S. & Haas, C.N. 2017. Amplicon-based taxonomic characterization of bacteria in urban and peri-urban roof-harvested rainwater stored in tanks. *Science of the Total Environment*. 576:326-334.
- Al-Salaymeh, A., Al-Khatib, I.A. & Arafat, H.A. 2011. Towards sustainable water quality: Management of rainwater harvesting cisterns in Southern Palestine. *Water Resources Management*. 25(6):1721–1736.
- Albrechtsen, H.J. 2002. Microbiological investigations of rainwater and graywater collected for toilet flushing. *Water Science and Technology*. 46(6–7):311–316.
- Allen, M.J., Edberg, S.C. & Reasoner, D.J. 2004. Heterotrophic plate count bacteria—what is their significance in drinking water?. *International Journal of Food Microbiology*. 92(3):265–274.
- Allen, N.S., Edge, M., Verran, J., Stratton, J., Maltby, J. & Bygott, C. 2008. Photocatalytic titania based surfaces: environmental benefits. *Polymer Degradation and Stability*. 93(9):1632-1646.
- Alrousan, D.M.A., Polo-López, M.I., Dunlop, P.S.M., Fernández-Ibáñez, P. & Byrne, J.A. 2012. Solar photocatalytic disinfection of water with immobilised titanium dioxide in re-circulating flow CPC reactors. *Applied Catalysis B: Environmental*. 128:126-134.
- Amin, M.T. & Han, M.Y. 2009. Roof-harvested rainwater for potable purposes: Application of solar collector disinfection (SOCO-DIS). *Water Research*. 43(20):5225–5235.
- Amin, M.T., Nawaz, M., Amin, M.N. & Han, M. 2014. Solar disinfection of *Pseudomonas aeruginosa* in harvested rainwater: A step towards potability of rainwater. *PLoS ONE*. 9(3):1–10.
- Arvanitidou, M., Kanellou, K. & Vagiona, D.G. 2005. Diversity of *Salmonella* spp. and fungi in Northern Greek rivers and their correlation to faecal pollution indicators. *Environmental Research*. 9:278–284.

- Baker, S., Grygorcewicz, E., Opperman, G. & Ward, V. 2007. *Rainwater harvesting in informal settlements of Windhoek, Namibia*. An interactive qualifying project report. Massachusetts, USA: Worcester Polytechnic Institute. Available: <https://web.wpi.edu/Pubs/E-project/Available/E-project-051207-152911/unrestricted/report.pdf> [2017, July 27].
- Benson, R.F. & Fields, B.S. 1998. Classification of the genus *Legionella*. *Seminars in Respiratory Infection*. 13(2):90-99.
- Borella, P., Guerrieri, E., Marchesi, I., Bondi, M. & Messi, P. 2005. Water ecology of *Legionella* and protozoan: environmental and public health perspectives. *Biotechnology Annual Review*. 11:355–380.
- Botes, A. 2012. *Green building interventions for low cost housing demonstrated by Cato Manor Green Street project*. Available: <http://urbanearth.co.za/articles/green-building-interventions-low-cost-housing-demonstrated-cato-manor-green-street-project> [2017, July 30].
- Broadhead, A.N., Negron-Alvira, A., Baez, L.A., Hazen, T.C. & Canoy, M.J. 1988. Occurrence of *Legionella* species in tropical rainwater cisterns. *Caribbean Journal of Science*. 24:71–73.
- Bruin, J.P., Ijzerman, E.P., Den Boer, J.W., Mouton, J.W. & Diederens, B.M. 2012. Wild- type MIC distribution and epidemiological cut-off values in clinical *Legionella pneumophila* Serogroup 1 isolates. *Diagnostic Microbiology and Infectious Disease*. 72(1):103-108.
- Burch, J.D. & Thomas, K.E. 1998. Water disinfection for developing countries and potential for solar thermal pasteurization. *Solar Energy*. 64(1):87-97.
- Butala, M., Žgur-Bertok, D. & Busby, S.J. 2009. The bacterial LexA transcriptional repressor. *Cellular and Molecular Life Sciences*. 66(1):82-93.
- Cabral, J. 2010. Water microbiology. Bacterial pathogens and water. *International Journal of Environmental Research and Public Health*. 7(10):3657-3703.
- Campese, C., Roche, D., Clément, C., Fierobe, F., Jarraud, S., De Waelle, P., Perrin, H. & Che, D. 2010. Cluster of Legionnaires disease associated with a public whirlpool spa, France, April–May 2010. *Eurosurveillance*. 15(26):1-3.
- Castro-Alfárez, M., Polo-López, M.I. & Fernández-Ibáñez, P. 2016. Intracellular mechanisms of solar water disinfection. *Scientific Reports*. 6:38145.
- Centers for Disease Control and Prevention. 2016. *Campylobacter - Technical information*. Available: <https://www.cdc.gov/foodsafety/diseases/campylobacter/technical.html> [2017, March 11].

Chapman, H., Cartwright, T., Huston, R. & O'Toole, J. 2008. *Water quality and health risks from urban rainwater tanks. Cooperative Research Centre for Water Quality and Treatment Research Report No 42*. Salisbury, Australia: Cooperative Research Centre for Water Quality and Treatment. ISBN 18766166787.

Chen, J. & Poon, C.S. 2009. Photocatalytic construction and building materials: from fundamentals to applications. *Building and Environment*. 44(9):1899-1906.

Chen, W.J., Tsai, P.J. & Chen, Y.C. 2008. Functional Fe₃O₄/TiO₂ core/shell magnetic nanoparticles as photokilling agents for pathogenic bacteria. *Small*. 4(4):485-491.

Chidamba, L. 2015. Microbial quality of rainwater harvested from rooftops, for domestic use and homestead food gardens. Ph.D. Dissertation. University of Pretoria.

Chidamba, L. & Korsten, L. 2015. Pyrosequencing analysis of roof-harvested rainwater and river water used for domestic purposes in Luthengele village in the Eastern Cape Province of South Africa. *Environmental Monitoring and Assessment*. 187(41):1-17.

City of Cape Town. 2017. *Water Dashboard*. Available: http://resource.capetown.gov.za/documentcentre/Documents/City_%20research_%20reports_%20and_%20review/damlevels.pdf [2017, Desember 06].

Coetzee, N., Duggal, H., Hawker, J., Ibbotson, S., Harrison, T.G., Phin, N., Laza-Stanca, V., Johnston, R., Iqbal, Z., Rehman, Y. & Knapper, E. 2012. An outbreak of Legionnaires' disease associated with a display spa pool in retail premises, Stoke-on-Trent, United Kingdom, July 2012. *Eurosurveillance*. 17(37):1-4.

Cole, R.S. 1970. Light-induced cross-linking of DNA in the presence of a furocoumarin (psoralen): Studies with phage λ , *Escherichia coli*, and mouse leukemia cells. *Biochimica et Biophysica Acta (BBA)-Nucleic Acids and Protein Synthesis*. 217(1):30-39.

Conroy, R.M., Elmore-Meegan, M., Joyce, T., McGuigan, K.G. & Barnes, J. 1996. Solar disinfection of drinking water and diarrhoea in Maasai children: a controlled field trial. *The Lancet*. 348(9043):1695-1697.

Conroy, R.M., Meegan, M.E., Joyce, T., McGuigan, K. & Barnes, J. 1999. Solar disinfection of water reduces diarrhoeal disease: an update. *Archives of Disease in Childhood*. 81(4):337-338.

Conroy, R.M., Meegan, M.E., Joyce, T., McGuigan, K. & Barnes, J. 2001. Solar disinfection of drinking water protects against cholera in children under 6 years of age. *Archives of Disease in Childhood*. 85(4):293-295.

- Coutinho, H.D.M., Falcão-Silva, V.S. & Gonçalves, F.G. 2008. Pulmonary bacterial pathogens in cystic fibrosis patients and antibiotic therapy: a tool for the health workers. *International Archives of Medicine*. 1(24):1-7.
- Crabtree, K.D., Ruskin, R.H., Shaw, S.B. & Rose, J.B. 1996. The detection of *Cryptosporidium* oocysts and *Giardia* cysts in cistern water in the U.S. Virgin Islands. *Water Research*. 30(1):208–216.
- Curtis, T.P., Mara, D.D. & Silva, S.A. 1992. Influence of pH, oxygen, and humic substances on ability of sunlight to damage faecal coliforms in waste stabilization pond water. *Applied and Environmental Microbiology*. 58(4):1335-1343.
- Dall'Acqua, F., Marciani, S., Vedaldi, D. & Rodighiero, G. 1974. Studies on the photoreactions (365 nm) between DNA and some methylpsoralens. *Biochimica et Biophysica Acta (BBA)-Nucleic Acids and Protein Synthesis*. 353(3):267-273.
- Davidson, F.W., Whitney, H.G. & Tahlan, K. 2015. Genome sequences of *Klebsiella variicola* isolates from dairy animals with bovine mastitis from Newfoundland, Canada. *Genome Announcements*. 3(5):1-2.
- De Kwaadsteniet, M., Dobrowsky, P.H., Van Deventer, A., Khan, W. & Cloete, T.E. 2013. Domestic rainwater harvesting: Microbial and chemical water quality and point-of-use treatment systems. *Water, Air, and Soil Pollution*. 224(7):1-19.
- Department of Water Affairs and Forestry (DWAf). 1996. *South African water quality guidelines 2nd ed.* Volume 1: Domestic Water Use. Pretoria: CSIR Environmental Services.
- Despins, C., Farahbakhsh, K. & Leidl, C. 2009. Assessment of rainwater quality from rainwater harvesting systems in Ontario, Canada. *Journal of Water Supply: Research and Technology—AQUA*. 58(2):117-134.
- Dillaha, T.A. & Zolan, W.J. 1985. Rainwater catchment water quality in Micronesia. *Water Research*. 19:741–746.
- Dobrowsky, P.H., Mannel, D., De Kwaadsteniet, M., Prozesky, H., Khan, W. & Cloete, T.E. 2014a. Quality assessment and primary uses of harvested rainwater in Kleinmond, South Africa. *Water SA*. 40(3):401–406.
- Dobrowsky, P.H., De Kwaadsteniet, M., Cloete, T.E. & Khan, W. 2014b. Distribution of indigenous bacterial pathogens and potential pathogens associated with roof-harvested rainwater. *Applied and Environmental Microbiology*. 80(7):2307–2316.

- Dobrowsky, P.H., Carstens, M., De Villiers, J., Cloete, T.E. & Khan, W. 2015. Efficiency of a closed-coupled solar pasteurization system in treating roof-harvested rainwater. *Science of the Total Environment*. 536:206–214.
- Dobrowsky, P.H., Khan, S., Cloete, T.E. & Khan, W. 2017a. Microbial and physico-chemical characteristics associated with the incidence of *Legionella* spp. and *Acanthamoeba* spp. in rainwater harvested from different roofing materials. *Water, Air & Soil Pollution*. 228(85):1-13.
- Dobrowsky, P.H., Khan, S. & Khan, W. 2017b. Resistance of *Legionella* and *Acanthamoeba mauritaniensis* to heat treatment as determined by relative and quantitative polymerase chain reactions. *Environmental Research*. 158:82-93.
- Donhauser, S.C., Niessner, R. & Seidel, M. 2011. Sensitive quantification of *Escherichia coli* O157:H7, *Salmonella enterica*, and *Campylobacter jejuni* by combining stopped polymerase chain reaction with chemiluminescence flow-through DNA microarray analysis. *Analytical Chemistry*. 83(8):3153-3160.
- Downes, A. & Blunt, T.P. 1877. Researches on the effect of light upon bacteria and other organisms. *Proceedings of the Royal Society of London*. 26(179-184):488-500.
- Doyle, K.C. 2008. Sizing the first flush and its effect on the storage-reliability-yield behaviour of rainwater harvesting in Rwanda. Ph.D. Dissertation. Massachusetts Institute of Technology.
- Dusserre, E., Ginevra, C., Hallier-Soulier, S., Vandenesch, F., Festoc, G., Etienne, J., Jarraud, S. & Molmeret, M. 2008. A PCR-based method for monitoring *Legionella pneumophila* in water samples detects viable but noncultivable legionellae that can recover their cultivability. *Applied and Environmental Microbiology*. 74(15):4817-4824.
- Eberhart-Philips, J., Walker, N., Garrett, N., Bell, D., Sinclair, D., Rainger, W. & Bates, M. 1997. Campylobacteriosis in New Zealand: results of a case-control study. *Journal of Epidemiology and Community Health*. 51(6):686–691.
- Edberg, S.C., Rice, E.W., Karlin, R.J. & Allen, M.J. 2000. *Escherichia coli*: the best biological drinking water indicator for public health protection. *Journal of Applied Microbiology*. 88(S1):106–116.
- Edelstein, P. & Cianciotto, N. 2010. *Legionella. Principles and practice of infectious diseases*. 7th ed. G.L. Mandell, J.E. Bennett & R. Dolin, Eds. Elsevier Churchill Livingstone, Philadelphia, PA. 2:2969-2984.
- Egodawatta, P., Thomas, E. & Goonetilleke, A. 2009. Understanding the physical processes of pollutant build-up and wash-off on roof surfaces. *Science of the Total Environment*. 407(6):1834-1841.

- Enniful, J. 2013. Rainwater Harvesting: A sustainable practice for low- income housing in South Africa. Ph.D. Dissertation. University of Witwatersrand.
- Evans, C.A., Coombes, P.J. & Dunstan, R.H. 2006. Wind, rain and bacteria: The effect of weather on the microbial composition of roof-harvested rainwater. *Water Research*. 40(1):37–44.
- Feachem, R.E., Bradley, D.J., Garelick, H. & Mara, D.D. 1983. *Sanitation and disease: Health aspects of excreta and wastewater management*. New York, USA: Wiley.
- Fields, B.S. 1996. The molecular ecology of Legionellae. *Trends in Microbiology*. 4(7):286–290.
- Fields, B.S., Benson, R.F. & Besser, R.E. 2002. *Legionella* and Legionnaires' disease: 25 years of investigation. *Clinical Microbiology Reviews*. 15(3):506-526.
- Fisher, M.B. 2004. Speeding up solar disinfection: Effects of hydrogen peroxide, temperature, and copper plus ascorbate on the photoinactivation of *E. coli* in Charles River water. Ph.D. Dissertation. Massachusetts Institute of Technology.
- Fisher, M.B., Keenan, C. R., Nelson, K.L. & Voelker, B.M. 2008. Speeding up solar disinfection (SODIS): effects of hydrogen peroxide, temperature, pH, and copper plus ascorbate on the photoinactivation of *E. coli*. *Journal of Water and Health*. 6(1):35-51.
- Fittipaldi, M., Nocker, A. & Codony, F. 2012. Progress in understanding preferential detection of live cells using viability dyes in combination with DNA amplification. *Journal of Microbiological Methods*. 91(2):276-289.
- Foster, H.A., Ditta, I.B., Varghese, S. & Steele, A. 2011. Photocatalytic disinfection using titanium dioxide: spectrum and mechanism of antimicrobial activity. *Applied Microbiology and Biotechnology*. 90(6):1847-1868.
- Franklin, L.J., Fielding, J.E., Gregory, J., Gullan, L., Lightfoot, D., Poznanski, S.Y. & Vally, H. 2009. An outbreak of *Salmonella typhimurium* 9 at a school camp linked to contamination of rainwater tanks. *Epidemiology and Infection*. 137(3):434-440.
- Fujioka, R.S., Inserra, S.G. & Chinn, R.D. 1991. The bacterial content of cistern waters in Hawaii. In: *Proceedings of the Fifth International Conference on Rain Water Cistern Systems*. Keelung, Taiwan. 33-45.
- Garau, J., Fritsch, A., Arvis, P. & Read, R. 2010. Clinical efficacy of moxifloxacin versus comparator therapies for community-acquired pneumonia caused by *Legionella* spp. *Journal of Chemotherapy*. 22(4):264-266.

- Gauthier, F. & Archibald, F. 2001. The ecology of “fecal indicator” bacteria commonly found in pulp and paper mill water systems. *Water Research*. 35:2207–2218.
- Giamarellou, H. 2002. Prescribing guidelines for severe *Pseudomonas* infections. *Journal of Antimicrobial Chemotherapy*. 49(2):229-233.
- Gikas, G.D. & Tsihrintzis, V.A. 2012. Assessment of water quality of first-flush roof runoff and harvested rainwater. *Journal of Hydrology*. 466–467:115-126.
- Gligorovski, S., Strekowski, R., Barbati, S. & Vione, D. 2015. Environmental implications of hydroxyl radicals (\bullet OH). *Chemical Reviews*. 115(24): 13051-13092.
- Global Development Research Centre. 2017. *Rainwater harvesting and utilisation*. Available: <http://www.gdrc.org/uem/water/rainwater/rainwaterguide.pdf> [2017, July 18].
- Gordon, D.M. & FitzGibbon, F. 1999. The distribution of enteric bacteria from Australian mammals: host and geographical effects. *Microbiology*. 145:2663–2671.
- Gould, J. & Nissen-Petersen, E. 1999. *Rainwater catchment systems for domestic supply: design, construction and implementation*. London: Intermediate Technology Publications.
- Gourmelon, M., Cillard, J. & Pommepeuy, M. 1994. Visible light damage to *Escherichia coli* in seawater: oxidative stress hypothesis. *Journal of Applied Microbiology*. 77(1):105-112.
- Graman, P.S., Quinlan, G.A. & Rank, J.A. 1997. Nosocomial legionellosis traced to a contaminated ice machine. *Infection Control & Hospital Epidemiology*. 18(9):637-640.
- Grimont, F., Grimont, P.A.D. & Richard, C. 2003. The genus *Klebsiella*. In: *The prokaryotes: an evolving electronic resource for the microbiological community*, 3rd ed. M. Dworkin, S. Falkow, E. Rosenberg. Eds. New York, NY, USA: Springer-Verlag.
- Grimont, P.A.D. & Grimont, F. 2005. Genus *Klebsiella*. In: *Bergey’s manual of systematic bacteriology*, 2nd ed. D.J. Brenner, N.R. Krieg, J.T. Staley. Eds. New York, NY, USA: Springer: Vol 2, Part B. 685–693.
- Guillard, C., Bui, T.H., Felix, C., Moules, V., Lina, B. & Lejeune, P. 2008. Microbiological disinfection of water and air by photocatalysis. *Comptes Rendus Chimie*. 11(1):107-113.
- Guion, C.E., Ochoa, T.J., Walker, C.M., Barletta, F. & Cleary, T.G. 2008. Detection of diarrheagenic *Escherichia coli* by use of melting-curve analysis and real-time multiplex PCR. *Journal of Clinical Microbiology*. 46(5):1752-1757.
- Gwenzi, W., Dunjana, N., Pisa, C., Tauro, T. & Nyamadzawo, G. 2015. Water quality and public

health risks associated with roof rainwater harvesting systems for potable supply: Review and perspectives. *Sustainability of Water Quality and Ecology*. 6:107–118.

Haas, C.N., Rose, J.B. & Gerba, C.P. 1999. *Quantitative microbial risk assessment*. USA: John Wiley & Sons, Inc.

Handia, L., Tembo, J.M. & Mwiindwa, C. 2003. Potential of rainwater harvesting in urban Zambia. *Physics and Chemistry of the Earth*. 28(20–27):893–896.

Handia, L. 2005. Operational paper comparative study of rainwater quality in urban Zambia. *Journal of Water Supply: Research and Technology- AQUA*. 54:55–64.

Harding, A.S. & Schwab, K.J. 2012. Using limes and synthetic psoralens to enhance solar disinfection of water (SODIS): a laboratory evaluation with norovirus, *Escherichia coli*, and MS2. *The American Journal of Tropical Medicine and Hygiene*. 86(4):566-572.

Helmreich, B. & Horn, H. 2009. Opportunities in rainwater harvesting. *Desalination*. 248(1–3):118–124.

Huhulescu, S., Simon, M., Lubnow, M., Kaase, M., Wewalka, G., Pietzka, A.T., Stöger, A., Ruppitsch, W. & Allerberger, F. 2011. Fatal *Pseudomonas aeruginosa* pneumonia in a previously healthy woman was most likely associated with a contaminated hot tub. *Infection*. 39(3):265-269.

Huston, R., Chan, Y.C., Gardner, T., Shaw, G. & Chapman, H. 2009. Characterisation of atmospheric deposition as a source of contaminants in urban rainwater tanks. *Water Research*. 43(6):1630–1640.

Huston, R., Chan, Y.C., Chapman, H., Gardner, T. & Shaw, G. 2012. Source apportionment of heavy metals and ionic contaminants in rainwater tanks in a subtropical urban area in Australia. *Water Research*. 46(4):1121–1132.

Ibraimo, N. & Munguambe, P. 2007. *Rainwater harvesting technologies for small scale rainfed agriculture in arid and semi-arid areas*. Limpopo Basin (Project CP 17). Maputo, Mozambique: Department of Rural Engineering, University Eduardo Mondlane.

International Agency for Research on Cancer. 1973. *Certain polycyclic aromatic hydrocarbons and heterocyclic compounds*. IARC Monographs on the Evaluation of Carcinogenic Risk of Chemicals to Humans, Vol 3. 5-11 December 1972. Lyon, France: International Agency for Research on Cancer.

Ishii, S., Segawa, T. & Okabe, S. 2013. Simultaneous quantification of multiple food and waterborne pathogens by use of microfluidic quantitative PCR. *Applied and Environmental Microbiology*. 79(9):2891-2898.

- Islam, M.F. & Johnston, R.B. 2006. Household pasteurization of drinking-water: the Chulli water-treatment system. *Journal of Health, Population and Nutrition*. 24(3): 356-362.
- Jaksik, R., Iwanaszko, M., Rzeszowska-Wolny, J. & Kimmel, M., 2015. Microarray experiments and factors which affect their reliability. *Biology Direct*. 10(46):1-14.
- Jaluria, P., Konstantopoulos, K., Betenbaugh, M. & Shiloach, J. 2007. A perspective on microarrays: current applications, pitfalls, and potential uses. *Microbial Cell Factories*. 6(4):1-14.
- Kapuscinski, R.B. & Mitchell, R. 1981. Solar radiation induces sub-lethal injury in *Escherichia coli* in seawater. *Applied and Environmental Microbiology*. 41(3):670-674.
- Kaushik, R., Balasubramanian, R. & De La Cruz, A.A. 2012. Influence of air quality on the composition of microbial pathogens in fresh rainwater. *Applied and Environmental Microbiology*. 78:2813-2818.
- Kim, B., Kim, D., Cho, D. & Cho, S. 2003. Bactericidal effect of TiO₂ photocatalyst on selected food-borne pathogenic bacteria. *Chemosphere*. 52(1):277-281.
- Kittinger, C., Lipp, M., Baumert, R., Folli, B., Koraimann, G., Toplitsch, D., Liebmann, A., Grisold, A.J., Farnleitner, A.H., Kirschner, A. & Zarfel, G. 2016. Antibiotic resistance patterns of *Pseudomonas* spp. isolated from the River Danube. *Frontiers in Microbiology*. 7:1–8.
- Knezevic, P., Obreht, D. & Petrovic, O. 2009. Isolation of *Pseudomonas aeruginosa* specific phages with broad activity spectra. *Current Microbiology*. 59(2):173-180.
- Kostić, T., Weilharter, A., Rubino, S., Delogu, G., Uzzau, S., Rudi, K., Sessitsch, A. & Bodrossy, L. 2007. A microbial diagnostic microarray technique for the sensitive detection and identification of pathogenic bacteria in a background of non-pathogens. *Analytical Biochemistry*. 360(2):244-254.
- Kozlova, E.A., Safatov, A.S., Kiselev, S.A., Marchenko, V.Y., Sergeev, A.A., Skarnovich, M.O., Emelyanova, E.K., Smetannikova, M.A., Buryak, G.A. & Vorontsov, A.V. 2010. Inactivation and mineralization of aerosol deposited model pathogenic microorganisms over TiO₂ and Pt/TiO₂. *Environmental Science & Technology*. 44(13):5121-5126.
- Kreuzer, K.N. 2013. DNA damage responses in prokaryotes: regulating gene expression, modulating growth patterns, and manipulating replication forks. *Cold Spring Harbor Perspectives in Biology*. 5(11):pa012674.
- Kühn, K.P., Chaberny, I.F., Massholder, K., Stickler, M., Benz, V.W., Sonntag, H.G. & Erdinger, L. 2003. Disinfection of surfaces by photocatalytic oxidation with titanium dioxide and UVA light. *Chemosphere*. 53(1):71-77.

- Kurosawa, H., Fujita, M., Kobatake, S., Kimura, H., Ohshima, M., Nagai, A., Kaneko, S., Iwasaki, Y. & Kozawa, K. 2010. A case of *Legionella pneumonia* linked to a hot spring facility in Gunma Prefecture, Japan. *Japanese Journal of Infectious Diseases*. 63(1):78-79.
- Law, J.W.F., Ab Mutalib, N.S., Chan, K.G. & Lee, L.H. 2014. Rapid methods for the detection of foodborne bacterial pathogens: principles, applications, advantages and limitations. *Frontiers in Microbiology*. 5:1-19.
- Lee, H., Lau, S.L., Kayhanian, M. & Stenstrom, M.K. 2004. Seasonal first flush phenomenon of urban storm water discharges. *Water Research*. 38(19):4153-4163.
- Lee, J.Y., Yang, J.S., Han, M. & Choi, J. 2010. Comparison of the microbiological and chemical characterization of harvested rainwater and reservoir water as alternative water resources. *Science of the Total Environment*. 408(4):896–905.
- Lee, K., Lee, K.M., Go, J., Ryu, J.C., Ryu, J.H. & Yoon, S.S. 2016. The ferrichrome receptor A as a new target for *Pseudomonas aeruginosa* virulence attenuation. *FEMS Microbiology Letters*. 363(11):1-8.
- Lévesque, B., Pereg, D., Watkinson, E., Maguire, J.S., Bissonnette, L., Gingras, S., Dewailly, E. 2008. Assessment of microbiological quality of drinking water from household tanks in Bermuda. *Canadian Journal Microbiology*. 54(6):495–500.
- Li, Z., Boyle, F. & Reynolds, A. 2010. Rainwater harvesting and greywater treatment systems for domestic application in Ireland. *Desalination*. 260(1–3):1–8.
- Lin, Z., Li, Z., Wang, X., Fu, X., Yang, G., Lin, H. & Meng, C. 2006. Inactivation efficiency of TiO₂ on H1N1 influenza virus. *Chemical Journal of Chinese Universities*. 27(4):721-725.
- Llamas, M.A., Sparrius, M., Kloet, R., Jiménez, C.R., Vandenbroucke-Grauls, C. & Bitter, W. 2006. The heterologous siderophores ferrioxamine B and ferrochrome activate signalling pathways in *Pseudomonas aeruginosa*. *Journal of Bacteriology*. 188(5):1882-1891.
- Lungu, B., Waltman, W.D., Berghaus, R.D. & Hofacre, C.L. 2012. Comparison of a real-time PCR method with a culture method for the detection of *Salmonella enterica* serotype *enteritidis* in naturally contaminated environmental samples from integrated poultry houses. *Journal of Food Protection*. 75(4):743-747.
- Luo, L., Miao, L., Tanemura, S. & Tanemura, M. 2008. Photocatalytic sterilization of TiO₂ films coated on Al fiber. *Materials Science and Engineering: B*. 148(1):183-186.

- Lutz, J.K. & Lee, J. 2011. Prevalence and antimicrobial-resistance of *Pseudomonas aeruginosa* in swimming pools and hot tubs. *International Journal of Environmental Research and Public Health*. 8(2):554-564.
- Lyczak, J.B., Cannon, C.L. & Pier, G.B. 2000. Establishment of *Pseudomonas aeruginosa* infection: lessons from a versatile opportunist. *Microbes and Infection*. 2(9):1051–1060.
- Lye, D. 1987. Bacterial levels in cistern water systems of northern Kentucky. *Water Resources Bulletin*. 23:1063–1068.
- Lye, D. J. 2002. Health risks associated with consumption of untreated water from household roof catchment system. *Journal of the American Water Resources Association*. 38(5):1301–1306.
- Lye, D.J. 2009. Rooftop runoff as a source of contamination: a review. *Science of the Total Environment*. 407(21):5429-5434.
- Malema, S., Abia, L.K.A., Mwenge Kahinda, J. & Ubomba-Jaswa, E. 2016. Gaining a better understanding of the factors that influence the quality of harvested rainwater in South Africa – a review. *WISA 2016 Biennial conference*. 16 - 19 May 2016. Durban, South Africa.
- Malik, A. & Aleem, A. 2011. Incidence of metal and antibiotic resistance in *Pseudomonas* spp. from the river water, agricultural soil irrigated with wastewater and groundwater. *Environmental Monitoring and Assessment*. 178(1):293-308.
- Mamane, H., Shemer, H. & Linden, K.G. 2007. Inactivation of *E. coli*, *B. subtilis* spores, and MS2, T4, and T7 phage using UV/H₂O₂ advanced oxidation. *Journal of Hazardous Materials*. 146(3):479-486.
- Mandel, B., Biswas, B., Banerjee, A., Mukherjee, T.K., Nandi, J. & Biswas, D. 2011. Breeding propensity of *Anopheles stephensi* in chlorinated and rainwater containers in Kolkata City, India. *Journal of Vector Borne Diseases*. 48(1):58–60.
- Marley Pipesystems. 2016. *Gutter Systems*. Available: <http://www.marleypipesystems.co.za/building-plastic-pipe-manufacturers/plumbing-plastic-pipe-and-fittings/upvc-gutter-systems> [2016, August 16].
- Marston, B.J., Lipman, H.B. & Breiman, R.F. 1994. Surveillance for legionnaires' disease: risk factors for morbidity and mortality. *Archives of Internal Medicine*. 154(21):2417–2422.
- Mazurier, S., Merieau, A., Bergeau, D., Decoin, V., Sperandio, D., Crépin, A., Barbey, C., Jeannot, K., Vicro-Gibouin, M., Plésiat, P. & Lemanceau, P. 2015. Type III secretion system and virulence markers highlight similarities and differences between human-and plant-associated pseudomonads

related to *Pseudomonas fluorescens* and *P. putida*. *Applied and Environmental Microbiology*. 81(7):2579-2590.

McGuigan, K.G., Méndez-Hermida, F., Castro-Hermida, J.A., Ares-Mazás, E., Kehoe, S.C., Boyle, M., Sichel, C., Fernández-Ibáñez, P., Meyer, B.P., Ramalingham, S. & Meyer, E. A. 2006. Batch solar disinfection inactivates oocysts of *Cryptosporidium parvum* and cysts of *Giardia muris* in drinking water. *Journal of Applied Microbiology*. 101(2):453-463.

McGuigan, K.G., Conroy, R.M., Mosler, H., Du Preez, M., Ubomba-Jaswa, E. & Fernandez-Ibanez, P. 2012. Solar water disinfection (SODIS): A review from bench- top to roof-top. *Journal of Hazardous Materials*. 235:29–46.

McLaughlin, G., Bajwa, V., Shukla, M., Hall, K. & Saxena, P. 2016. Inactivation of *E. coli* by copper and silver wire in the presence of synthetic sunlight for safe drinking water. *Journal of Water Sanitation and Hygiene for Development*. 6(4):576-583.

Mena, K.D. & Gerba, C.P. 2009. Risk assessment of *Pseudomonas aeruginosa* in water. In *Reviews of Environmental Contamination and Toxicology*. US: Springer. 201:71-115.

Mendez, C.B., Klenzendorf, J.B., Afshar, B.R., Simmons, M.T., Barrett, M.E., Kinney, K.A. & Kirisits, M.J. 2011. The effect of roofing material on the quality of harvested rainwater. *Water Research*. 45(5):2049-2059.

Mercante, J.W. & Winchell, J.M. 2015. Current and emerging *Legionella* diagnostics for laboratory and outbreak investigations. *Clinical Microbiology Reviews*. 28(1):95-133.

Merritt, A., Miles, R. & Bates, J. 1999. An outbreak of *Campylobacter enteritis* on an island resort, North Queensland. *Communicable Diseases Intelligence*. 23:215–219.

Meyer, M. & Kircher, M. 2010. Illumina sequencing library preparation for highly multiplexed target capture and sequencing. *Cold Springs Harbor Protocols*. 2010: pdb.prot5448.

Mingeot-Leclercq, M.P., Glupczynski, Y. & Tulkens, P.M. 1999. Aminoglycosides: activity and resistance. *American Society for Microbiology*. 43(4):727-737.

Ministry of Health. 1995. *Drinking Water Standards for New Zealand*. National Drinking-Water Standards Review Expert Working Group. Ministry of Health. Wellington, New-Zealand.

Mishra, A., Singh, A.K., Singh, K.A., Pandey, P., Yadav, S., Khan, A.H. & Barman, S.C. 2012. Urban air pollution and their effects on rain water characteristics in Lucknow city, India. *Journal of Environmental Research and Development*. 6(4):1127-1132.

Mo, A.C., Xu, W., Xian, S.Q., Li, Y.B. & Bai, S. 2007. Antibacterial activity of silver-hydroxyapatite/titania nanocomposite coating on titanium against oral bacteria. *Key Engineering Materials*. 330-332:455-458.

Murga, R., Forster, T.S., Brown, E., Pruckler, J.M., Fields, B.S. & Donlan, R.M. 2001. Role of biofilms in the survival of *Legionella pneumophila* in a model potable-water system. *Microbiology*. 147(11):3121-3126.

Mwabi, J.K., Adeyemo, F.E., Mahlangu, T.O., Mamba, B.B., Brouckaert, B.M., Swartz, C.D., Offringa, G., Mpenyana-Monyatsi, L. & Momba, M.N.B. 2011. Household water treatment systems: a solution to the production of safe drinking water by the low-income communities of Southern Africa. *Physics and Chemistry of the Earth, Parts A/B/C*. 36(14):1120-1128.

Mwenge Kahinda, J., Taigbenu, A.E. & Boroto, J.R. 2007. Domestic rainwater harvesting to improve water supply in rural South Africa. *Physics and Chemistry of the Earth, Parts A/B/C*. 32(15–18):1050–1057.

Mwenge Kahinda, J. & Taigbenu, A.E. 2011. Rainwater harvesting in South Africa: Challenges and opportunities. *Physics and Chemistry of the Earth, Parts A/B/C*. 36(14–15):968–976.

Naidoo, S. (2011). *Green projects put Cato Manor streets ahead*. *Clevergreen*. Available: <http://www.clevergreen.co.za/2011/11/05/green-project-puts-cato-manor-streets-ahead/> [2016, July 8].

National Health and Medical Research Council & National Resource Management Ministerial Council (NHMRC & NRMMC). 2004. *Australian drinking water guidelines 6, Volume 1. National water quality management strategy*. National Health and Medical Research Council, National Resource Management Ministerial Council Commonwealth of Australia. Canberra, Australia: NHMRC & NRMMC.

National Health and Medical Research Council & National Resource Management Ministerial Council (NHMRC & NRMMC). 2011. *Australian drinking water guidelines 6, Volume 1. National water quality management strategy*. National Health and Medical Research Council, National Resource Management of Australia. Canberra, Australia: NHMRC & NRMMC.

Navntoft, C., Ubomba-Jaswa, E., McGuigan, K.G. & Fernández-Ibáñez, P. 2008. Effectiveness of solar disinfection using batch reactors with non-imaging aluminium reflectors under real conditions: Natural well-water and solar light. *Journal of Photochemistry and Photobiology B: Biology*. 93(3):155-161.

- Nawaz, M., Amin, M.T., Han, M., Alazba, A.A., Manzoor, U. & Amin, M.N. 2014. Variation of *Pseudomonas aeruginosa* in rainwater harvesting systems: Effects of seasons, catchments and storage conditions. *CLEAN–Soil, Air, Water*. 42(7):893-900.
- Nega, H. & Kimeu, P.M. 2002. *Low-cost methods of rainwater storage: results from field trials in Ethiopia and Kenya*. Nairobi: Regional Land Management Unit. ISBN 9966-896-64-3.
- Nieuwoudt, M.N. & Mathews, E.H. 2005. A mobile solar water heater for rural housing in Southern Africa. *Building and Environment*. 40(9):1217-1234.
- Noble, R.C. & Overman, S.B. 1994. *Pseudomonas stutzeri* infection a review of hospital isolates and a review of the literature. *Diagnostic Microbiology and Infectious Disease*. 19(1):51-56.
- Noble, R., Moore, D., Leecaster, M., McGee, C. & Weisberg, S. 2003. Comparison of total coliform, fecal coliform, and enterococcus bacterial indicator response for ocean recreational water quality testing. *Water Research*. 37(7):1637–1643.
- Osawa, K., Shigemura, K., Abe, Y., Jikimoto, T., Yoshida, H., Fujisawa, M. & Arakawa, S. 2014. A case of nosocomial *Legionella pneumonia* associated with a contaminated hospital cooling tower. *Journal of Infection and Chemotherapy*. 20(1):68-70.
- Oxford Gene Technology. 2014. *Understanding and measuring variations in DNA sample quality*. Available:
https://www.oqt.com/resources/literature/483_understanding_and_measuring_variations_in_dna_sample_quality. [2017, March 26].
- Özen, A.I. & Ussery, D.W. 2012. Defining the *Pseudomonas* genus: where do we draw the line with *Azotobacter*? *Microbial Ecology*. 63(2):239-248.
- Palmore, T.N., Stock, F., White, M., Bordner, M., Michelin, A., Bennett, J.E., Murray, P.R. & Henderson, D.K. 2009. A cluster of cases of nosocomial legionnaires disease linked to a contaminated hospital decorative water fountain. *Infection Control & Hospital Epidemiology*. 30(8):764-768.
- Peller, J.R., Whitman, R.L., Griffith, S., Harris, P., Peller, C. & Scalzitti, J. 2007. TiO₂ as a photocatalyst for control of the aquatic invasive alga, *Cladophora*, under natural and artificial light. *Journal of Photochemistry and Photobiology A: Chemistry*. 186(2):212-217.
- Peters, A.J., Weidner, K.L. & Howley, C.L. 2008. The chemical water quality in roof-harvested water cisterns in Bermuda. *Journal of Water Supply: Research and Technology – AQUA*. 57(3):153–163.
- Pinfold, J.V., Horan, N.J., Wirojanagud, W. & Mara, D. 1993. The bacteriological quality of rainjar

water in rural northeast Thailand. *Water Research*. 27(2):297–302.

Pitkänen, T., Paakkari, P., Miettinen, I.T., Heinonen-Tanski, H., Paulin, L. & Hänninen, M.L. 2007. Comparison of media for enumeration of coliform bacteria and *Escherichia coli* in non-disinfected water. *Journal of Microbiological Methods*. 68(3):522-529.

Ramalingam, N., Rui, Z., Liu, H.B., Dai, C.C., Kaushik, R., Ratnaharika, B. & Gong, H.Q. 2010. Real-time PCR-based microfluidic array chip for simultaneous detection of multiple waterborne pathogens. *Sensors and Actuators B: Chemical*. 145(1):543-552.

Ratnam, S., Hogan, K., March, S.B. & Butler, R.W. 1986. Whirlpool-associated folliculitis caused by *Pseudomonas aeruginosa*: report of an outbreak and review. *Journal of Clinical Microbiology*. 23(3):655-659.

Raveendhra, D., Faruqui, S. & Saini, P. 2014. Transformer less FPGA controlled 2-stage isolated grid connected PV system. In *Power and Energy Systems Conference: Towards Sustainable Energy, 2014*. Bangalore, India: IEEE. ISBN: 978-1-4799-3421-8.

Reed, R.H. 2004. The inactivation of microbes by sunlight: solar disinfection as a water treatment process. *Advances in Applied Microbiology*. 54:333-365.

Reyneke, B., Dobrowsky, P.H., Ndlovu, T., Khan, S. & Khan, W. 2016. EMA-qPCR to monitor the efficiency of a closed-coupled solar pasteurization system in reducing *Legionella* contamination of roof-harvested rainwater. *Science of the Total Environment*. 553:662–670.

Ribeiro, A.F., Bodilis, J., Alonso, L., Buquet, S., Feuilleley, M., Dupont, J.P. & Pawlak, B. 2014. Occurrence of multi-antibiotic resistant *Pseudomonas* spp. in drinking water produced from karstic hydrosystems. *Science of the Total Environment*. 490:370-378.

Río-Álvarez, I., Rodríguez-Herva, J.J., Martínez, P.M., González-Melendi, P., García-Casado, G., Rodríguez-Palenzuela, P. & López-Solanilla, E. 2014. Light regulates motility, attachment and virulence in the plant pathogen *Pseudomonas syringae* pv tomato DC3000. *Environmental Microbiology*. 16(7):2072-2085.

Rodríguez, S.M., Gálvez, J.B., Rubio, M.M., Ibáñez, P.F., Padilla, D.A., Pereira, M.C., Mendes, J.F. & De Oliveira, J.C. 2004. Engineering of solar photocatalytic collectors. *Solar Energy*. 77(5):513-524.

Rompré, A., Servais, P., Baudart, J., De-Roubin, M.R. & Laurent, P. 2002. Detection and enumeration of coliforms in drinking water: current methods and emerging approaches. *Journal of Microbiological Methods*. 49(1):31-54.

- Rose, A., Roy, S., Abraham, V., Holmgren, G., George, K., Balraj, V., Abraham, S., Muliylil, J., Joseph, A. & Kang, G. 2006. Solar disinfection of water for diarrhoeal prevention in southern India. *Archives of Disease in Childhood*. 91(2):139-141.
- Rous, C.J., Fishman, M. & Filiatrault, M.J. 2016. Interactions among rsm ncRNAs and Rsm RNA-binding proteins in the plant pathogen *Pseudomonas syringae* DC3000. *The FASEB Journal*. 30 (1 Supplement):1054.
- Safapour, N. & Metcalf, R.H. 1999. Enhancement of solar water pasteurization with reflectors. *Applied and Environmental Microbiology*. 65(2):859-861.
- Sánchez, A.S., Cohim, E. & Kalid, R.A. 2015. A review on physicochemical and microbiological contamination of roof-harvested rainwater in urban areas. *Sustainability of Water Quality and Ecology*. 6:119–137.
- Savill, M.G., Hudson, J.A., Ball, A., Klena, J.D., Scholes, P., Whyte, R.J. & Jankovic, D. 2001. Enumeration of *Campylobacter* in New Zealand recreational and drinking waters. *Journal of Applied Microbiology*. 91(1):38–46.
- Sazakli, E., Alexopoulos, A. & Leotsinidis, M. 2007. Rainwater harvesting, quality assessment and utilization in Kefalonia Island, Greece. *Water Research*. 41(9):2039–2047.
- Schlech, W.F., Gorman, G.W., Payne, M.C. & Broome, C. 1985. Legionnaires's disease in Caribbean: an outbreak associated with a resort hotel. *Archives of Internal Medicine*. 145:2076–2079.
- Scott, B.R., Pathak, M.A. & Mohn, G.R. 1976. Molecular and genetic basis of furocoumarin reactions. *Mutation Research/Reviews in Genetic Toxicology*. 39(1):29-74.
- Sichel, C., Blanco, J., Malato, S. & Fernandez-Ibanez, P. 2007. Effects of experimental conditions on *E. coli* survival during solar photocatalytic water disinfection. *Journal of Photochemistry and Photobiology A: Chemistry*. 189(2):239-246.
- Silby, M.W., Winstanley, C., Godfrey, S.A., Levy, S.B. & Jackson, R.W. 2011. *Pseudomonas* genomes: diverse and adaptable. *FEMS Microbiology Reviews*. 35(4):652-680.
- Simmons, G., Hope, V., Lewis, G., Whitmore, J. & Gao, W. 2001. Contamination of potable roof-collected rainwater in Auckland, New Zealand. *Water Research*. 35(6):1518–1524.
- Simmons, G., Jury, S., Thornley, C., Harte, D., Mohiuddin, J. & Taylor, M. 2008. A Legionnaires' disease outbreak: A water blaster and roof-collected rainwater systems. *Water Research*. 42(6–7):1449–1458.

- Social Housing Foundation. 2010. *Social housing trends*. Available: www.shf.org.za [2016, July 8].
- Sökmen, M., Değerli, S. & Aslan, A. 2008. Photocatalytic disinfection of *Giardia intestinalis* and *Acanthamoeba castellanii* cysts in water. *Experimental Parasitology*. 119(1):44-48.
- Sommer, B., Mariño, A., Solarte, Y., Salas, M.L., Dierolf, C., Valiente, C. & Wegelin, M. 1997. SODIS - An emerging water treatment process. *Journal of Water Supply: Research and Technology – AQUA*. 46(3):127-137.
- South African Bureau of Standards (SABS). 2005. South African National Standards (SANS) 241: In *Drinking water quality management guide for water services authorities*. 6th ed. Annexure 1. ISBN 0-626-17752-9
- Spinks, J., Phillips, S., Robinson, P. & Van Buynder, P. 2006. Bushfires and tank rainwater quality: A cause for concern? *Journal of Water and Health*. 4(1):21–28.
- Stout, J.E., Rihs, J.D. & Yu, V.L. 2003. *Legionella*. In: *Manual of clinical microbiology*. Rev. 8th ed. P.R. Murray, Ed. Washington, DC., USA: American Society for Microbiology Press: 809–823.
- Strauss, A., Dobrowsky, P.H., Ndlovu, T., Reyneke, B. & Khan, W. 2016. Comparative analysis of solar pasteurization versus solar disinfection for the treatment of harvested rainwater. *BMC Microbiology*. 16(1):289.
- Sturm, M., Zimmermann, M., Schütz, K., Urban, W. & Hartung, H. 2009. Rainwater harvesting as an alternative water resource in rural sites in central northern Namibia. *Physics and Chemistry of the Earth*. 34(13–16):776–785.
- Sullivan, J.H. & Worsley, D.A. 2002. Zinc runoff from galvanised steel materials exposed in industrial/marine environment. *British Corrosion Journal*. 37(4):282-288.
- Sunflower Solar. 2017. *Solar water heaters, thermosiphon tubular solar water heater, solar geyser, stainless steel*. Available: http://www.sunflower-solar.com/index.php?act=content&scheduler_id=54 [2017, July 22].
- Suto, M.J., Domagala, J.M., Roland, G.E. Mailloux, G.B. & Cohen, M.A. 1992. Fluoroquinolones: relationships between structural variations, mammalian cell cytotoxicity, and antimicrobial activity. *Journal of Medicinal Chemistry*. 35(25):4745–50.
- Suzuki, Y., Kajii, S., Nishiyama, M. & Iguchi, A. 2013. Susceptibility of *Pseudomonas aeruginosa* isolates collected from river water in Japan to antipseudomonal agents. *Science of the Total Environment*. 450:148-154.

Swiss Federal Institute for Environmental Science and Technology (SFIEST). 2002. *Solar water disinfection: a guide for the application of SODIS. Department Water & Sanitation in Developing Countries (SANDEC) Report No 06/ 02.* Duebendorf, Switzerland: SFIEST.

Tenson, T., Lovmar, M. & Ehrenberg, M. 2003. The mechanism of action of macrolides, lincosamides and streptogramin B reveals the nascent peptide exit path in the ribosome. *Journal of Molecular Biology.* 330(5):1005-1014.

Texas Totes & Barrels. 2016. *First flush.* Available: <http://www.texastotesandbarrels.com/blog/archives/04-2016>. [2017, July 30].

Thomas, P.R. & Greene, G.R. 1993. Rainwater quality from different roof catchments. *Water Science & Technology.* 28:48–53.

Uba, B.N. & Aghogho, O. 2000. Rainwater quality from different roof-catchments in the Port Harcourt District, Rivers State, Nigeria. *Journal of Water Supply: Research and Technology-AQUA.* 49:281–288.

Ubomba-Jaswa, E., Navntoft, C., Polo-Lopez, M.I., Fernandez-Ibáñez, P. & McGuigan, K.G., 2009. Solar disinfection of drinking water (SODIS): an investigation of the effect of UV-A dose on inactivation efficiency. *Photochemical & Photobiological Sciences.* 8(5):587-595.

Ubomba-Jaswa, E., Fernández-Ibáñez, P. & McGuigan, K.G., 2010a. A preliminary Ames fluctuation assay assessment of the genotoxicity of drinking water that has been solar disinfected in polyethylene terephthalate (PET) bottles. *Journal of Water and Health.* 8(4):712-719.

Ubomba-Jaswa, E., Fernández-Ibáñez, P., Navntoft, C., Polo-López, M.I. & McGuigan, K.G. 2010b. Investigating the microbial inactivation efficiency of a 25 L batch solar disinfection (SODIS) reactor enhanced with a compound parabolic collector (CPC) for household use. *Journal of Chemical Technology and Biotechnology.* 85(8):1028-1037.

United Nations (UN). 2010. *The human right to water and sanitation.* Available: http://www.un.org/waterforlifedecade/pdf/human_right_to_water_and_sanitation_media_brief.pdf [2017, July 13].

United Nations (UN). 2015a. *The Millenium Development Goals Report 2015.* Available: [http://www.un.org/millenniumgoals/2015_MDG_Report/pdf/MDG %202015 %20rev %20 %28July %201 %29.pdf](http://www.un.org/millenniumgoals/2015_MDG_Report/pdf/MDG%202015%20rev%20%28July%201%29.pdf) [2016, August 8].

United Nations (UN). 2015b. *Sustainable development goals - water and sanitation.* Available: <http://www.un.org/sustainabledevelopment/water-and-sanitation/>. [2016, August 8].

United Nations Environment Programme (UNEP). 2016. *Rainwater harvesting and utilisation*. Available: <http://www.unep.or.jp/ietc/Publications/Urban/UrbanEnv-2/6.asp> [2016, August 5].

Uygur, N., Karaca, F. & Alagha, O. 2010. Prediction of sources of metal pollution in rainwater in Istanbul, Turkey using factor analysis and long-range transport models. *Atmospheric Research*. 95(1):55–64.

Vaz-Moreira, I., Nunes, O.C. & Manaia, C.M. 2012. Diversity and antibiotic resistance in *Pseudomonas* spp. from drinking water. *Science of the Total Environment*. 426:366-374.

Venkobachar, C., Leela, L. & Prabhakara, R. 1977. Mechanism of disinfection: Effect of chlorine on cell membrane functions. *Water Research*. 11(8):727-729.

Verrinder, G. & Keleher, H. 2001. Domestic drinking water in rural areas: are tanks on farms a health hazard? *Environmental Health*. 1(3):51–56.

Waso, M., Ndlovu, T., Dobrowsky, P.H., Khan, S. & Khan, W. 2016. Presence of microbial and chemical source tracking markers in roof-harvested rainwater and catchment systems for the detection of faecal contamination. *Environmental Science and Pollution Research*. 23(17):16987-17001.

Water Conservation Systems. 2016. *Superhead Rainwater Tank Filter*. Available: <http://www.watercon.co.za/superhead-rain-water-tank-filter.html> [2016, October 7].

Water Stewardship Information Series. 2007. *Total, fecal & E. coli bacteria in groundwater*. Available: [http://www.env.gov.bc.ca/wsd/plan_protect_sustain/groundwater/library/ground_fact_sheets/pdfs/colliform\(020715\)_fin2.pdf](http://www.env.gov.bc.ca/wsd/plan_protect_sustain/groundwater/library/ground_fact_sheets/pdfs/colliform(020715)_fin2.pdf) [2017, March 10].

Waweru, C. 2014. Effects of dust on the well being of people in learning institutions and businesses along Wamagana-ihithe road in Nyeri county. M.Sc. thesis. University of Nairobi.

Wegelin, M., Canonica, S., Alder, C., Marazuela, D., Suter, M.F., Bucheli, T.D., Haefliger, O.P., Zenobi, R., McGuigan, K.G., Kelly, M.T. & Ibrahim, P. 2001. Does sunlight change the material and content of polyethylene terephthalate (PET) bottles? *Journal of Water Supply: Research and Technology-AQUA*. 50(3):125-135.

Williams, M.T., Ndlovu, T., Dobrowsky, P.H., Waso, M. & Khan, W. 2015. Quality profile of fresh rainwater versus rainwater harvested from various catchment materials. Unpublished Honours. Thesis, Stellenbosch University.

World Health Organisation (WHO). 1984. *Guidelines for drinking-water quality. Volume 1. Recommendations*. World Health Organisation. Geneva, Switzerland: WHO Press. ISBN 92-4-

154638-7

World Health Organisation (WHO). 2003. *Heterotrophic plate counts and drinking-water safety – the significance of HPCs for water quality and human health*. London, United Kingdom: IWA Publishing. ISBN 1-84339-025-6.

World Health Organisation (WHO). 2007. *Legionella and the prevention of legionellosis*. World Health Organisation. Geneva, Switzerland. WHO Press:ISBN 92-4-156297-8.

World Health Organisation (WHO). 2011. *Guidelines for drinking-water quality*. Rev. 4th ed. World Health Organisation. Geneva, Switzerland: WHO Press. ISBN: 978-92-4- 154815-1.

Yao, Y., Ohko, Y., Sekiguchi, Y., Fujishima, A. & Kubota, Y. 2008. Self-sterilization using silicone catheters coated with Ag and TiO₂ nanocomposite thin film. *Journal of Biomedical Materials Research Part B: Applied Biomaterials*. 85(2):453-460.

Yayan, J., Ghebremedhin, B. & Rasche, K. 2015. Antibiotic resistance of *Pseudomonas aeruginosa* in pneumonia at a single university hospital center in Germany over a 10-year period. *Plos ONE*. 10(10):1-20.

Yaziz, M.I., Gunting, H., Sapari, N. & Ghazali, A.W. 1989. Variations in rainwater quality from roof catchments. *Water Research*. 23(6):761–765.

Yu, V.L., Plouffe, J.F., Pastoris, M.C., Stout, J.E., Schousboe, M., Widmer, A., Summersgill, J., File, T., Heath, C.M., Paterson, D.L. & Cheresky, A. 2002. Distribution of *Legionella* species and serogroups isolated by culture in patients with sporadic community-acquired Legionellosis: an international collaborative survey. *The Journal of Infectious Diseases*. 186(1):127-128.

Žgur-Bertok, D. 2013. DNA damage repair and bacterial pathogens. *PLoS Pathogens*. 9(11):p.e1003711.

Zimmer, J.L. & Slawson, R.M. 2002. Potential repair of *Escherichia coli* DNA following exposure to UV radiation from both medium-and low-pressure UV sources used in drinking water treatment. *Applied and Environmental Microbiology*. 68(7):3293-3299.

Chapter 2:

(Chapter 2 is compiled in the format of the Water Research journal and UK spelling is employed.)

Compound parabolic collector solar disinfection system for the treatment of harvested rainwater

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Abstract

A cost-effective solar disinfection (SODIS) system fitted with a compound parabolic collector (CPC) (SODIS-CPC) was designed for the treatment of rainwater. One SODIS-CPC system (SODIS-CPC-1) was installed next to a 2000 L rainwater harvesting tank without pre-treatment (Tank 1) and a second system (SODIS-CPC-2) was installed next to a 2000 L tank connected to a first flush (FF) diverter [Tank 2 (FF)]. To analyse the efficiency of the SODIS-CPC systems for the treatment of rainwater, various physicochemical (e.g. pH, turbidity), chemical (e.g. anions, cations) and microbial [e.g. indicator counts including total and faecal coliforms, *Escherichia coli* (*E. coli*), enterococci and heterotrophic bacteria (HPC)] parameters were investigated. The anion and cation concentrations analysed in all the samples were within drinking water guidelines as stipulated by national and international water associations. The *E. coli* counts were reduced to below the detection limit in all samples collected from the SODIS systems, while the HPC counts recorded in Tanks 1 (mean of 1.9×10^6 CFU/100 mL) and 2 (FF) (mean of 7.1×10^4 CFU/100 mL) were reduced to within the drinking water guideline of $< 1.0 \times 10^4$ CFU/100 mL following SODIS treatment. Similarly, the total coliform counts recorded in Tanks 1 and 2 (FF) during sampling sessions 1 to 4 were reduced to below the detection limit (< 1 CFU/100 mL) after SODIS treatment however, counts with a mean ranging from 1 to 75 CFU/100 mL were still recorded in the SODIS samples collected during sampling sessions 5 to 7. For both SODIS-CPC systems, ethidium monoazide bromide quantitative polymerase chain reaction (EMA-qPCR) analysis revealed a mean reduction of 99.5 % in viable *Legionella* copy numbers when a maximum mean UV-A radiation of 29.5 W/m^2 was observed. Similarly, for both SODIS-CPC systems, EMA-qPCR analysis revealed a mean reduction of 99.8 % in viable *Pseudomonas* copy numbers when the rainwater temperatures were $\geq 52 \text{ }^\circ\text{C}$ in the SODIS treated samples after eight hrs and a maximum mean UV-A radiation of 20.5 W/m^2 was recorded on the sampling day. Moreover, based on the chemical and microbial results obtained, the pre-filtration first flush diverter effectively reduced the level of contamination in roof-harvested rainwater. The point-of-use treatment system designed in the current study can thus be implemented on site where standard water infrastructure cannot be employed and it is recommended that the treated rainwater be utilised as a supplementary water source for domestic purposes.

Keywords: Rainwater harvesting; SODIS-CPC; first flush diverter; microbial indicators; EMA-qPCR

2.1. Introduction

Despite the success of the Millennium Development Goals (MDG), in 2015 the World Health Organisation (WHO) estimated that 663 million people worldwide still lacked access to improved drinking water sources, with 156 million people located in Africa utilising sub-standard water supplies. To compound the problem, sub-Saharan African countries, such as South Africa, are facing severe water shortages and drought conditions. The Sustainable Development Goals (SDG) were thus assembled in 2015 as a successor to the MDG, with 17 goals stipulated and the sixth goal set to ensure global access to clean water and sanitation services by 2030 [United Nations (UN), 2015]. In line with the targets for the sixth SDG goal, numerous countries are adopting strategies to achieve equitable access to safe drinking water sources. Domestic rainwater harvesting, which refers to the catchment and storage of rainwater from specifically rooftops during a rain event (Pacey & Cullis, 1986), has subsequently been implemented in countries around the globe as a sustainable water source (Amin & Han, 2009; De Kwaadsteniet et al. 2013). Correspondingly, rainwater harvesting has been earmarked by the custodian of South Africa's water sources, the Department of Water and Sanitation, as an alternative water supply and for food production [Mwenge Kahinda et al. 2007; Department of Water Affairs (DWA), 2009; 2013].

As rainwater is utilised by many developing and developed countries to supplement potable and domestic water supplies, numerous studies have investigated the quality of this water source (Ahmed et al. 2010; 2011; 2012; Dobrowsky et al. 2014a; 2014b; Kaushik et al. 2012; Lee et al. 2010; Lévesque et al. 2008; Sazakli et al. 2007). While the chemical quality of harvested rainwater generally adheres to guidelines stipulated by the WHO (2011), the microbial quality is substandard as various indicator organisms [e.g. *E. coli*, enterococci, heterotrophic plate counts (HPC), faecal and total coliforms] as well as pathogenic bacteria, viruses and fungi have been detected in rainwater (Ahmed et al. 2008; 2010; 2012; De Kwaadsteniet et al. 2013; Dobrowsky et al. 2014a; Gikas & Tsihrintzis, 2012; Lee et al. 2010; Reyneke et al. 2016; Strauss et al. 2016).

It has thus been concluded that stored harvested rainwater is not suitable for potable purposes due to the microbial quality in particular not complying with drinking water standards as established by various water associations [Australian Drinking Water Guidelines (ADWG), Department of Water Affairs and Forestry (DWAFF) and the WHO] and it is recommended that harvested rainwater should be treated prior to use (Amin et al. 2014; Dobrowsky et al. 2014b). However, as dust, debris and bird and animal faecal matter may accumulate on the rainwater catchment area (e.g. rooftop), the first consortium of run-off water, also called the first flush, generally has the highest concentration of pollutants. Filtration using a first flush diverter is thus recommended as pre-treatment, as previous studies have shown that the implementation of these systems reduces the initial contamination in the storage tanks and effectively also limits sludge build up in the tank which improves the harvested rainwater's quality (Gardner et al. 2004; Huston et al. 2009). Conflicting results on the efficiency of first flush diverters have however been reported with Gikas and Tsihrintzis (2012) indicating that

while these devices improved the physicochemical quality of the harvested rainwater, the concentration of microbial pollutants was not sufficiently reduced.

Primary treatment methods employed for the disinfection of harvested rainwater include solar pasteurization (SOPAS) and solar disinfection (SODIS), amongst other techniques (Amin et al. 2014; Dowbrowsky et al. 2015a; Reyneke et al. 2016; Strauss et al. 2016). While SOPAS and SODIS have successfully been employed to reduce the level of microbial contamination in water sources (Amin & Han, 2009; Amin et al. 2014; McGuigan et al. 2012; Strauss et al. 2016; Ubomba-Jaswa et al. 2010), SODIS is considered more cost-effective and is promoted by the WHO as a water treatment method. Solar disinfection is based on the synergistic effect of direct ultra-violet (UV) radiation and solar mild-heat to inactivate microorganisms. The sun's rays (consisting of UV-A and UV-B radiation) penetrates the water, whereafter UV-B light will interfere directly with a cell's genetic material (causing mutations) leading to cell death. In contrast, UV-A light is absorbed by photosensitiser molecules within the cell, which reacts with molecular oxygen, amongst other molecules, causing the production of reactive oxygen species (ROS). These ROS react with cellular constituents such as proteins, deoxyribonucleic acids (DNA), ribonucleic acids (RNA) and the cell membrane (especially membrane lipids by lipid peroxidation) (Castro-Alf rez et al. 2016; Gourmelon et al. 1994). This results in an increased membrane permeability followed by a disruption of membrane potential gradients leading to cell death due to adenosine triphosphate (ATP) exhaustion. Cells are however, able to counter the production of ROS and protect themselves against solar radiation by the production of antioxidant enzymes such as catalase and superoxide dismutase. However, it has been shown that these antioxidant systems are also UV sensitive and will be inactivated once irradiated with a substantial dose of UV-A radiation (Kapusinski & Mitchell, 1981). In addition, as this system depends on the direct penetration of UV radiation, an increased turbidity [Nephelometric Turbidity Units (NTU) > 30] of the water may also influence this system's efficiency (Dawney & Pearce, 2012).

Numerous studies have however, indicated that simple SODIS systems (2 L bottles filled with contaminated water and exposed to direct sunlight) have the potential to inactivate a wide range of microbial pathogens (Boyle et al. 2008; McGuigan et al. 2006). As polyethylene terephthalate (PET) is readily available and cost-effective, bottles constructed from this material are often utilised as reactors in traditional SODIS systems. However, PET bottle size availability (manufacturer moulds) restricts the treatment volume to a maximum of 5 L. As indicated, SODIS mainly employs solar radiation (UV-B: 281 - 320 nm; UV-A: 321 – 400 nm) for microbial inactivation and the combination of UV-A and UV-B radiation leads to improved disinfection efficiencies. Polyethylene terephthalate has an optical lower limit of 330 nm (Ubomba-Jaswa et al. 2010) and is thus opaque to UV-B and only transmits a portion of UV-A radiation, resulting in limited treatment efficiencies. Borosilicate glass, which is also used for SODIS treatment, transmits wavelengths as low as 280 nm

(recommended for efficient SODIS treatment as it transmits UV-A and UV-B radiation). However, this material is considered less robust and is more costly than standard PET.

To optimise and upscale the treatment volume of traditional SODIS systems, continuous flow- and batch reactors have been designed, where a batch reactor treats a fixed volume of stagnant water, while a continuous flow reactor continuously pumps water through the system in order to treat larger quantities of water. These batch reactor SODIS systems consist mainly of two parts, namely a transparent reactor and a solar collector. Although a continuous flow reactor treats larger quantities of water (range from 2 L/min to 10 L/min) (Sichel et al. 2007), the low flow rate employed in these systems negatively affects microbial inactivation (Ubomba-Jaswa et al. 2009). Re-circulated systems also require additional maintenance and materials as well as an electrical pump to circulate the water, which increases treatment costs. Research has indicated that a compound parabolic collector (CPC), fitted to a SODIS system, may be utilised to enhance the SODIS effect (Ubomba-Jaswa et al. 2010). In principle, a CPC tracks the sun's rays throughout the day to optimally concentrate UV radiation onto the reactor, effectively enhancing the UV dose the water is exposed to and decreasing the treatment time, even under cloudy weather conditions (Navntoft et al. 2008; Ubomba-Jaswa et al. 2010). Ubomba-Jaswa et al. (2010) then investigated the effect of a CPC fitted to a SODIS batch reactor for the inactivation of *E. coli* (spiked in well water). In addition, the disinfection efficiency of reactors constructed from polymethyl methacrylate (trade name – Plexiglas/Perspex) and borosilicate glass were compared. Results obtained in the study indicated that *E. coli* (spiked in well water) were completely inactivated (< 1 CFU/mL) within the first two hrs of solar exposure in the borosilicate glass reactor, while six hrs of solar exposure in the polymethyl methacrylate reactor was required before the lower limit of detection (< 1 CFU/mL) was achieved. In addition, under cloudy conditions, the borosilicate glass reactor completely inactivated *E. coli* (< 1 CFU/mL) within the first two hrs, while a 2-log *E. coli* concentration was still recorded in the polymethyl methacrylate reactor after six hrs of treatment (Ubomba-Jaswa et al. 2010).

A poor correlation however, exists between the presence of indicator organisms, such as *E. coli* and pathogens and opportunistic pathogens in harvested rainwater (Ahmed et al. 2010; Dobrowsky et al. 2014a; Harwood et al. 2005; Reyneke et al. 2016; Savichtcheva & Okabe, 2006; Wilkes et al. 2009). For example, viable *Legionella* and *Pseudomonas* spp. were detected in roof-harvested rainwater when indicator counts within drinking water standards were recorded (Reyneke et al. 2016; Strauss et al. 2016). In addition, Oliver (2010) conducted a review study and indicated that opportunistic pathogenic bacteria (including *E. coli*, *Legionella pneumophila*, *Mycobacterium tuberculosis*, *Pseudomonas aeruginosa*, *Vibrio cholerae*, amongst others) are able to enter a viable but non-culturable state under unfavourable conditions such as high temperatures (Maalej et al. 2004), lowered osmotic concentrations (Asakura et al. 2008) and nutrient starvation (Cook & Bolster, 2007). Furthermore, *Legionella* and *Pseudomonas* spp. are thermotolerant and the presence of heat shock proteins such as Hsp60 and DnaK, respectively, allow these genera to survive at temperatures

greater than 50 °C (Fields et al. 2002; Vervaeren et al. 2006). Reyneke et al. (2016) utilised ethidium monoazide bromide quantitative polymerase chain reaction (EMA-qPCR) assays to quantify the number of viable *Legionella* present in SOPAS treated rainwater and reported a mean concentration of 1.4×10^4 *Legionella* copies/mL after treatment (71.5 °C to 95 °C). Similarly, Strauss et al. (2016) utilised EMA-qPCR to quantify the viable number of *Pseudomonas* spp. present in SODIS (solar cooker with 2 L PET bottles as reactors) treated rainwater and reported that a mean concentration of 1.66×10^7 and 1.46×10^7 *Pseudomonas* copy numbers were still present after six (52 °C to 89 °C) and eight (63 °C to 86°C) hrs of SODIS treatment, respectively. It is thus imperative to accurately monitor pathogen viability in rainwater after the implementation of treatment strategies.

The primary aim of this study was to design and construct a SODIS system, fitted with a CPC (SODIS-CPC), which is (i) cost-effective, (ii) robust in nature, (iii) requires minimum maintenance and (iv) exhibits increased treatment efficiency. Two SODIS-CPC systems were connected to two separate 2 000 L rainwater harvesting tanks which were mounted onto metal stands to ensure the passive flow of harvested rainwater from the tanks into the SODIS-CPC systems. One rainwater harvesting tank was utilised without pre-treatment, while the second SODIS system was installed next to a rainwater harvesting tank connected to a first flush diverter. To determine the efficiency of the low-cost SODIS-CPC systems, the chemical (anion and cation concentrations as well as turbidity) and microbial quality (indicator organisms including *E. coli*, HPC, enterococci, total and faecal coliforms) of untreated and SODIS treated rainwater samples, were assessed. In addition, the viable *Legionella* and *Pseudomonas* population present in the untreated and SODIS treated rainwater was determined using EMA-qPCR assays.

2.2. Materials and methods

2.2.1. Solar disinfection unit

2.2.1.1. UV transmittance

As one objective of this study was to upscale the traditional SODIS reactor system (treatment volume of ~2 L) using cost-effective materials, the UV transmittance of locally supplied borosilicate glass and polymethyl methacrylate was determined. These materials were selected as large volume tube reactors (1 L to 17 L) of borosilicate glass and polymethyl methacrylate are available in South Africa. For the UV transmittance measurement, 12 mm × 50 mm sections of each material was analysed, in duplicate, using a Cintra 101 UV-Vis spectrometer (GBC Scientific Equipment Ltd., Australia).

2.2.1.2. Design and construction of a SODIS–CPC

As indicated, a contemporary SODIS system consists mainly of two parts, namely a transparent reactor and a solar collector or CPC (enhanced SODIS effect). For the construction of the CPC, an arch profile was designed to reflect all available solar rays directly onto the reactor tube. For the

construction of the arch profile, two arches (mirror images) were designed as a blueprint (**Figure 2.1**) using the computer software AutoCAD® 2016. The semi-angle of acceptance for the current SODIS-CPC was 81.5° , which falls within the 60 to 90° range considered optimal for photocatalytic applications (Malato Rodríguez et al. 2004). The profile was 780 mm in length and 305 mm in height and consisted of a variable radius of 105 mm and 275 mm, as indicated on **Figure 2.1**. Two dimensional and 3-D diagrams of the arch profile are represented in **Appendix A, Figure A1**.

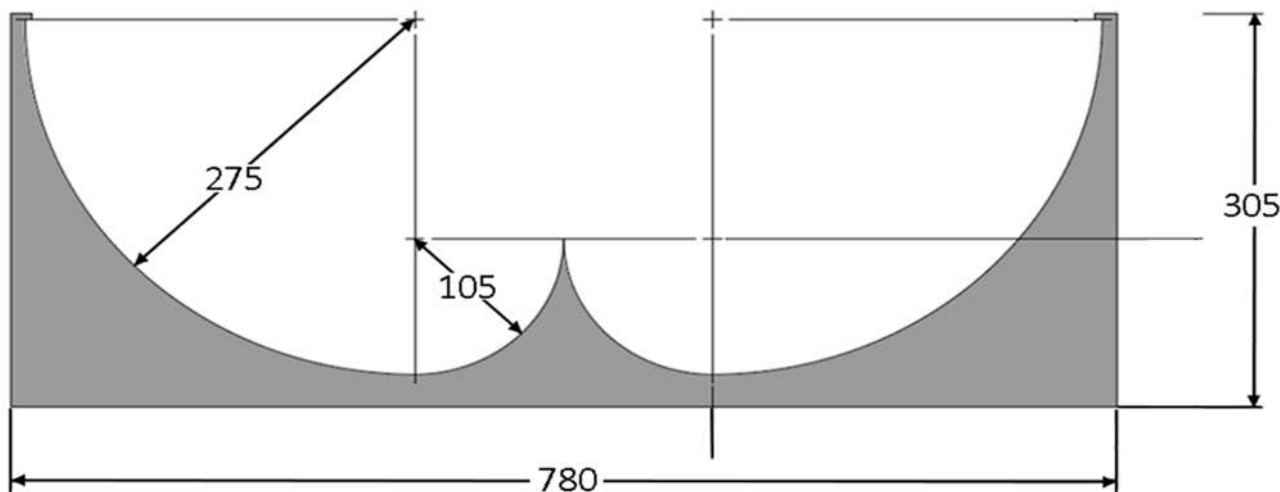


Figure 2.1: Schematic diagram of the arch profile with a variable radii which was used to construct the CPC of the SODIS-CPC system. All measurements are presented in mm.

The schematic diagrams of the arch profile of the CPC were sent to Fabrinox (Pty) Ltd. (Paarl, South Africa) for manufacturing. The respective curves of the arch profile were cut from a 2 mm thick stainless steel (grade 304) plate as this material is robust, corrosion resistant, commercially available and cost-effective. Four of the arch profiles [**Figure 2.2 (A)**] and two end plates [**Figure 2.2 (B)**] (305 mm \times 780 mm, stainless steel grade 304) were cut and aligned and were connected to three 25 mm \times 2 mm flat bars (stainless steel grade 304) which served as side panels [**Figure 2.2 (C)**]. After the frame (arch profiles, end plates and flat bars) was constructed, a highly reflective 0.5 mm thick stainless steel [grade 430 bright annealed (BA)] sheet (596 mm \times $1\ 500$ mm) was bent and superimposed onto the end plates to fit the curves of the arch profiles (**Appendix A, Figures A2 and A3**).

Based on the UV transmittance data, the transparent reactor was constructed from a 2.5 mm thick borosilicate glass cylinder with a length of 1.5 m. Sommer et al. (1997) conducted a study on the effectiveness of SODIS and indicated that at a depth of 100 mm, the UV-A transmittance was 75% for water with a turbidity value of 1 NTU. In addition, Ubomba-Jaswa et al. (2010) utilised a SODIS-CPC system, where the reactor was made of methacrylate with an outside diameter of 200 mm, and indicated a complete inactivation of *E. coli* (initial concentration of 10^6 CFU/mL) after six hrs of SODIS treatment. The outside diameter of the borosilicate reactor tube in the current study was however 100 mm (due to availability and price of the reactor), resulting in a treatment volume of 10.6 L. Both

ends of the reactor were sealed with a polymethyl methacrylate plug containing a rubber O-ring (**Appendix A, Figure A4**). A hole with a radius of 10 mm was drilled into the centre of the plugs as well as into the end plates [**Figure 2.2 (D)**] and was fitted with a polyvinyl chloride (PVC) socket screw [**Appendix A Figure A4**], which was further connected to a PVC ball valve tap.

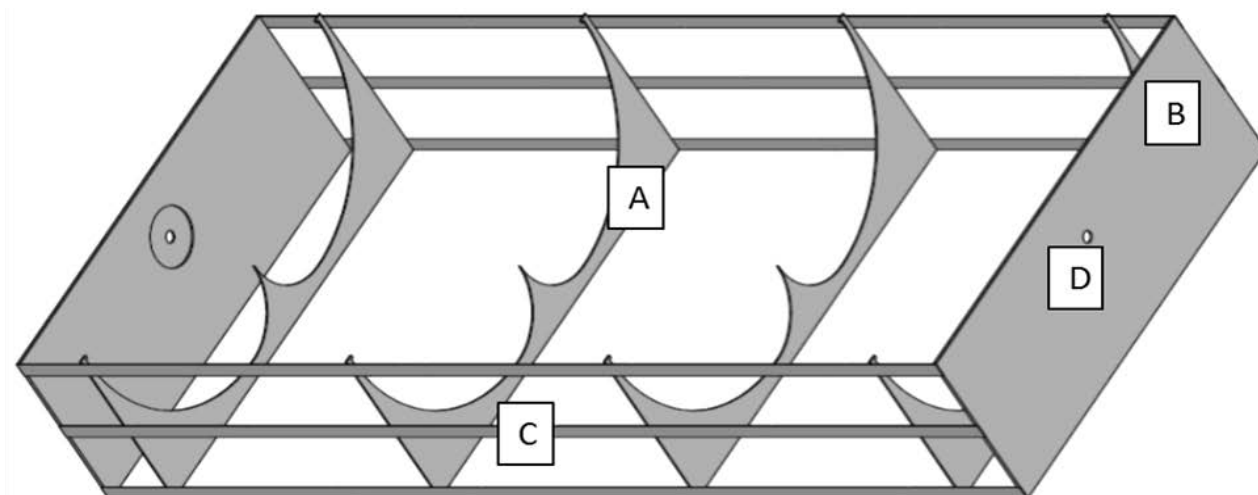


Figure 2.2: Schematic diagram of the CPC frame consisting of four arch profiles (A) lined up with a side plate (B) at each end, which was connected with three flat bars (C) at both vertical sides. The side plate contains a hole (D) (radius of 10 mm) which was used to fit the socket of the reactor through.

The transparent reactor (borosilicate glass cylinder) [**Figure 2.3i (A)**] was then positioned in the centre of the CPC [**Figure 2.3i (B)**], where the two arches connect axially along the linear focus of the CPC reflector. Each SODIC-CPC system thus consisted of two mirrored arches with variable radii, lined with a reflective stainless steel sheet to reflect the sun's rays directly onto the reactor (borosilicate glass cylinder). In principle, the sun's rays would be reflected directly onto the reactor throughout the course of the day without having to adjust or tilt the system, resulting in an enhancement of the SODIS effect.

As indicated, ball valve taps [**Figure 2.3i (C)**] were installed at both ends of the borosilicate glass cylinder reactor where the top influent tap was connected by a pipe to the rainwater harvesting tank (installed on a metal stand) to allow for the passive flow of rainwater from the harvesting tank into the reactor (no pumps or electricity was required), while the effluent tap at the bottom of the reactor was used for sample collection. A polypropylene cover was then attached to the top (open) side of the SODIS-CPC system to prevent dust particles from accumulating on the reactor and aid in heat retention in the systems. Furthermore, to obtain optimum performance throughout the year, extension poles [**Figure 2.3ii (D)**] were fitted onto the bottom of the frame in order to adjust the angle at which the upright system faces the sun. Additional information outlining the design of the SODIS-CPC system is included in **Appendix A, Figures A1 to Figure A4**. A cost analysis for the construction of a single system is also outlined in **Appendix A, Table A1**.

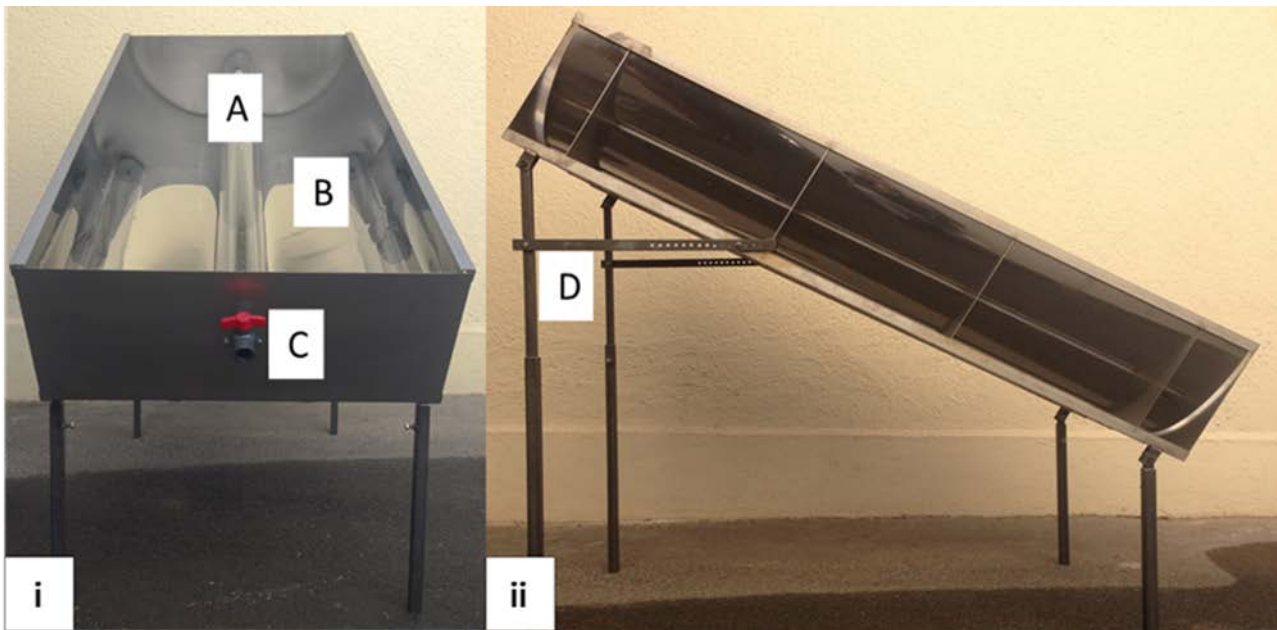


Figure 2.3: The SODIS–CPC system designed and used in this study. i) Front view of the system showing the reactor (A) which was linearly positioned along the focus of the CPC (B), with the PVC ball valve tap (C) on the outside of the system. ii) Side view of the system showing the extendable poles (D) which were used to tilt the system to obtain a perpendicular angle towards the sun.

2.2.2. Sampling site

Rainwater harvesting tanks were installed at the Welgevallen experimental farm, Stellenbosch University, South Africa (GPS co-ordinates: 33° 56' 36.19"S, 18° 52' 6.08"E) in June 2012 (**Figure 2.4**). Each polyethylene rainwater tank (2 000 L) was connected to an asbestos roof (catchment area) with a Chrysotile (white asbestos) conveyance system. These tanks were installed at separate ends of the conveyance system onto metal stands to allow for the passive flow of the rainwater into the respective treatment systems. Furthermore, the sampling site is situated on the periphery of Stellenbosch, next to a dairy farm where gravel roads pass the sampling site which is also surrounded by trees. However, no tree branches obstructed the catchment area.

A first flush (FF) diverter (Superhead[®] rainwater filter) was installed by Project Pumps[®], South Africa, to the conveyance system leading into Tank 2 (FF) (**Appendix A, Figure A5**). Thus, Tank 2 (FF) was connected to a first flush diverter, while Tank 1 was directly connected to the catchment area via the conveyance system. Tanks 1 and 2 (FF) were connected to the SODIS-CPC-1 and SODIS-CPC-2 (FF) systems, respectively. The reactor of both SODIS-CPC systems was placed in a Northern direction with an incline angle of 33° (with respect to the ground), for maximum solar irradiation exposure.



Figure 2.4: Rainwater harvesting systems located on Welgevallen experimental farm, Stellenbosch University, South Africa. The asbestos roof catchment area, is connected to the two tanks [Tank 1 and Tank 2 (FF)] via the conveyance system. Harvested rainwater flows passively from Tank 1 and Tank 2 (FF) into the SODIS-CPC-1 and SODIS-CPC-2 (FF) systems, respectively.

2.2.3. Sample collection

The reactors (borosilicate glass cylinders) of the respective SODIS-CPC systems were filled with harvested rainwater from the respective rainwater harvesting tanks on the morning of a sampling session. The rainwater was then exposed to direct sunlight for eight hrs (Amin & Han, 2009; Strauss et al. 2016), after which a total of four samples were collected. Samples were collected directly from Tank 1 and Tank 2 (FF), respectively and were designated as the untreated rainwater samples ($n = 2$). In addition, treated samples ($n = 2$) were collected from the SODIS-CPC-1 and the SODIS-CPC-2 (FF) reactors, respectively. Samples were collected in 5 L sterile bottles and a total of seven sampling sessions were conducted from March to April 2017, resulting in 28 samples ($n = 28$) collected in total during the course of this study (**Appendix A, Table A2**). For the presentation of results in the tables, sampling sessions 1 to 7 are designated as #1 (sampling session 1), #2 (sampling session 2), etc.

The pH and temperature of each untreated and SODIS treated sample (after the eight hr exposure period) was measured on site, using a hand-held pH meter (Milwaukee Instruments, Inc., USA) and mercury thermometer (ALLA® France, France), respectively. The maximum daily temperature and solar irradiance data [maximum UV-A and UV-B radiation and the maximum direct normal irradiance

(DNI⁴) were obtained from the Stellenbosch Weather Services, Stellenbosch University, Faculty of Engineering ([http:// weather.sun.ac.za/](http://weather.sun.ac.za/)).

2.2.4. Chemical analysis of SODIS treated and untreated rainwater samples

The chemical quality (cation and anion concentrations) of the untreated and SODIS treated samples (eight hrs) was determined. Before each sampling session, for the cation and metal concentration analysis, Falcon™ 50 mL high-clarity polypropylene tubes (Corning Life Sciences, USA), including the polyethylene caps, were pre-treated with 1 % nitric acid. The cation and metal ion concentrations [aluminium (Al), calcium (Ca), copper (Cu), iron (Fe), lead (Pb), magnesium (Mg), mercury (Hg), potassium (K), zinc (Zn), amongst others] were then determined after acidification (1 % ultrapure nitric acid) using inductively coupled plasma mass spectrometry (Agilent 7700 ICP-MS). This analysis was completed by the Central Analytical Facility (CAF), Stellenbosch University. The total water hardness was then calculated using the mean Ca and Mg concentrations as indicated in **Equation 1**.

$$\text{Total water hardness} = 2.497 \times [\text{mg Ca}] + 4.118 \times [\text{mg Mg}] \quad (1)$$

The anion analyses of representative samples [Tank1: #3, #6, #7; SODIS-CPC-1: #3, #6, #7; Tank 2 (FF): #3, #6, #7 and SODIS-CPC-2 (FF): #3, #6, #7] (nomenclature of samples is outlined in **Appendix A, Table A2**) was performed by Bemlabs (Cape Town, South Africa). All anions including, chloride (Cl), fluoride (F) sulphate (SO₄), nitrate (NO₃), nitrite (NO₂) and phosphate (PO₄) were measured utilising a Thermo Scientific Gallery™ Automated Photometric Analyser. The turbidity of all untreated and treated samples was also analysed by Bemlabs (Cape Town, South Africa).

2.2.5. Microbial analysis of SODIS treated and untreated rainwater samples

2.2.5.1. Enumeration of traditional indicator bacteria

A serial dilution was prepared ($10^{-1} - 10^{-3}$) for each rainwater sample collected ($n = 28$) during the sampling period and using the spread plate method, 100 µL of the undiluted rainwater sample and each dilution ($10^{-1} - 10^{-3}$) was cultured in duplicate onto the respective media. Briefly, Slanetz and Bartley Agar (Oxoid, Hampshire, England) (incubated for 24 - 48 hrs at 35 ± 2 °C), m-FC Agar (Merck, Darmstadt, Germany) (incubated for 22 – 24 hrs at 44 ± 2 °C) and R2A Agar (Oxoid) (incubated for 72 – 96 hrs at 35 ± 2 °C) were used to enumerate enterococci, faecal coliforms and HPC bacteria, respectively. *Escherichia coli* and total coliforms were enumerated by filtering a total volume of 100 mL (undiluted, 10^{-1} and 10^{-2}) through a sterile GN-6 Metricel® S-Pack Membrane Disc Filter (Pall Life Sciences, Michigan, USA) with a pore size of 0.45 µm and a diameter of 47 mm, at a

⁴ Amount of solar radiation received per square area by a surface that is perpendicular to direct sunrays.

filtration flow rate of approximately ≥ 65 mL/min/cm² at 0.7 bar (70 kPa), in duplicate. The membrane filters were then incubated on Membrane Lactose Glucuronide Agar (MLGA) (Oxoid) at 35 ± 2 °C for 18 - 24 hrs.

2.2.5.2. Rainwater concentration and filtration, EMA treatment and DNA extraction

For each sampling session, 1 L of the untreated [Tank 1 and Tank 2 (FF)] and treated [SODIS-CPC-1 and SODIS-CPC-2 (FF)] samples were concentrated by filtering as outlined in Dobrowsky et al. (2015b). The concentrated rainwater samples were utilised for *Legionella* and *Pseudomonas* spp. quantification and were treated with 2.5 µg/mL ethidium monoazide bromide (EMA) as previously described by Delgado-Viscogliosi et al. (2009) and Strauss et al. (2016), respectively. Following the addition of EMA to the concentrated rainwater samples, the samples were incubated on ice for 10 min followed by a 15 min halogen light exposure (keeping the samples on ice to avoid overheating during the photoactivation step). The EMA treated samples were washed with 1 mL NaCl (0.85 %) followed by centrifugation (16 000 × g for 5 min). Deoxyribonucleic acid was extracted using the Soil Microbe DNA MiniPrep™ Kit (Zymo Research, Irvine, USA) as per manufacturer's instructions by first re-suspending the obtained pellet in the lysis solution and transferring the mixture to the ZR BashingBead™ Lysis Tubes.

2.2.5.3. Detection of *Legionella* and *Pseudomonas* spp. by EMA-qPCR

Following the EMA treatment and DNA extractions, EMA-qPCR was performed on a LightCycler®96 (Roche Applied Science, Mannheim, Germany) using the FastStart Essential DNA Green Master Mix (Roche Applied Science). To a final reaction volume of 20 µL, the following was added: 10 µL FastStart Essential DNA Green Master Mix (2x), 5 µL template DNA (diluted by 10 fold) and 0.4 µL of each primer (final concentration 200 nM) as previously described by Roosa et al. (2014) for *Pseudomonas* spp. and by Herpers et al. (2003) for *Legionella* spp.

The primer set PS1 (5'-ATGAACAACGTTCTGAAATTC-3') and PS2 (5'-CTGCGGC TGGCTTTTCCAG-3') was utilised to amplify a 249 bp product of the *Pseudomonas* lipoprotein *oprI* gene (Roosa et al. 2014). The amplification conditions for *Pseudomonas* spp. were as follows: initial denaturation at 95 °C for 10 min, followed by 50 cycles of denaturation at 94 °C for 30 s, annealing at 58 °C for 30 s and extension at 72 °C for 30 s. For *Legionella* spp., the primer set LegF (5'-CTAATTGGCTGATTGTCTTGAC-3') and LegR (5'-CAATCGGAGTTCTTC GTG-3') was utilised to amplify a 259 bp product of the 23S rRNA gene (Herpers et al. 2003). The amplification conditions for *Legionella* spp. were as follows: initial denaturation at 95 °C for 10 min, followed by 50 cycles of denaturation at 95 °C for 15 s, annealing at 60 °C for 15 s and extension at 72 °C for 11 s.

The standard curves for the *Pseudomonas* spp. SYBR green real-time PCR assays were produced by conventional PCR by amplifying the lipoprotein *oprI* gene of *P. aeruginosa* ATCC 27853, using primer set PS1 and PS2. In addition, the standard curves for the *Legionella* spp. SYBR green real-

time PCR assays were produced by amplifying the 23S rRNA gene of *Legionella pneumophila* ATCC 33152, using primers LegF and LegR. These PCR products were purified using the DNA Clean & Concentrator™-5 Kit (Zymo Research) and were verified by DNA sequencing (by CAF, Stellenbosch University) using the BigDye Terminator Version 3.1 Sequencing Kit (Applied Biosystems®, Foster City, USA). Sequencing results were analysed by using FinchTV version 1.4.0 and sequence identification was completed using the Basic Local Alignment Search Tool (BLAST) of the National Centre for Biotechnology Information (NCBI) available at <https://blast.ncbi.nlm.nih.gov/Blast.cgi>. The DNA concentration was then determined in triplicate, using the NanoDrop® ND-1000 (Nanodrop Technologies Inc., Wilmington, Delaware, USA). A serial 10-fold dilution (10^8 to 10^1) of the PCR products was prepared in order to generate a standard curve, with the regression coefficient (R^2) maintained between 0.94 and 1.00 for each experiment. For *Pseudomonas* and *Legionella* spp. detection, a concentration of 1.00×10^8 gene copies/ μL was prepared for the dilution with the highest copy number, while a concentration of 1.00×10^1 gene copies/ μL was prepared for the dilution with the lowest copy number. The standard curves were generated by plotting the quantitative cycle (Cq) values versus the log concentrations of standard DNA, as previously described by Chen and Chang (2010). Melt curve analysis were included for the *Pseudomonas* and *Legionella* spp. SYBR green real-time PCR assays in order to verify the specificity of the primer set by ramping the temperature from 65 to 97 °C at a rate of 0.2 °C/s with a continuous fluorescent signal acquisition at 5 readings/°C.

2.2.6. Statistical analysis

The Microsoft Excel® version 15.31 (Microsoft Corporation, Redmond, WA, USA) and Statistica version 13.2.92 (Dell Inc., Tulsa, OK, USA) software package were used for the evaluation of the microbial analysis and physicochemical correlations. To test the significance of the data set, a paired t-test was performed on the mean data per analysis. A significance level of 5 % was used as a standard in the hypothesis tests (Dunn and Clark, 1974), while in all tests a p-value of < 0.05 was considered statistically significant. Furthermore, bacterial removal efficiency after treatment was determined using **Equation 2**, as previously described in Brözel and Cloete (1991). The log difference between the two tanks [Tank 1 and Tank 2 (FF)] and the two SODIS systems (SODIS-CPC-1 and SODIS-CPC-2 (FF)] as well as the log reduction after SODIS treatment was calculated using **Equation 3**.

$$\text{Percentage reduction} = 100 - \frac{\text{survival count}}{\text{initial count}} \times 100 \quad (2)$$

$$\text{Log reduction} = \text{Log}_{10} \text{bacterial count}_{\text{before treatment}} - \text{Log}_{10} \text{bacterial count}_{\text{after treatment}} \quad (3)$$

2.3. Results

2.3.1. UV transmittance

Borosilicate glass (2.5 mm thick) and polymethyl methacrylate (2.5 mm thick) were selected as possible reactor materials as they are cost-effective and large volume tube reactors (1 L to 17 L) of these materials are commercially available in South Africa. The transmission spectra of borosilicate glass and polymethyl methacrylate was determined with results indicated in **Figure 2.5**. The percentage transmittance (y-axis value) of light with a specific wavelength (x-axis value) through the respective materials was measured. As indicated, polymethyl methacrylate had an optical lower transmittance limit of light at a wavelength of ≥ 378 nm, while borosilicate glass had an optical lower transmittance limit of light at a wavelength of ≥ 273 nm (**Figure 2.5**). While polymethyl methacrylate is more robust than borosilicate glass, the borosilicate transmitted lower wavelengths making it more suitable for SODIS application, which is directly dependant on the combined effect of UV-A and UV-B radiation.

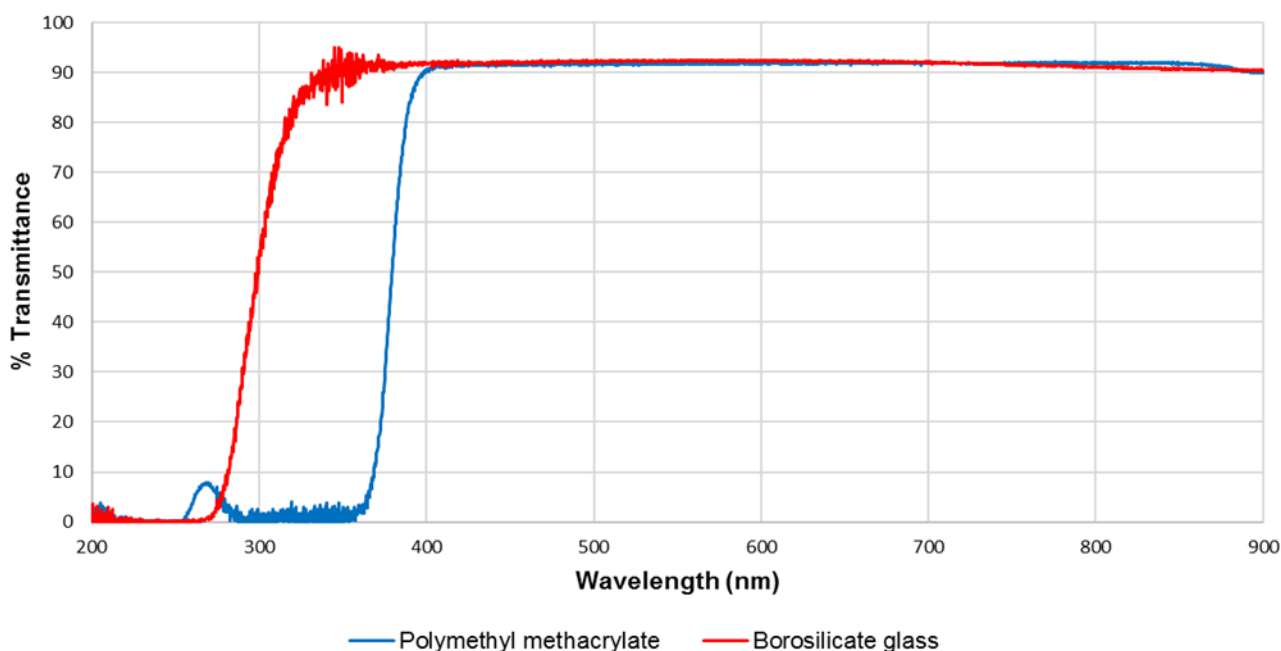


Figure 2.5: Percentage optical transmittance of light, with wavelengths ranging from 200 to 900 nm, through polymethyl methacrylate (blue line) and borosilicate glass (red line).

2.3.2. Physicochemical parameters

The temperatures and pH values recorded during March to April 2017 for the untreated rainwater samples [Tank 1 and Tank 2 (FF)] and the corresponding treated rainwater samples [SODIS-CPC-1 and SODIS-CPC-2 (FF)], which were collected after eight hrs of direct solar exposure, are outlined in **Table 2.1**. The temperatures of the rainwater samples collected from Tank 1 (untreated) ($n = 7$) ranged from a minimum of 23 °C to a maximum of 26 °C. The pH values ($n = 7$) recorded for these samples then ranged from a minimum of 6.55 to a maximum of 7.10. For the corresponding SODIS-

CPC-1 rainwater samples ($n = 7$), the temperatures ranged from a minimum of 45 °C to a maximum of 59 °C. The pH values ($n = 7$) recorded for these samples ranged from minimum of 6.48 to a maximum of 7.27.

For Tank 2 (untreated with FF installed) the temperatures of the rainwater samples ($n = 7$) ranged from a minimum of 19 °C to a maximum of 24 °C. The pH values ranged from a minimum of 6.28 to a maximum of 7.06. For the corresponding SODIS-CPC-2 (FF) rainwater samples ($n = 7$), the temperatures ranged from a minimum of 39 °C to a maximum of 53 °C, while the pH values ranged from a minimum of 6.42 to a maximum of 6.94.

The turbidity of all samples ($n = 28$) collected during the sampling period was also recorded (**Table 2.1**). The turbidity of rainwater samples collected from Tank 1 ranged from a minimum of 0.00 NTU to a maximum of 1.50 NTU, while the turbidity in the rainwater samples collected from SODIS-CPC-1 ranged from a minimum of 0.54 NTU to a maximum of 2.36 NTU.

The turbidity of the rainwater samples collected from Tank 2 (FF) then ranged from a minimum of 0.00 NTU to a maximum of 1.17 NTU, while the turbidity of the treated rainwater samples collected from SODIS-CPC-2 (FF) ranged from a minimum of 0.00 NTU to a maximum of 1.93 NTU. Overall, no significant differences ($p = 0.508$) were observed in the mean turbidity values recorded in Tank 1 versus Tank 2 (FF) as well as the mean turbidity values recorded for the SODIS treated rainwater samples collected from SODIS-CPC-1 and SODIS-CPC-2 (FF) ($p = 0.301$). Moreover, based on the fluctuation in data and the range of NTU values recorded (**Table 2.1**), statistically no significant differences were observed in the turbidity values recorded in Tank 1 versus SODIS-CPC-1 ($p = 0.113$) and Tank 2 (FF) versus SODIS-CPC-2 (FF) ($p = 0.209$).

The maximum daily ambient temperature and maximum solar irradiance data was also recorded throughout the sampling period (**Figure 2.6**). The daily ambient temperature ranged from a minimum of 27.7 °C (23/04/2017) to a maximum of 36.2 °C (20/03/2017). The UV-A radiation recorded throughout the sampling period then ranged from a minimum of 18.84 W/m² (24/04/2017) to a maximum of 31.13 W/m² (10/03/2017), while the UV-B radiation ranged from a minimum of 3.00 W/m² (24/04/2017) to a maximum of 4.15 W/m² (10/03/2017). In addition, the daily DNI ranged from a minimum of 918.26 W/m² (25/04/2017) to a maximum of 1028.65 W/m² (10/03/2017).

Table 2.1: Temperature, pH and turbidity values of SODIS treated [SODIS-CPC-1 and SODIS-CPC-2 (FF)] and the corresponding untreated rainwater samples.

Sampling date	Tank 1			SODIS-CPC-1			Tank 2 (FF)			SODIS-CPC-2 (FF)		
	Temp (°C)	pH	Turbidity (NTU)	Temp (°C)	pH	Turbidity (NTU)	Tem. (°C)	pH	Turbidity (NTU)	Temp (°C)	pH	Turbidity (NTU)
#1 - 10/03/2017	26	6.55	1.50	51	6.71	1.04	23	6.28	0.00	50	6.65	1.13
#2 - 20/03/2017	25	6.68	0.79	51	6.48	0.54	24	6.63	1.17	47	6.43	1.93
#3 - 19/04/2017	23	6.59	0.22	58	7.10	2.36	21	6.58	0.42	52	6.94	0.17
#4 - 20/04/2017	25	6.72	0.00	59	6.87	1.36	22	6.46	0.00	53	6.48	0.00
#5 - 23/04/2017	23	6.79	0.87	45	6.90	1.23	19	6.45	0.84	39	6.42	0.73
#6 - 24/04/2017	24	7.10	0.61	48	7.27	1.03	20	7.06	0.11	45	6.80	1.05
#7 - 25/04/2017	25	7.02	0.03	52	7.17	0.92	23	6.39	0.27	52	6.45	0.03

- Sampling session number

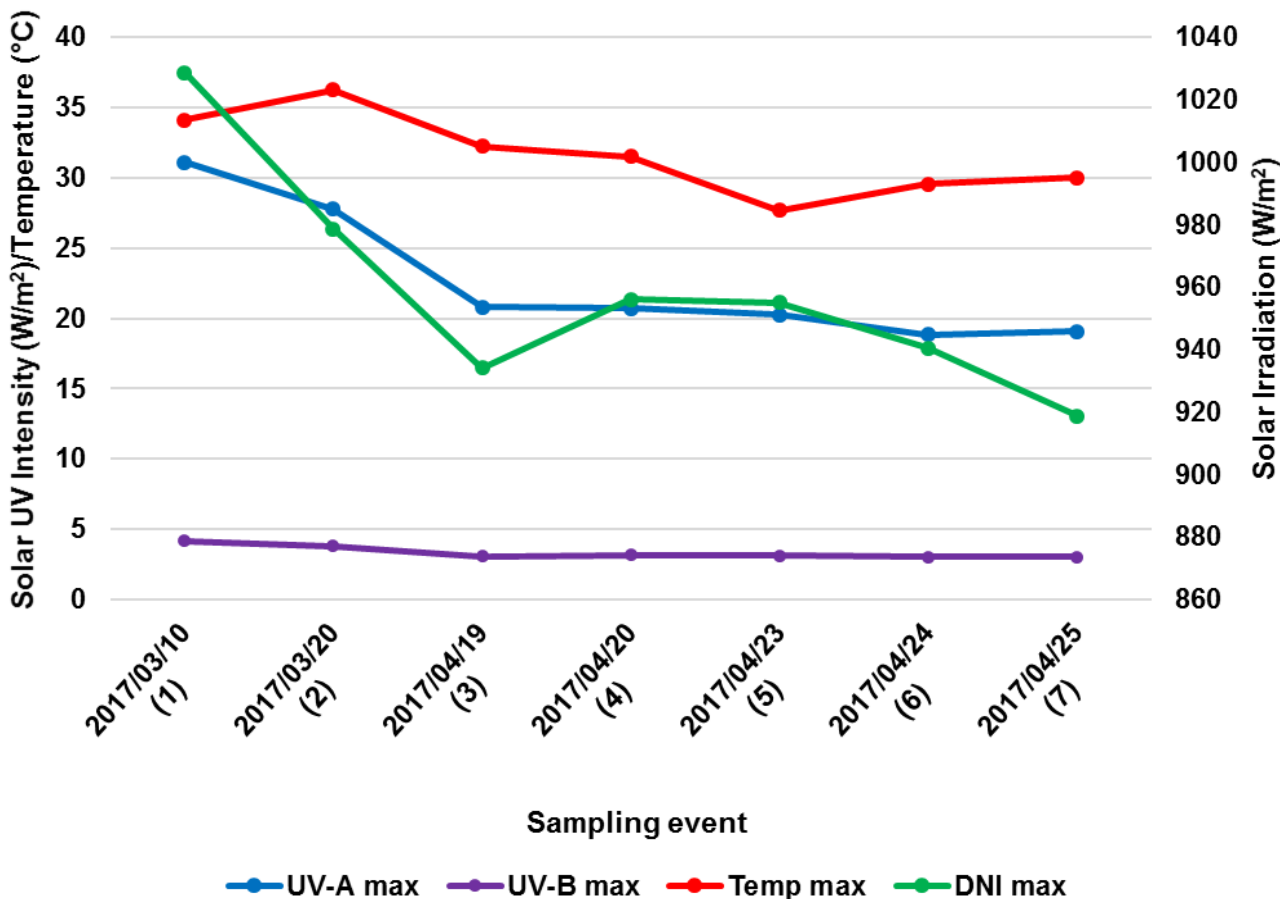


Figure 2.6: Solar data (maximum UV-A and UV-B radiation, and maximum DNI) and maximum daily ambient temperature recorded throughout the sampling period.

2.3.3. Chemical analysis

The anion concentration results for all the samples analysed are represented in **Table 2.2**. As indicated, representative samples of Tank 1 (#3; #6; #7) and SODIS-CPC-1 (#3; #6; #7) as well of Tank 2 (FF) [#3; #6; #7] and SODIS-CPC-2 (FF) (#3; #6; #7) were analysed. All anion concentrations were within drinking water guidelines as stipulated by DWAF (DWAF, 1996), South African National Standards (SANS) 241 [South African Bureau of Standards (SABS), 2015], ADWG [National Health and Medical Research Council & National Resource Management Ministerial Council (NHMRC & NRMCC), 2011] and the WHO (WHO, 2011). Based on the results obtained, overall, the concentrations of Cl, SO₄, NO₃ and PO₄ were significantly ($p < 0.05$) higher in the rainwater samples collected from Tank 1 (#3, #6 and #7), in comparison to the concentrations recorded in rainwater samples collected from Tank 2 (FF) (#3, #6 and #7). For example, the NO₃ concentrations recorded in rainwater samples collected from Tank 1 ranged from a minimum of 3.52 mg/L to a maximum of 3.73 mg/L (mean of 3.62 mg/L), while the NO₃ concentrations recorded for rainwater samples collected from Tank 2 (FF) were significantly lower ($p = 0.0004$) and ranged from 1.78 mg/L to 1.93 mg/L (mean of 1.88 mg/L).

Table 2.2: Anion concentrations (mg/L) of samples collected from Tank 1 and Tank 2 (FF) with their corresponding treated samples, compared to various drinking water guidelines.

Anion (mg/L)	Tank 1 #3	SODIS-CPC-1 #3	Tank 1 #6	SODIS-CPC-1 #6	Tank 1 #7	SODIS-CPC-1 #7	Tank 2 (FF) #3	SODIS-CPC-2 (FF) #3	Tank 2 (FF) #6	SODIS-CPC-2 (FF) #6	Tank 2 (FF) #7	SODIS-CPC-2 (FF) #7	Drinking Water Guidelines			
													DWAF (1996)	SANS 241 (2015)	ADWG (2011)	WHO (2011)
Chloride (Cl)	14.80	13.0	13.30	15.90	13.00	14.5	12.2	10.4	11.1	10.7	11.9	10.4	100	400	250	-
Sulphate (SO ₄)	7	6	6	6	6	6	5	5	4	5	5	5	200	200	250	-
Nitrate (NO ₃)	3.52	3.01	3.61	3.03	3.73	3.31	1.78	1.96	1.93	1.28	1.93	1.83	6	10	50	50
Nitrite (NO ₂)	0.08	0.06	0.08	0.07	0.05	0.05	0.04	0.04	0.05	0.07	0.04	0.06	6	10	50	50
Fluoride (F)	0.1	0.1	0.1	0.1	0.1	0.0	0.2	0.1	0.1	0.1	0.1	0.1	1	1	1.5	1.5
Phosphate (PO ₄)	1.16	0.86	1.44	0.89	1.29	1.13	0.64	0.07	1.01	0.28	0.73	0.64	-	-	-	-

Similarly, a significant difference ($p < 0.05$) in the concentrations in Cl, SO₄, NO₃ and PO₄ was observed between the rainwater samples collected from SODIS-CPC-1 and SODIS-CPC-2 (FF). For example, the NO₃ concentrations recorded for SODIS-CPC-1 ranged from 3.01 mg/L to 3.31 mg/L (mean of 3.12 mg/L), while the NO₃ concentrations of rainwater samples collected from SODIS-CPC-2 (FF) were significantly lower ($p = 0.020$) and ranged from 1.28 mg/L to 1.96 mg/L (mean of 1.69 mg/L). Furthermore, with the exception of the NO₃ concentrations which were significantly ($p = 0.008$) lower in SODIS-CPC-1 rainwater samples, no significant differences ($p > 0.05$) in the anion concentrations were recorded for Tank 1 versus SODIS-CPC-1. Similarly, no significant differences ($p > 0.05$) in anion concentrations were observed between Tank 2 (FF) and SODIS-CPC-2 (FF).

All cation concentrations recorded in samples collected from Tank 1 and the corresponding SODIS treated rainwater samples (SODIS-CPC-1) (**Table 2.3**; representative cations presented) were within the drinking water guidelines stipulated by ADWG (NHMRC & NRMCC, 2011), DWAF (DWAF, 1996), SANS 241 (SABS, 2015), and the WHO (WHO, 2011). Similarly, the cation concentrations of rainwater samples collected from Tank 2 (FF) and the corresponding SODIS treated rainwater samples [SODIS-CPC-2 (FF)], were within the stipulated drinking water guidelines throughout the sampling period (**Table 2.4**).

Furthermore, with the exception of Zn and Hg, no significant differences ($p > 0.05$) in the cation concentrations (e.g. Al, Fe, Cu, As, Pb, Ca, K, Mg, Na and P) was observed between the rainwater samples collected from Tank 1 versus SODIS-CPC-1. Similarly, with the exception of Pb, no significant difference ($p > 0.05$) in the cation concentrations (e.g. Al, Fe, Cu, As, Zn, Hg, Ca, K, Mg, Na and P) was observed between the rainwater samples collected from Tank 2 (FF) versus SODIS-CPC-2 (FF). In contrast, with the exception of the Pb and Hg concentrations, significant differences ($p < 0.05$) were observed in the cation concentrations (Al, Fe, Cu, Zn, As, Ca, K, Mg, Na and P), recorded in samples collected from Tank 1 (**Table 2.3**) versus Tank 2 (FF) (**Table 2.4**). For example, the Fe concentrations recorded for Tank 1 ranged from 10.52 µg/L to 16.79 µg/L (mean of 12.44 µg/L) and was significantly higher ($p = 0.0004$) than the Fe concentrations recorded in rainwater samples collected from Tank 2 (FF), which ranged from 4.42 µg/L to 8.14 µg/L (mean of 7.04 µg/L). While still within standards, the overall mean concentrations of Al (7.13 µg/L), K (3.05 mg/L), Mg (1.22 mg/L), Na (5.14 mg/L) and P (0.33 mg/L) collected from Tank 2 (FF) were then significantly ($p < 0.05$) higher than the overall mean Al (3.22 µg/L), K (1.65 mg/L), Mg (0.92 mg/L), Na (3.83 mg/L) and P (0.18 mg/L) concentrations recorded in Tank 1.

Correspondingly, with the exception of the Hg concentrations, a significant difference ($p < 0.05$) in the cation concentrations (e.g. Al, Fe, Cu, Zn, As, Pb, Ca, K, Mg, Na and P) was observed between the rainwater samples collected from SODIS-CPC-1 versus SODIS-CPC-2 (FF).

Table 2.3: Cation concentrations of rainwater samples collected from Tank 1 and the corresponding SODIS treated rainwater samples (SODIS-CPC-1) during sampling sessions 1 to 7, compared to various drinking water guidelines.

Cation/ Metal	Tank 1 #1	SODIS-CPC-1 #1	Tank 1 #2	SODIS-CPC-1 #2	Tank 1 #3	SODIS-CPC-1 #3	Tank 1 #4	SODIS-CPC-1 #4	Tank 1 #5	SODIS-CPC-1 #5	Tank 1 #6	SODIS-CPC-1 #6	Tank 1 #7	SODIS-CPC-1 #7	Drinking Water Guidelines			
															DWAF (1996)	SANS 241 (2015)	WHO (2011)	ADWG (2011)
Aluminium as Al ($\mu\text{g/L}$)	3.07	1.98	7.73	1.98	1.98	1.98	1.98	2.81	2.96	1.98	2.84	1.98	1.98	1.98	150	300	-	100
Iron as Fe ($\mu\text{g/L}$)	16.79	14.78	13.74	13.13	11.03	10.05	11.70	11.60	11.19	11.68	10.52	11.05	12.07	11.45	100	300	-	300
Copper as Cu ($\mu\text{g/L}$)	30.01	6.89	7.87	7.26	7.23	7.28	7.31	9.70	9.18	9.06	8.36	9.83	9.47	8.97	1000	2000	2000	2000
Arsenic as As ($\mu\text{g/L}$)	1.20	1.15	1.19	1.14	1.22	1.19	1.23	1.20	1.23	1.26	1.23	1.22	1.20	1.34	10	10	10	10
Zinc as Zn ($\mu\text{g/L}$)	9.78	53.72	13.37	22.97	24.53	22.75	26.51	36.26	14.33	31.34	13.43	38.13	21.52	33.59	3000	5000	-	3000
Mercury as Hg ($\mu\text{g/L}$)	<0.02	<0.005	<0.02	<0.007	<0.004	<0.004	<0.004	<0.004	<0.02	<0.004	<0.02	<0.004	<0.004	<0.004	1	6	6	1
Lead as Pb ($\mu\text{g/L}$)	<0.2	<0.29	<0.2	<0.27	<0.01	<0.58	<0.01	<0.23	0.20	<0.06	<0.20	<0.010	<0.24	<0.01	10	10	10	10
Calcium as Ca (mg/L)	11.42	11.16	11.42	10.90	11.31	11.21	11.67	11.60	11.40	11.59	11.86	11.31	11.39	11.31	32	-	-	200
Potassium as K (mg/L)	1.62	1.69	1.68	1.63	1.59	1.71	1.66	2.28	1.66	1.67	1.64	1.66	1.70	1.79	50	-	-	-
Magnesium as Mg (mg/L)	0.92	0.91	0.92	0.89	0.91	0.93	0.93	0.94	0.92	0.93	0.94	0.91	0.93	0.96	30	-	-	-
Sodium as Na (mg/L)	3.89	3.81	3.86	3.74	3.73	3.93	3.78	4.32	3.84	3.85	3.85	3.79	3.89	4.12	100	200	-	180
Phosphorus as P (mg/L)	0.17	0.19	0.18	0.17	0.20	0.20	0.19	0.13	0.17	0.20	0.18	0.20	0.18	0.19	-	-	-	-

Table 2.4: Cation concentrations of rainwater samples collected from Tank 2 (FF) and the corresponding SODIS treated rainwater samples [SODIS-CPC-2 (FF)] during sampling sessions 1 to 7, compared to various drinking water guidelines.

Cation/ Metal	Tank 2 (FF) #1	SODIS-CPC-2 (FF) #1	Tank 2 (FF) #2	SODIS-CPC-2 (FF) #2	Tank 2 (FF) #3	SODIS-CPC-2 (FF) #3	Tank 2 (FF) #4	SODIS-CPC-2 (FF) #4	Tank 2 (FF) #5	SODIS-CPC-2 (FF) #5	Tank 2 (FF) #6	SODIS-CPC-2 (FF) #6	Tank 2 (FF) #7	SODIS-CPC-2 (FF) #7	Drinking Water Guidelines			
															DWAF (1996)	SANS 241 (2015)	WHO (2011)	ADWG (2011)
Aluminium as Al (µg/L)	8.98	5.92	10.21	5.48	6.73	9.17	6.50	7.57	8.24	12.54	5.44	12.14	3.81	7.28	150	300	-	100
Iron as Fe (µg/L)	7.46	7.30	8.08	7.04	8.14	8.61	7.12	8.35	7.16	9.96	4.42	9.21	6.89	8.14	100	300	-	300
Copper as Cu (µg/L)	25.13	4.91	3.36	2.86	3.44	3.35	3.27	3.33	4.56	3.79	3.88	3.16	8.99	4.11	1000	2000	2000	2000
Arsenic as As (µg/L)	1.10	1.06	1.06	1.10	1.05	1.12	1.10	1.11	1.10	1.15	1.14	1.16	1.22	1.14	10	10	10	10
Zinc as Zn (µg/L)	5.99	16.98	3.59	15.17	7.21	5.44	7.11	5.62	3.63	28.19	7.49	21.52	5.85	8.36	3000	5000	-	3000
Mercury as Hg (µg/L)	<0.02	<0.004	<0.02	<0.004	<0.004	<0.006	<0.004	<0.004	<0.02	<0.004	<0.02	<0.004	<0.006	<0.004	1	6	6	1
Lead as Pb (µg/L)	<0.2	<0.04	<0.2	<0.01	<0.01	<0.01	<0.01	<0.01	<0.2	<0.01	<0.2	<0.01	<0.24	<0.01	10	10	10	10
Calcium as Ca (mg/L)	9.44	9.31	9.68	9.26	10.37	10.33	10.59	10.38	10.82	10.89	10.93	10.42	10.40	10.42	32	-	-	200
Potassium as K (mg/L)	3.03	2.94	2.94	2.94	3.13	3.04	3.17	3.06	2.96	3.09	2.99	3.14	3.16	3.13	50	-	-	-
Magnesium as Mg (mg/L)	1.15	1.10	1.16	1.12	1.23	1.23	1.24	1.23	1.25	1.24	1.26	1.24	1.25	1.25	30	-	-	-
Sodium as Na (mg/L)	4.54	4.32	4.44	4.44	5.34	5.30	5.48	5.27	5.31	5.37	5.36	5.49	5.52	5.43	100	200	-	180
Phosphorus as P (mg/L)	0.32	0.31	0.33	0.32	0.33	0.33	0.31	0.34	0.33	0.36	0.32	0.34	0.33	0.35	-	-	-	-

For example, the Fe concentrations recorded for SODIS-CPC-1 ranged from 10.05 µg/L to 14.78 µg/L (mean of 11.96 µg/L) and was significantly higher ($p = 0.007$) than the Fe concentrations recorded in rainwater samples collected from SODIS-CPC-2 (FF), which ranged from 7.04 µg/L to 9.96 µg/L (mean of 8.37 µg/L). The overall mean concentrations of Al (8.59 µg/L), K (3.05 mg/L), Mg (1.20 mg/L), Na (5.09 mg/L) and P (0.34 mg/L) collected from SODIS-CPC-2 (FF) were however also significantly ($p < 0.05$) higher than the overall mean Al (2.10 µg/L), K (1.78 mg/L), Mg (0.92 mg/L), Na (3.94 mg/L) and P (0.19 mg/L) concentrations recorded in SODIS-CPC-1. As indicated all the cation concentrations recorded were within drinking water standards.

The total water hardness of rainwater samples (before and after SODIS treatment) was subsequently determined using the mean Mg and Ca concentrations as indicated in **Equation 1** and is expressed as CaCO₃ mg/L. A mean water hardness of 32.51 CaCO₃ mg/L was calculated for Tank 1, which then decreased to 32.02 CaCO₃ mg/L after SODIS treatment (SODIS-CPC-1). Similarly, the mean total water hardness of rainwater samples collected from Tank 2 (FF) was calculated as 30.78 CaCO₃ mg/L, which then decreased to 30.28 CaCO₃ mg/L after SODIS treatment [SODIS-CPC-2 (FF)].

2.3.4. Indicator bacteria detected in untreated and SODIS treated rainwater

2.3.4.1. Tank 1 and SODIS-CPC-1 rainwater samples

In order to assess the general microbial quality of the untreated and SODIS treated rainwater samples, indicator organisms including enterococci, faecal coliforms, total coliforms, *E. coli* and HPC were enumerated. For Tank 1 and the corresponding SODIS-CPC-1 samples, enterococci counts were within the < 1 CFU/100 mL guideline limit as stipulated by ADWG (NHMRC & NRMMC, 2011), as no enterococci were detected in any of the samples analysed (results not shown). Similarly, the faecal coliform counts were within the respective drinking water guideline limits of < 1 CFU/100 mL [DWAF (DWAF, 1996), SANS 241 (SABS, 2015), ADWG (NHMRC & NRMMC, 2011) and WHO (2011)], as no faecal coliforms were detected in the samples analysed for Tank 1 and SODIS-CPC-1 (results not shown).

In contrast, the *E. coli* counts exceeded the drinking water guidelines (< 1 CFU/100 mL) stipulated by ADWG (NHMRC & NRMMC, 2011), DWAF (DWAF, 1996), WHO (WHO, 2011) and SANS 241 (SABS, 2015) in all the samples analysed for Tank 1 (**Table 2.5**). *Escherichia coli* counts then ranged from a minimum of 2 CFU/100 mL to a maximum of 2.0×10^1 CFU/100 mL in Tank 1 rainwater samples, which then significantly decreased ($p < 0.05$) to below the detection limit (< 1 CFU/100 mL) after treatment in the SODIS-CPC-1 reactor. Similarly, the total coliform counts exceeded the drinking water guidelines as stipulated by DWAF (DWAF, 1996) and SANS 241 (SABS, 2015) of < 5 CFU/100 mL and < 10 CFU/100 mL, respectively in all the samples analysed for Tank 1 (**Table 2.5**). The total coliform counts ranged from a minimum of 1.57×10^2 CFU/100 mL to a

maximum of 1.0×10^4 CFU/100 mL and decreased significantly ($p < 0.05$) to below the detection limit (< 1 CFU/100 mL; exception of the counts recorded in sampling sessions 5 to 7) after treatment in the SODIS-CPC-1 rainwater samples. However, while a mean reduction of 2.60-log was recorded overall, total coliform counts enumerated in the rainwater samples collected from SODIS-CPC-1 for sampling session 5, 6 and 7, were recorded as 1 CFU/100 mL, 7.5×10^1 CFU/100 mL and 5.1×10^1 CFU/100 mL, respectively.

Table 2.5: Total coliform (TC) and *E. coli* counts recorded before (Tank 1) and after SODIS treatment (SODIS-CPC-1) at various UV-A irradiances and temperatures.

Sampling session	*UV-A (W/m ²)	**SODIS Temp (°C)	Indicator	Tank 1 (CFU/100 mL)	SODIS-CPC-1 (CFU/100 mL)	% reduction
1	31.1	51	<i>E. coli</i>	8	BDL	> 99.9
			TC	1.0×10^4	BDL	> 99.9
2	27.8	51	<i>E. coli</i>	3	BDL	> 99.9
			TC	1.7×10^3	BDL	> 99.9
3	20.8	58	<i>E. coli</i>	4	BDL	> 99.9
			TC	7.3×10^3	BDL	> 99.9
4	20.7	59	<i>E. coli</i>	2	BDL	> 99.9
			TC	3.4×10^3	BDL	> 99.9
5	20.3	45	<i>E. coli</i>	2	BDL	>99.9
			TC	1.57×10^2	1	99.4
6	18.8	48	<i>E. coli</i>	3	BDL	>99.9
			TC	2.26×10^3	7.5×10^1	96.7
7	19.1	52	<i>E. coli</i>	2.0×10^1	BDL	>99.9
			TC	5.7×10^3	5.1×10^1	99.1

BDL – below detection limit (< 1 CFU/100 mL)

* - Maximum ambient UV-A data; ** - Temperature recorded in SODIS treated rainwater sample after eight hrs

The HPC results recorded for the rainwater samples collected from Tank 1 and SODIS-CPC-1 are depicted in **Figure 2.7**. The heterotrophic bacteria counts in all the rainwater samples collected from Tank 1 exceeded the drinking water guideline of $< 1.0 \times 10^4$ CFU/100 mL as stipulated by DWAF (DWAF, 1996), with counts ranging from a minimum of 3.0×10^5 CFU/100 mL to a maximum of 8.3×10^6 CFU/100 mL, with an overall mean of 1.9×10^6 CFU/100 mL recorded. However, with the exception of the samples collected during sampling sessions 5 and 6, the HPC decreased significantly ($p < 0.05$) to below the detection limit (< 1 CFU/100 mL) after treatment in the SODIS-CPC-1 reactor. During sampling sessions 5 and 6, while a HPC of 3.0×10^5 CFU/100 mL was recorded in the untreated samples collected from Tank 1, heterotrophic bacteria counts of 5.7×10^3 CFU/100 mL and 3.3×10^3 CFU/100 mL, respectively, were still recorded after SODIS treatment. The counts recorded were however, within the DWAF (1996) guideline of $< 1.0 \times 10^4$ CFU/100 mL. It should however be noted that overall, in comparison to the HPC recorded in the untreated rainwater samples collected from Tank 1, a mean reduction of 3.17-log was observed in HPC after treatment in the SODIS-CPC-1 rainwater samples.

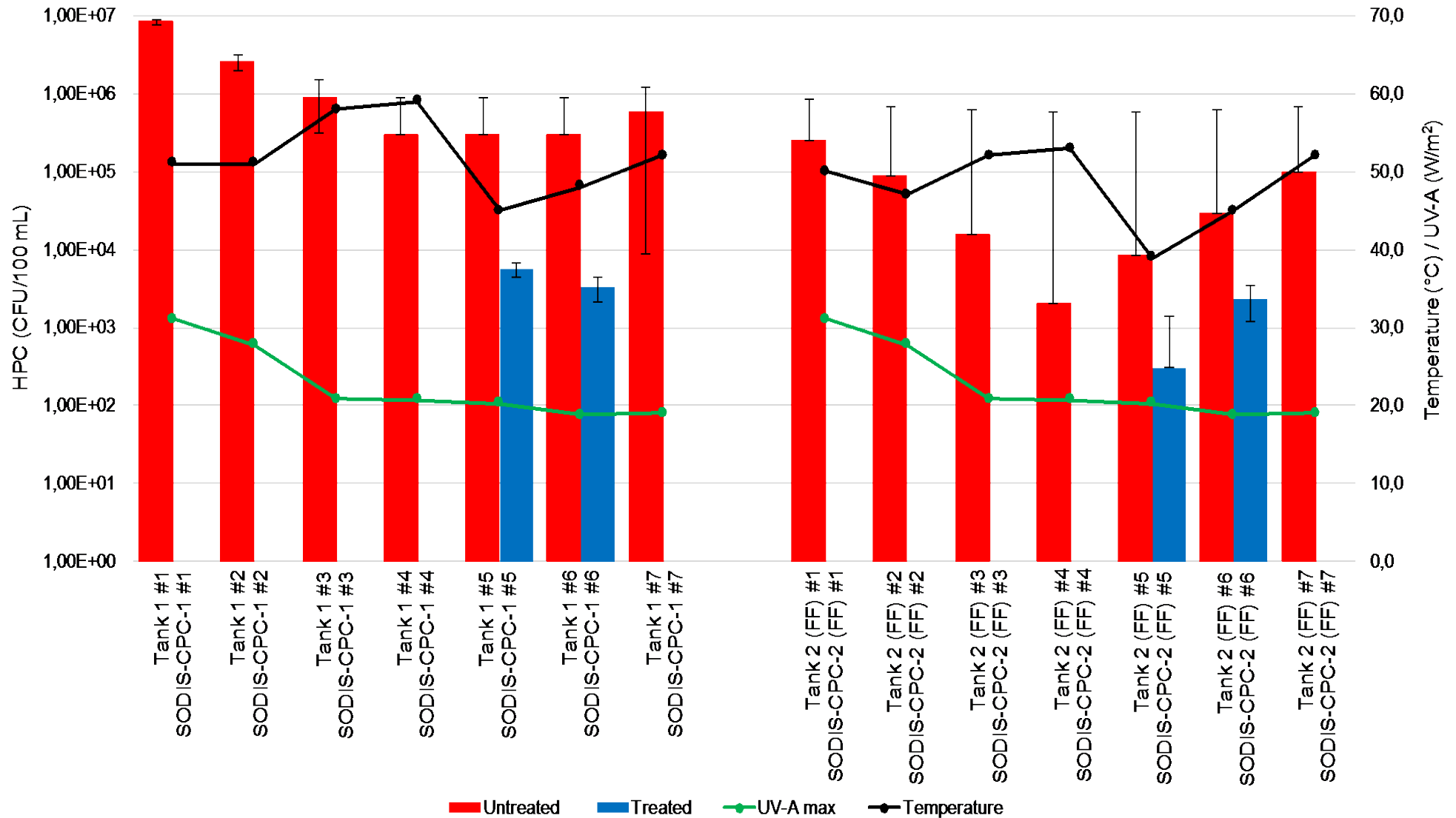


Figure 2.7: Heterotrophic plate count bacteria detected, using culture based analysis, in Tank 1 and Tank 2 (FF) rainwater samples (red), with their corresponding SODIS treated [SODIS-CPC-1 and SODIS-CPC-2 (FF), respectively] rainwater samples (blue). The black and green lines represent the temperature recorded in the SODIS treated sample after eight hrs and the maximum ambient UV-A radiation recorded on the sampling day, respectively.

2.3.4.2. Tank 2 (FF) and SODIS-CPC-2 (FF) rainwater samples

Untreated rainwater samples were collected from Tank 2 (FF), while SODIS treated rainwater samples were collected from SODIS-CPC-2 (FF) in order to assess the microbial quality. Indicator bacteria including enterococci, faecal coliform, total coliform, HPC and *E. coli* counts were monitored and compared to the various drinking water guidelines. Similar to results obtained for Tank 1 and SODIS-CPC-1, enterococci and faecal coliform counts were below the detection limit (< 1 CFU/100 mL) in all samples analysed and complied with the respective drinking water guidelines as stipulated by ADWG (NHMRC & NRMCC, 2011), DWAF (DWAF, 1996), WHO (WHO, 2011) and SANS 241 (SABS, 2015) (results not shown).

In contrast, the *E. coli* counts exceeded the drinking water guidelines of < 1 CFU/100 mL as stipulated by ADWG (NHMRC & NRMCC, 2011), DWAF (DWAF, 1996), WHO (WHO, 2011) and SANS 241 (SABS, 2015) in all Tank 2 (FF) rainwater samples, with the exception of results recorded during sampling event one, where *E. coli* counts were below the detection limit (< 1 CFU/100 mL) (**Table 2.6**). For the untreated rainwater samples collected from Tank 2 (FF), the *E. coli* counts then ranged from below the detection limit to a maximum of 2.1×10^1 CFU/100 mL, with an overall mean of 8 CFU/100 mL recorded. However, all *E. coli* counts were significantly reduced ($p < 0.05$) to below the detection limit (< 1 CFU/100 mL) after SODIS treatment in the SODIS-CPC-2 (FF) reactor and were within the drinking water guidelines (DWAF, 1996; NHMRC & NRMCC, 2011; SABS, 2015; WHO, 2011).

Table 2.6: Total coliform (TC) and *E. coli* counts recorded before [Tank 2 (FF)] and after SODIS treatment [SODIS-CPC-2 (FF)] at various UV-A irradiances and temperatures.

Sampling session	*UV-A (W/m ²)	**SODIS Temp (°C)	Indicator	Tank 2 (CFU/100 mL)	SODIS-CPC-2 (CFU/100 mL)	% reduction
1	31.1	50	<i>E. coli</i>	BDL	BDL	> 99.9
			TC	1.0×10^3	BDL	> 99.9
2	27.8	47	<i>E. coli</i>	6	BDL	> 99.9
			TC	1.1×10^1	BDL	> 99.9
3	20.8	52	<i>E. coli</i>	2.1×10^1	BDL	> 99.9
			TC	3.3×10^3	BDL	> 99.9
4	20.7	53	<i>E. coli</i>	2	BDL	> 99.9
			TC	7.1×10^2	BDL	> 99.9
5	20.3	39	<i>E. coli</i>	2	BDL	> 99.9
			TC	8.0×10^2	2.2×10^1	97.3
6	18.8	45	<i>E. coli</i>	3	BDL	> 99.9
			TC	1.9×10^3	1.1×10^1	99.4
7	19.1	52	<i>E. coli</i>	2.0×10^1	BDL	> 99.9
			TC	2.8×10^3	2	99.9

BDL – below detection limit (< 1 CFU/100 mL)

* - Maximum ambient UV-A data; ** - Temperature recorded in SODIS treated rainwater sample after eight hrs

In addition, the total coliform counts recorded in the rainwater samples collected from Tank 2 (FF) were above the drinking water guidelines of < 5 CFU/100 mL and < 10 CFU/100 mL as stipulated by DWAF (DWAF, 1996) and SANS 241 (SABS, 2015), respectively (**Table 2.6**). The total coliform counts then ranged from a minimum of 1.1×10^1 CFU/100 mL to a maximum of 3.3×10^3 CFU/100 mL, with an overall mean of 1.5×10^3 CFU/100 mL recorded in all Tank 2 (FF) rainwater samples. The total coliform counts were then significantly reduced ($p < 0.05$) to below the detection limit (< 1 CFU/100 mL) in SODIS-CPC-2 (FF) rainwater samples collected during sampling events 1 to 4. However, while a 1- to 2-log reduction was recorded in the total coliform counts recorded in the rainwater samples collected from SODIS-CPC-2 (FF) during sampling sessions 5 to 7, total coliform counts of 2.2×10^1 CFU/100 mL, 1.1×10^1 CFU/100 mL and 2 CFU/100 mL, were still recorded. Overall, in comparison to the total coliforms recorded in the untreated rainwater samples collected from Tank 2 (FF), a significant reduction ($p = 0.017$) (99.7 %) in total coliforms of 2.48-log was observed after treatment in the SODIS-CPC-2 rainwater samples.

Results for the enumeration of the HPC recorded in the rainwater samples collected from Tank 2 (FF) and SODIS-CPC-2 (FF) are also depicted in **Figure 2.7**. With the exception of results recorded for sampling sessions 4 (2.0×10^3 CFU/100 mL) and 5 (8.4×10^3 CFU/100 mL), all the HPC recorded for the rainwater samples collected from Tank 2 (FF) were above the drinking water guideline of $< 1.0 \times 10^4$ CFU/100 mL as stipulated by DWAF (DWAF, 1996). Overall the HPC counts ranged from a minimum of 2.0×10^3 CFU/100 mL to a maximum of 2.5×10^5 CFU/100 mL with an overall mean of 7.1×10^4 CFU/100 mL recorded. However, with the exception of the HPC recorded during sampling sessions 5 and 6, the heterotrophic bacteria counts decreased to below the detection limit (< 1 CFU/100 mL) after SODIS treatment in the SODIS-CPC-2 (FF) reactor and were within drinking water standards. During sample sessions 5 and 6, HPC of 8.4×10^3 CFU/100 mL and 3.0×10^4 CFU/100 mL were then recorded in the Tank 2 (FF) samples, respectively, which decreased by 1.45 and 1.12-log after SODIS treatment to 3.0×10^2 CFU/100 mL and 2.3×10^3 CFU/100 mL, respectively, which were within the DWAF (DWAF, 1996) guideline of $< 1.0 \times 10^4$ CFU/100 mL. It should however be noted that, in comparison to the HPC recorded in the untreated rainwater samples collected from Tank 2 (FF), an overall reduction of 2.28-log was observed in the SODIS-CPC-2 (FF) rainwater samples.

2.3.4. Quantitative PCR analysis of *Legionella* spp. in untreated and SODIS treated rainwater

Quantitative PCR analysis was used in conjunction with EMA pre-treatment in order to quantify the viable *Legionella* copy numbers present in the untreated and SODIS treated rainwater samples. A standard curve was generated with a linear range of 10^8 to 10^1 gene copies per μ L using the computer software LightCycler®96 Ver. 1.1.0.1320 (Roche Diagnostics International Ltd). Furthermore, an amplification efficiency of 1.83 (92 %) was obtained with a R^2 value of 1.00. The lower limit of detection (LLOD) was recorded as 77 gene copies per mL and using the standard curve

generated, *Legionella* copy numbers were quantified in all the rainwater samples collected before and after SODIS treatment and are represented as 23S rRNA gene copies per mL (**Figure 2.8**).

The *Legionella* copy numbers recorded in Tank 1 ranged from a minimum of 5.2×10^6 copies/mL to a maximum of 1.9×10^7 copies/mL (**Figure 2.8**). The *Legionella* copy numbers recorded in treated rainwater samples collected from the SODIS-CPC-1 system then ranged from a minimum of 1.9×10^4 copies/mL to a maximum of 6.9×10^6 copies/mL. The overall mean of 1.1×10^7 copies/mL, observed before treatment (Tank 1), then decreased significantly ($p = 0.007$) by 0.61-log (75.5 %) to an overall mean of 2.7×10^6 copies/mL after SODIS treatment in the SODIS-CPC-1 reactor. Furthermore, the greatest log reduction (2.74-log) was observed during the first sampling session where the highest ambient solar radiation of UV-A (31.1 W/m^2) and UV-B (4.2 W/m^2) was observed.

The EMA-qPCR results obtained for *Legionella* copy numbers recorded in Tank 2 (FF) then ranged from a minimum of 6.0×10^5 copies/mL to a maximum of 1.9×10^7 copies/mL (**Figure 2.8**), with the 23S rRNA *Legionella* gene copy numbers ranging from a minimum of 4.2×10^4 copies/mL to a maximum of 2.9×10^5 copies/mL in the treated rainwater samples collected from the SODIS-CPC-2 (FF) reactor. The overall mean of 3.9×10^6 copies/mL observed before treatment for samples collected from Tank 2 (FF), then decreased [albeit not significantly ($p = 0.195$) due to fluctuation in data and the range of copies/mL values recorded] by 1.42-log (96.2 %) to an overall mean of 1.5×10^5 copies/mL after SODIS treatment in the SODIS-CPC-2 (FF) rainwater samples. Moreover, the greatest log reduction (2.14-logs) in *Legionella* copy numbers was observed during sampling 2 when the ambient solar radiation was recorded as 27.8 W/m^2 and 3.8 W/m^2 for UV-A and UV-B (second highest solar radiation recorded in the current study), respectively.

2.3.5. Quantitative PCR analysis of *Pseudomonas* spp. in untreated and SODIS treated rainwater

Quantitative PCR analysis was used in conjunction with EMA pre-treatment in order to quantify the viable *Pseudomonas* copy numbers present in the untreated and SODIS treated rainwater samples. A standard curve was generated with a linear range of 10^8 to 10^1 gene copies per μL using the computer software LightCycler®96 Ver. 1.1.0.1320 (Roche Diagnostics International Ltd). Furthermore, a mean amplification efficiency of 1.87 (94%) was obtained with a R^2 mean value of 0.94. The LLOD was determined to be 20 to 21 gene copies per mL and using the standard curve generated, *Pseudomonas* copy numbers were quantified in the rainwater samples collected before and after SODIS treatment and are represented as lipoprotein *oprI* gene copies per mL (**Figure 2.9**).

For the rainwater samples collected from Tank 1, the *Pseudomonas* copy numbers recorded ranged from a minimum of 7.0×10^5 copies/mL to a maximum of 7.9×10^6 copies/mL. *Pseudomonas* copy numbers recorded in the SODIS-CPC-1 rainwater samples ranged from a minimum of 7.6×10^3 to a maximum of 4.5×10^5 copies/mL.

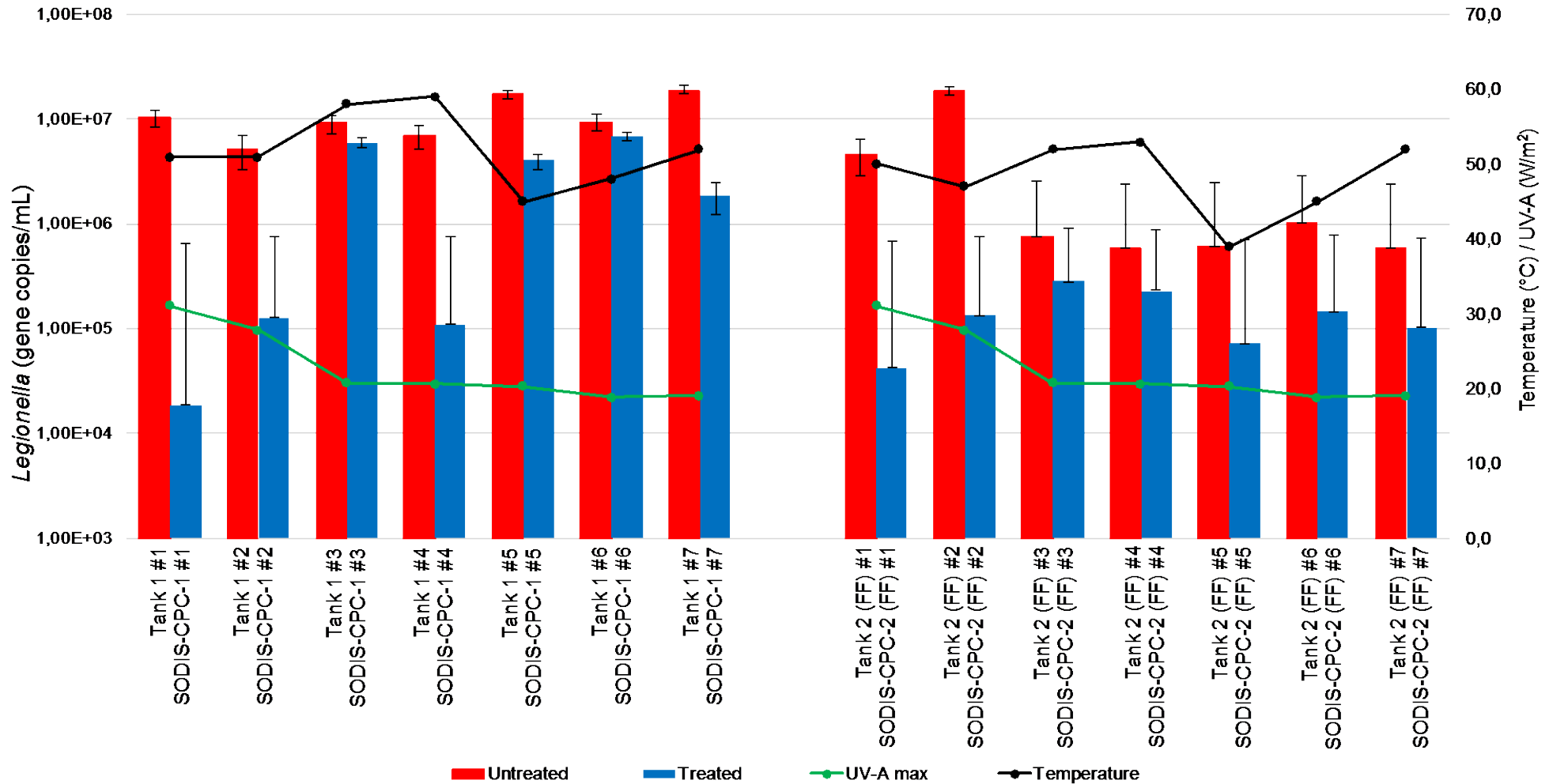


Figure 2.8: Viable *Legionella* spp. gene copy numbers (EMA-qPCR) recorded in untreated and corresponding SODIS treated rainwater samples collected after eight hrs of treatment. The untreated [Tank 1 and Tank 2 (FF)] rainwater samples are represented by the red bars, while the treated [SODIS-CPC-1 and SODIS-CPC-2 (FF)] rainwater samples are represented by the blue bars. The black and green lines represent the temperature recorded in the SODIS treated sample after eight hrs and the maximum ambient UV-A radiation recorded on the sampling day, respectively. Error bar: SE (1 SD) of duplicate samples analysed.

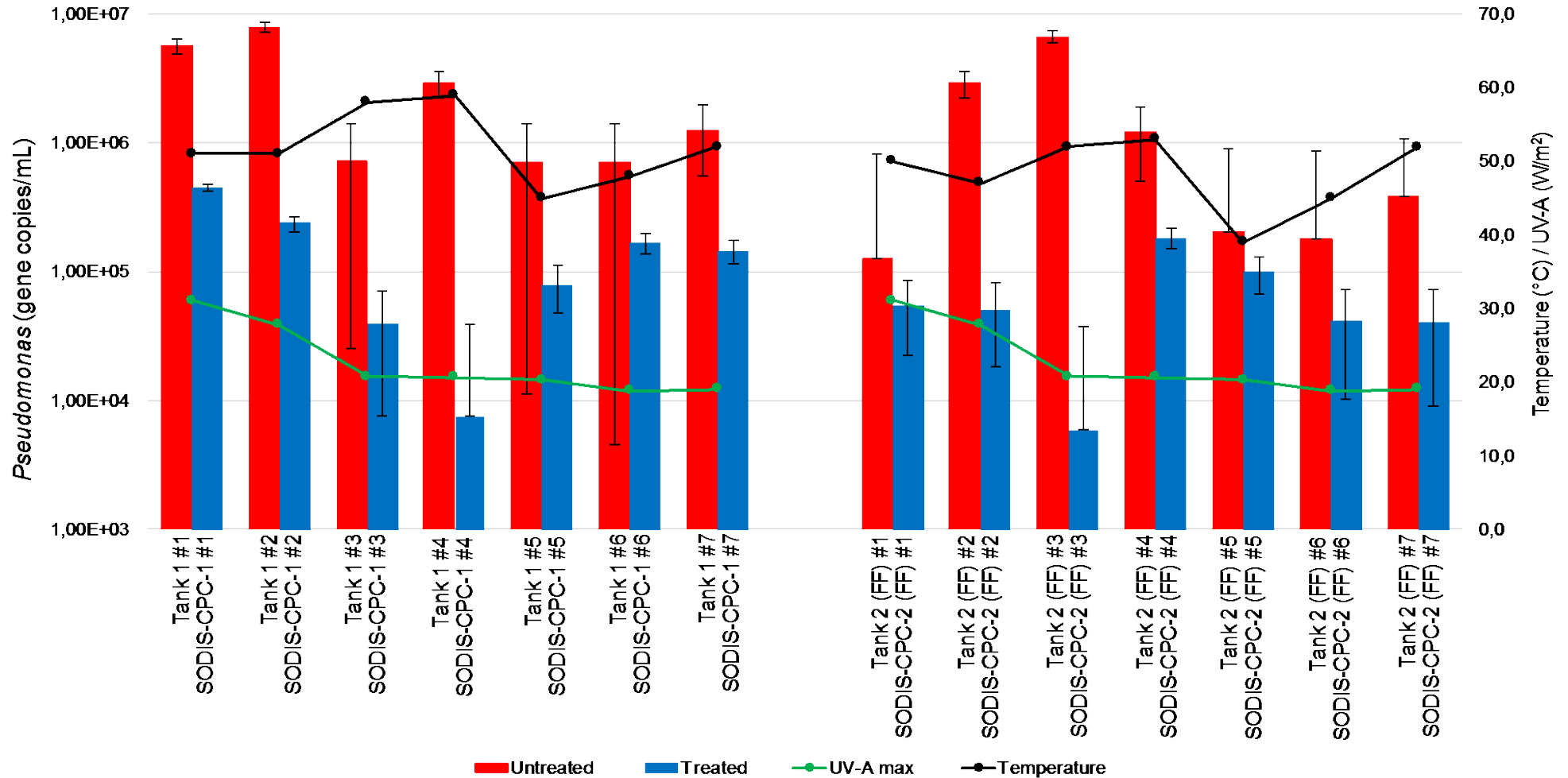


Figure 2.9: Viable *Pseudomonas* spp. gene copy numbers (EMA-qPCR) recorded in untreated and corresponding SODIS treated rainwater samples collected after eight hrs of treatment. The untreated [Tank 1 and Tank 2 (FF)] rainwater samples are represented by the red bars, while the treated [SODIS-CPC-1 and SODIS-CPC-2 (FF)] rainwater samples are represented by the blue bars. The black and green lines represent the temperature recorded in the SODIS treated sample after eight hrs and the maximum ambient UV-A radiation recorded on the sampling day, respectively. Error bar: SE (1 SD) of duplicate samples analysed.

The overall mean *Pseudomonas* copy numbers of 2.8×10^6 copies/mL recorded in Tank 1 rainwater samples then significantly ($p = 0.044$) decreased by 1.24-log (94.3 %) to 1.6×10^5 *Pseudomonas* copies/mL after SODIS treatment. The greatest log reduction (2.58-log) in *Pseudomonas* copy numbers was observed when a rainwater temperature of 59 °C was recorded after eight hrs in the treated sample collected during sampling session 4 (**Figure 2.9**), however the maximum ambient UV-A and UV-B were measured as 20.7 W/m² and 3.2 W/m² (fourth highest solar radiation values recorded in the current study), respectively on that sampling day (**Figure 2.6**).

The *Pseudomonas* copy numbers recorded in Tank 2 (FF) then ranged from a minimum of 1.3×10^5 copies/mL to a maximum of 6.6×10^6 copies/mL (**Figure 2.9**), with *Pseudomonas* gene copy numbers ranging from a minimum of 6.0×10^3 copies/mL to a maximum of 1.8×10^5 copies/mL in the treated rainwater samples collected from the SODIS-CPC-2 (FF) reactor. The overall mean of 1.7×10^6 copies/mL observed before treatment for samples collected from Tank 2 (FF), then decreased [albeit not significantly ($p = 0.133$) due to fluctuation in data and the range of copies/mL values recorded] by 1.39-log (95.9 %) to an overall mean of 6.8×10^4 copies/mL after SODIS treatment in the SODIS-CPC-2 (FF) rainwater samples. Moreover, the greatest log reduction (3.05-log) in *Pseudomonas* copy numbers was observed during sampling session 3 where a temperature of 52 °C was recorded in the SODIS treated sample after eight hrs [second highest temperature recorded for a SODIS-CPC-2 (FF) sample in this study] and a relatively high maximum ambient UV radiation of 20.3 W/m² and 3.1 W/m² for UV-A and UV-B, respectively was recorded (third highest ambient UV radiation recorded in this study) on that sampling day (**Figure 2.6**).

2.4. Discussion

To analyse the efficiency of the SODIS-CPC systems for the treatment of rainwater, various physicochemical (e.g. pH, turbidity), chemical (e.g. anions, cations) and microbial [e.g. indicator counts including total and faecal coliforms, *E. coli*, enterococci and heterotrophic bacteria] parameters were investigated. While a significant difference was observed between the mean pH values recorded in Tank 1 and Tank 2 (FF) ($p = 0.034$) and SODIS-CPC-1 and SODIS-CPC-2 (FF) ($p = 0.013$), the pH of all the samples fell within the target quality range for drinking water as stipulated by DWAF (DWAF, 1996) and SANS 241 (SABS, 2015), where pH values of 6.0 to 9.0 and 5.0 to 9.7 are recommended, respectively. It is imperative to routinely monitor the pH of rainwater as the leaching of toxic metals such as Pb and Cu, from the roofing material, could occur at pH values < 6.0 (DWAF, 1996). In contrast, pH values greater than 9.0 negatively affect the organoleptic properties and aesthetics of the water and toxic effects may be associated with the water source due to the formation of deprotonated species (ammonia from ammonium) (DWAF, 1996).

As research has indicated that an increase in turbidity significantly decreases the microbial inactivation efficiency of SODIS (Amin & Han, 2009; Amin et al. 2014; McGuigan et al. 2012), the turbidity of all rainwater samples collected during the study period was determined with results

indicating that no significant difference ($p > 0.05$) was observed in the turbidity values recorded in the untreated and SODIS-CPC treated rainwater samples. In addition, comparison of the turbidity values recorded in the untreated versus the corresponding treated [Tank 1 vs. SODIS-CPC-1; Tank 2 (FF) vs. SODIS-CPC-2 (FF)] rainwater samples did not yield any significant difference ($p > 0.05$). Tank 2 (FF) was connected to a pre-filtration first flush diverter, which should effectively reduce the initial contamination in the rainwater tank and correspondingly the turbidity levels. It should however, be noted that the collection point (ball valve tap) of the harvesting tanks is located ~50 cm from the bottom of the tank (**Figure 2.4**). It is thus hypothesised that sedimentation of solids occurred below the tank water collection point which could elucidate the similar NTU values recorded in the current study for samples collected from the respective rainwater tanks [Tank 1 and Tank 2 (FF)] and subsequently the SODIS-CPC [SODIS-CPC-1 and SODIS-CPC-2 (FF)] systems. The NTU values recorded in the current study were also within the aesthetic turbidity value (≤ 5 NTU) stipulated by SANS 241 for drinking water (SABS, 2015).

In the current study, the anion concentrations recorded in the representative untreated [Tank 1 and Tank 2 (FF)] and treated [SODIS-CPC-1 and SODIS-CPC-2 (FF)] rainwater samples were within the drinking water guidelines as stipulated by ADWG (NHMRC & NRMCC, 2011), DWAF (DWAF, 1996) SANS 241 (SABS, 2015) and the WHO (WHO, 2011). These results correlated to results obtained by Lee et al. (2010) where the microbial and chemical quality of harvested rainwater in Gangneung, South Korea was assessed. The authors indicated that while anions such as NO_3 , Cl and SO_4 were detected in the harvested rainwater samples, the concentrations recorded were well within the drinking water guidelines (not specified in the study). However, comparison of the anion concentrations obtained in Tank 1 versus Tank 2 (FF), indicated that a significant difference in Cl ($p = 0.048$), SO_4 ($p = 0.038$), NO_3 ($p = 0.0004$) and PO_4 ($p = 0.006$) concentrations was obtained. Similarly, when comparing the anion concentrations recorded for treated rainwater samples collected from SODIS-CPC-1 and the SODIS-CPC-2 (FF), a significant difference in Cl ($p = 0.034$), SO_4 ($p = 0.038$), NO_3 ($p = 0.020$) and PO_4 ($p = 0.019$) concentrations was observed. As the mean anion concentrations recorded in samples collected from Tank 2 (FF) and SODIS-CPC-2 (FF) were significantly lower than those recorded in samples collected from Tank 1 and SODIS-CPC-1 (with the exception of F and NO_2 concentrations), respectively, it is hypothesised that the levels of chemical pollutants (anions) in Tank 2 (FF) and the corresponding SODIS-CPC-2 (FF) samples were reduced after pre-filtration using the first flush diverter.

The cation concentrations recorded in all the untreated and SODIS-CPC treated rainwater samples ($n = 28$) were within drinking water guidelines stipulated by ADWG (NHMRC & NRMCC, 2011), DWAF (DWAF, 1996), SANS 241 (SABS, 2015) and the WHO (WHO, 2011). Furthermore, for the comparison of untreated versus SODIS treated rainwater, with the exception of Zn ($p = 0.024$) and Hg ($p = 0.026$), no significant differences ($p > 0.05$) were observed between the cation concentrations recorded in samples collected from Tank 1 versus SODIS-CPC-1. Similarly, with the

exception of Pb ($p = 0.009$), no significant differences ($p > 0.05$) were observed when comparing the cation concentrations recorded in samples collected from Tank 2 (FF) versus SODIS-CPC-2 (FF). It should be emphasised that the cation concentrations of Zn (significant increase; $p < 0.05$) and Hg (significant decrease; $p < 0.05$), which were significantly different after treatment in the SODIS-CPC-1 samples and Pb, which was significantly lower in the SODIS-CPC-2 (FF) samples, were well within national and international drinking water guidelines. The concentrations of these cations fluctuated throughout the sampling period and as no Zn components were utilised in the construction of the SODIS-CPC systems, it is hypothesised that this cation did not leach from the system during treatment. Elevated concentrations of Zn are however, usually linked to anthropogenic activities where Cuculić et al. (20019) indicated that specifically agricultural activities could significantly influence the biogeochemical cycles of trace metals and enhance their bioavailability. The pilot scale systems were constructed on Welgevallen experimental farm, where agricultural activities linked to dairy and vegetable farming are performed on a daily basis which could possibly elucidate the increased Zn concentrations observed in the current study.

The cation concentrations recorded in the untreated and treated rainwater samples fluctuated throughout the sampling period with the mean cation concentrations (except Al, K, Mg, Na and P concentrations) recorded in rainwater samples collected from Tank 1 being significantly higher ($p < 0.05$) than the concentrations recorded in samples analysed for Tank 2 (FF). In contrast, while still within standards, the Al, K, Mg, Na and P concentrations were significantly ($p < 0.05$) higher in the Tank 2 (FF) rainwater samples. Similarly, the mean cation concentrations recorded in the treated rainwater samples collected from SODIS-CPC-1 were significantly higher ($p < 0.05$) than the cation concentrations recorded in samples collected from SODIS-CPC-2 (FF), with the exception of Al, K, Mg, Na and P concentrations, which were significantly ($p < 0.05$) higher in the SODIS-CPC-2 (FF) rainwater samples. The chemical quality results obtained in the current study for the untreated and SODIS-CPC treated rainwater samples, correlated to results obtained by various research groups, where anion and cation concentrations recorded fluctuated irrespective of whether pre-filtration systems were employed (Gikas & Tsihrintzis, 2012; Huston et al. 2012; Sazakli et al. 2007). For example, Yaziz et al. (1989) constructed a simulated first flush system, where a plastic ball was used to block the respective sampling bottles in the series as they sequentially filled with rainwater. The authors concluded that the concentrations of specifically Pb and Zn, initially increased (sampling bottles one to four) as the first flush (hypothesised to contain more pollutants) was harvested and then decreased significantly in sampling bottle five, as less pollutants were collected. In a study conducted in Kleinmond, South Africa, the rainwater quality of 29 rainwater harvesting tanks were monitored where no pre-filtration first-flush diverter systems were installed (Water Research Commission, 2014). While the anion and cation concentrations recorded were within drinking water guidelines [ADWG (NHMRC & NRMCC, 2011); DWAF (DWAF, 1996); SANS 241 (SABS, 2015); WHO (WHO, 2011)], the cation concentrations (e.g. Al, Zn, K, Hg) fluctuated during the study period.

The total water hardness of all rainwater samples (untreated and treated) was calculated with results indicating that the rainwater collected from Tank 1, SODIS-CPC-1, Tank 2 (FF) and SODIS-CPC-2 (FF) could be classified as 'soft' (< 50 mg CaCO₃/L), based on DWAF (DWAF, 1996) criteria, as values of 32.51 mg CaCO₃/L, 32.02 mg CaCO₃/L, 30.78 mg CaCO₃/L and 30.28 mg CaCO₃/L, respectively, were obtained. Total water hardness is an important consideration for water quality, as high concentrations of Ca and Mg, in water classified as excessively "hard" (> 200 mg CaCO₃/L), could lead to the formation of a white lime precipitate on the surface of plumbing and heating systems, thus leading to economic losses and decreased system efficiencies. In contrast, "soft" water could contribute to the corrosion of Cu piping products used in water systems (DWAF, 1996). However, as no Cu products were used in the manufacture of the two SODIS-CPC systems, the 'soft' water did not negatively influence the system's efficiency.

Although numerous studies have concluded that the chemical quality of harvested rainwater generally complies with respective drinking water guidelines, the microbial quality is of particular concern and often exceeds drinking water guidelines (Ahmed et al. 2010; 2012; De Kwaadsteniet et al. 2013; Dobrowsky et al. 2014a; Reyneke et al. 2016). The microbial quality of all untreated and treated rainwater samples collected throughout the sampling period was thus analysed by screening for the traditional indicator organisms. These indicators are also generally employed to monitor the effectiveness of a treatment method, such as SODIS, to reduce the level of microorganisms in a water source (WHO, 2011).

The presence of enterococci, faecal coliforms and *E. coli* in rainwater generally indicates that bird, rodent and animal (which have access to the roof) faecal matter may be present on the roof catchment system, while total coliforms are generally isolated from soil, dust and debris and serve as an indication of disease causing organisms being present in a water source (Owusu-Boateng & Gadogbe, 2015). The faecal coliform and enterococci counts recorded in all the rainwater samples analysed for Tank 1 and Tank 2 (FF) and the corresponding SODIS treated rainwater samples were however, below the detection limit (< 1 CFU/mL) and were comparable to results obtained in previous studies where faecal coliforms and enterococci were not detected (Ahmed et al. 2009; 2012; Chapman et al. 2008; Dobrowsky et al. 2017; Evans et al. 2006; Reyneke et al. 2016). In contrast, results obtained for *E. coli* (except sample collected from Tank 2 during sampling 1 which was BDL), total coliforms and HPC indicated that the harvested rainwater collected from Tank 1 and Tank 2 (FF) was sub-standard and contravened drinking water guidelines stipulated by ADWG (NHMRC & NRMCC, 2011), DWAF (DWAF, 1996), SANS 241 (SABS, 2015) and WHO (WHO, 2011). It should however be noted that the *E. coli* counts recorded in Tank 1 and Tank 2 (FF) were effectively reduced after eight hrs of SODIS treatment to below the detection limit (< 1 CFU/100 mL) in the treated rainwater samples (**Tables 2.5 and 2.6**). In contrast, while the total coliform counts in samples collected during sampling sessions 1 to 4 were reduced by > 99.9% after SODIS treatment and were within drinking water standards (**Tables 2.5 and 2.6**), total coliforms were still detected in

SODIS-CPC-1 and SODIS-CPC-2 (FF) rainwater samples collected during sampling sessions 5 to 7.

With the exception of rainwater samples collected during sampling sessions 4 and 5 for Tank 2, where counts of 2.0×10^3 CFU/100 mL and 8.4×10^3 CFU/100 mL, respectively were recorded, results for the enumeration of heterotrophic bacteria exceeded the drinking water guideline of $< 1 \times 10^4$ CFU/100 mL in rainwater samples collected from Tank 1 and Tank 2 (FF) (DWAF, 1996). In addition, the mean HPC recorded in the Tank 2 (FF) rainwater samples was 1.43-logs lower than the HPC recorded in the Tank 1 rainwater samples, while the mean HPC recorded in the SODIS-CPC-2 (FF) rainwater samples was 0.54-logs lower than the HPC recorded in the SODIS-CPC-1 rainwater samples. After SODIS treatment, with the exception of rainwater samples collected during sampling sessions 5 and 6, for both SODIS-CPC-1 and SODIS-CPC-2 (FF) samples, all heterotrophic bacteria counts were reduced to below the detection limit (< 1 CFU/100 mL) and were within the drinking water guidelines (DWAF, 1996). The HPC counts in the treated rainwater samples collected during sampling sessions 5 and 6 were however, reduced to within the drinking water guideline of $< 1.0 \times 10^4$ CFU/100 mL (DWAF, 1996).

As indicated, total coliforms were still detected in the treated rainwater samples collected from SODIS-CPC-1 and SODIS-CPC-2 (FF) during sampling sessions 5 to 7, while HPC were still detected in these samples during sampling sessions 5 and 6. While SODIS is based on the synergistic effect of UV and heat to destroy microorganisms, numerous research groups have indicated that UV predominantly produces the bactericidal effect (Hoerter et al. 2005; Ubomba-Jaswa et al. 2009). As indicated in **Tables 2.5** and **2.6**, the temperature of the SODIS treated rainwater samples was lowest during sampling sessions 5 and 6, while the lowest solar radiation (UV-A and UV-B radiation and DNI) was recorded during sampling sessions 6 and 7. Thus, while the rainwater temperatures recorded in the samples collected from the two SODIS systems differed [lower temperature generally recorded for samples collected from SODIS-CPC-2 (FF)], it was noted that an increased mean solar exposure (UV-A: 25.1 W/m^2 ; UV-B: 3.6 W/m^2 ; DNI: 959.4 W/m^2) and an increased mean treated rainwater temperature [SODIS-CPC-1: $55 \text{ }^\circ\text{C}$ and SODIS-CPC-2 (FF): $51 \text{ }^\circ\text{C}$] recorded during sampling sessions 1 to 4 was required to effectively inactivate total coliforms to below the detection limit of < 1 CFU/100 mL and reduce the HPC count by 3.17-logs and 2.28-logs in the SODIS-CPC-1 and SODIS-CPC-2 (FF) systems, respectively. Furthermore, the heterotrophic bacteria were completely inactivated by temperatures as low as $50 \text{ }^\circ\text{C}$ if a higher solar radiation (UV-A: 31.1 W/m^2 ; UV-B: 4.2 W/m^2 ; DNI: 1028.6 W/m^2) dosage was obtained. These results correlate to the results obtained by Ubomba-Jaswa et al. (2010) where SODIS was used to treat well water spiked with *E. coli*. Temperatures of $> 45 \text{ }^\circ\text{C}$ resulted in the complete inactivation of *E. coli* if adequate solar exposure (UV-A: 26.9 W/m^2) was present, even during the winter season. Similarly, Strauss et al. (2016) treated rainwater in a solar cooker for six and eight hrs of UV exposure

and indicated that a minimum water temperature of 52 °C (solar radiation data was not reported) was required for the complete inactivation of heterotrophic bacteria.

It has however been documented that the presence of indicator microorganisms does not always correlate positively with the presence of opportunistic bacteria in roof-harvested rainwater (Harwood et al. 2005; Savichtcheva & Okabe, 2006; Wilkes et al. 2009). For example, numerous research groups have indicated that opportunistic bacteria, such as *Klebsiella* spp., *Staphylococcus* spp., *Serratia* spp., *Legionella* spp. and *Pseudomonas* spp. amongst others, persist in roof-harvested rainwater when low indicator counts are recorded (Ahmed et al. 2010; Dobrowsky et al. 2015a; Strauss et al. 2016). Ethidium monoazide bromide (EMA) and propidium monoazide bromide (PMA) are nucleic acid binding dyes that can be used to bind to the DNA of cells (after photoactivation) with damaged and/or permeable membranes (non-viable cells). The binding of the dye to the DNA prevents PCR amplification of the DNA and thereby leads to a strong signal reduction during qPCR as only the DNA from intact (viable) cells will be amplified (Fittipaldi et al. 2012; Reyneke et al. 2016). Reyneke et al. (2017) compared EMA-, PMA- and DNase qPCR as well as various concentrations of EMA and PMA for the accurate determination of microbial cell viability. *Legionella pneumophila* and *P. aeruginosa*, amongst other organisms, were used as test organisms in the respective viability assays with the authors concluding that EMA applied at a concentration of 6 µM could be utilised for the accurate quantification of viable bacterial cells. Consequently, EMA-qPCR (6 µM) was utilised in the current study to monitor the efficiency of the SODIS-CPC systems to reduce the levels of viable *Legionella* and *Pseudomonas* spp. in roof-harvested rainwater.

Ethidium monoazide bromide qPCR analysis indicated that viable *Legionella* spp. (reported as *Legionella* 23S rRNA gene copy numbers) were detected in all Tank 1 (mean of 1.1×10^7 copies/mL) and Tank 2 (FF) (mean of 3.9×10^6 copies/mL) rainwater samples, with an overall mean reduction in copy numbers of 0.61-logs and 1.42-logs recorded after SODIS treatment using the SODIS-CPC-1 and SODIS-CPC-2 (FF) systems, respectively. Similarly, utilising EMA-qPCR, Reyneke et al. (2016) indicated that *Legionella* spp. were still viable after SOPAS treatment at a temperature of 95 °C, while Strauss et al. (2016) indicated that viable *Legionella* spp. and *Pseudomonas* spp. were present after eight hrs of SODIS treatment (solar cooker with 2 L PET bottles as reactors) at a maximum temperature of 89 °C. Furthermore, while not significant ($p = 0.117$), comparison of results indicated that the mean *Legionella* copy numbers recorded in Tank 2 (FF) were 0.46-logs lower than the mean *Legionella* copy numbers recorded in Tank 1. Albeit not significant ($p = 0.057$), the mean *Legionella* copy numbers observed in SODIS-CPC-2 (FF) were also 1.27-logs lower than results obtained for SODIS-CPC-1.

The greatest reduction (2.74-logs) in viable *Legionella* copy numbers was obtained during sampling session 1 (**Figure 2.6**) after treatment in the SODIS-CPC-1 system where the highest ambient UV radiation data (UV-A: 31.1 W/m²; UV-B: 4.2 W/m²; DNI: 1028.6 W/m²) was recorded. For the SODIS-CPC-2 (FF) system, the greatest reduction (2.14-log) was obtained during sampling session 2

(**Figure 2.6**) where the second highest ambient UV irradiance data (UV-A: 27.8 W/m²; UV-B: 3.8 W/m²; DNI: 918, 6 W/m²) was recorded. In comparison, a 1.80-log and 0.41-log reduction was observed in viable *Legionella* copy numbers when temperatures of 59 °C (sampling 4) and 53 °C (sampling 4) were recorded after eight hrs of SODIS treatment in the rainwater samples collected from the SODIS-CPC-1 and SODIS-CPC-2 (FF) systems, respectively. Thus, based on the results obtained in the current study, solar exposure or UV radiation yielded an increased disinfection efficiency as a greater reduction in viable *Legionella* copy numbers was obtained. The survival and proliferation of *Legionella* spp. after UV disinfection is however, a matter of serious concern as this bacterium enters the body by inhalation of aerosolised contaminated water, whereafter it is able to replicate and infect the alveoli, resulting in Legionnaires' disease or a mild flu-like disease called Pontiac fever (Fields et al. 2002; WHO, 2007). Muraca et al. (1987) compared the disinfection efficiency of chlorination, heat, ozonation and UV light in a model plumbing system which was spiked with *L. pneumophila* (10⁷ CFU/mL) and concluded that while continuous UV irradiation resulted in a 5-log reduction in viable *L. pneumophila* cells after 20 minutes, a 2-log cell concentration was still recorded after six hrs of continuous UV irradiation exposure. Oguma et al. (2004) also investigated the photoreactivation⁵ of *L. pneumophila* after UV inactivation. Results indicated that while a 3-log reduction in *L. pneumophila* cells was obtained after UV treatment, the process of photoreactivation in *L. pneumophila* effectively only resulted in an overall reduction of 0.5-logs. Thus, through the process of photoreactivation the *L. pneumophila* cells were able to repair the DNA damage caused by the UV treatment, ultimately yielding a lower disinfection efficiency.

Results obtained for the *Pseudomonas* EMA-qPCR analysis indicated that viable *Pseudomonas* spp. were detected in rainwater samples collected from Tank 1 (mean of 2.8 × 10⁶ copies/mL) and Tank 2 (FF) (mean of 1.7 × 10⁶ copies/mL), which correlated to results obtained in previous studies (Albrechtsen, 2002; Amin et al. 2014; Dobrowsky et al. 2014b; Kaushik et al. 2012). A reduction of 1.24-log and 1.39-log in viable *Pseudomonas* copy numbers was then observed after treatment using the SODIS-CPC-1 and SODIS-CPC-2 (FF) systems, respectively. Furthermore, while not significant ($p = 0.445$), comparison of results indicated that the mean *Pseudomonas* copy numbers recorded in Tank 2 (FF) was 0.23-log lower than the mean *Pseudomonas* copy numbers recorded in Tank 1. Similarly, while not significant ($p = 0.213$), the mean *Pseudomonas* copy numbers observed in SODIS-CPC-2 (FF) were 0.38-logs lower than *Pseudomonas* copy numbers obtained in SODIS-CPC-1 samples. Thus, as observed for the mean *Legionella* gene copy numbers, *Pseudomonas* gene copy numbers were lower in the untreated [Tank 2 (FF)] and treated [SODIS-CPC-2 (FF)] rainwater samples exposed to pre-filtration.

⁵ Recovery from biological damage caused by UV radiation by the subsequent treatment of light with shorter wavelengths.

For the SODIS-CPC-1 rainwater samples, the greatest reduction (2.58-logs) in *Pseudomonas* copy numbers was observed when a rainwater temperature of 59 °C (sampling 4) was recorded after eight hrs in the sample collected during sampling session 4 (**Figure 2.9**), while the second highest reduction (1.52-logs) was obtained when high UV irradiance data of UV-A: 27.8 W/m²; UV-B: 3.8 W/m² and DNI: 918.6 W/m² was logged during sampling 2. Similarly for the SODIS-CPC-2 (FF) rainwater samples, the greatest reduction (3.05-logs) in *Pseudomonas* copy numbers was observed when a rainwater temperatures of 52 °C [second highest temperature recorded for samples collected from SODIS-CPC-2 (FF)] was recorded after eight hrs of SODIS treatment in the sample collected during sampling session 3, while the second highest reduction (1.77-logs) was observed during high UV irradiance exposure (UV-A: 27.8 W/m²; UV-B: 3.8 W/m² and DNI: 918.6 W/m²; second highest solar radiation data recorded during this study) recorded during sampling session 2. Thus, based on the results obtained in the current study, *Pseudomonas* spp. are susceptible to high temperatures (> 52 °C) as well as to the effect of solar exposure (UV-A > 27.8 W/m²; UV-B > 3.8 W/m² and DNI > 918.6 W/m²). However, viable *Pseudomonas* copy numbers were still observed after treatment in both SODIS systems, which correlates to results obtained in previous studies where UV treatment was utilised for the disinfection of *Pseudomonas* spp. For example, Amin et al. (2014) applied SODIS to treat harvested rainwater and subsequently determined the percentage survival of *P. aeruginosa* using culture based methods. The authors reported that *P. aeruginosa* was completely inactivated after nine hrs when temperature of > 50 °C and solar irradiance (DNI) of > 700 W/m² was obtained. The inactivation of *Pseudomonas* spp. was however only monitored using culture based methods and molecular viability or metabolic assessment assays are required to accurately determine the disinfection efficiency.

Results obtained in the current study thus also indicate that *Pseudomonas* was viable after SODIS treatment when a rainwater temperature of 59 °C (highest temperature recorded in a treated sample after eight hrs) and solar irradiance (DNI) of 956.1 W/m² was obtained. It is recognised that *Pseudomonas* can enter a viable but non-culturable state which allows it to survive adverse conditions (Dwidjosiswojo et al. 2011). Additionally, Srivastava et al. (2008) indicated that the overexpression of the sigma factor *algT*, protects the *Pseudomonas* spp. from heat stress and allows these organisms to persist during unfavourable conditions. *Pseudomonas* can also form associations with protozoa where they exist as intracellular parasites (Thomas et al. 2010). Furthermore, *Pseudomonas* spp. are able to enter the human body through a skin wound or during surgery where it is absorbed into the blood stream leading to bacteraemia that could cause endocarditis, pneumonia, gastrointestinal and urinary tract infections (Lyczak et al. 2000; Mena & Gerba, 2000).

2.5. Conclusion

Chemical analysis of the untreated and SODIS-CPC system treated rainwater indicated that all cation and anion concentrations were within drinking water guidelines as stipulated by various

national and international water associations. In addition, while the cation concentrations in the untreated and treated rainwater samples fluctuated throughout the study period, the FF diverter effectively reduced the anion concentrations (Cl, SO₄, NO₃ and PO₄) in Tank 2 (FF) rainwater samples. Similarly, the total coliform and HPC counts, as well as *Legionella* and *Pseudomonas* copy numbers were 0.68-log, 1.43-log, 0.46-log and 0.23-log, respectively lower in Tank 2 (FF) rainwater samples, in comparison to values recorded in Tank 1 rainwater samples, which indicates that pre-filtration using the FF diverter effectively reduced the initial microbial contamination load.

The SODIS-CPC systems were also able to effectively reduce the level of microbial contamination in roof-harvested rainwater as an overall mean reduction of 0.87-log, 2.61-log, 2.73-log, 1.02-log and 1.32-log in *E. coli*, total coliforms and HPC as well as *Legionella* and *Pseudomonas* spp. copy numbers, respectively were observed in the treated rainwater samples [SODIS-CPC-1 and SODIS-CPC-2 (FF)], in comparison to the values recorded in the untreated rainwater. It should however be noted that while the *E. coli* and HPC were within the drinking water guidelines as stipulated by various national and international water associations after SODIS treatment, the total coliform counts recorded during sampling sessions 5 to 7 exceeded the drinking water guidelines after SODIS treatment. Furthermore, viable *Legionella* and *Pseudomonas* spp. were detected using EMA-qPCR in the SODIS treated rainwater. Based on these results, it is recommended that the treated rainwater should primarily be used as a supplementary water source for domestic purposes.

Future research should thus focus on quantitative microbial risk assessment studies to determine the health risks associated with the consumption of harvested rainwater where viable opportunistic pathogens such as *Legionella* and *Pseudomonas* are detected. Moreover, it is imperative that next generation sequencing techniques such as pyrosequencing or Illumina sequencing are employed in conjunction with viability assays, to fully elucidate the range of pathogenic microorganisms persisting after treatment. This information is crucial as it allows for the accurate monitoring of the microbial disinfection efficiency of novel treatment methods.

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2.7. References

Ahmed, W., Huygens, F., Goonetilleke, A., Gardner, T., 2008. Real-time PCR detection of pathogenic microorganisms in roof-harvested rainwater in Southeast Queensland, Australia. *Appl. Environ. Microbiol.* 74(17), 5490–5496.

- Ahmed, W., Sawant, S., Huygens, F., Goonetilleke, A., Gardner, T., 2009. Prevalence and occurrence of zoonotic bacterial pathogens in surface waters determined by quantitative PCR. *Water Res.* 43(19), 4918–4928.
- Ahmed, W., Vieritz, A., Goonetilleke, A., Gardner, T., 2010. Health risk from the use of roof-harvested rainwater in Southeast Queensland, Australia, as potable or non-potable water, determined using quantitative microbial risk assessment. *Appl. Environ. Microbiol.* 76, 7382–91.
- Ahmed, W., Gardner, T., Toze, S., 2011. Microbiological quality of roof-harvested rainwater and health risks: a review. *J. Environ. Qual.* 40, 13-21.
- Ahmed, W., Hodgers, L., Sidhu, J.P.S., Toze, S., 2012. Fecal indicators and zoonotic pathogens in household drinking water taps fed from rainwater tanks in Southeast Queensland, Australia. *Appl. Environ. Microbiol.* 78(1), 219–226.
- Albrechtsen, H.J., 2002. Microbiological investigations of rainwater and graywater collected for toilet flushing. *Wat. Sci. Tech.* 46(6–7), 311–316.
- Amin, M.T., Han, M.Y., 2009. Roof-harvested rainwater for potable purposes: application of solar collector disinfection (SOCO-DIS). *Water Res.* 43(20), 5225–5235.
- Amin, M.T., Nawaz, M., Amin, M.N., Han, M., 2014. Solar disinfection of *Pseudomonas aeruginosa* in harvested rainwater: a step towards potability of rainwater. *PloS One.* 9(3), 1–10.
- Asakura, H., Kawamoto, K., Haishima, Y., Igimi, S., Yamamoto, S., Makino, S.I., 2008. Differential expression of the outer membrane protein W (OmpW) stress response in entero- hemorrhagic *Escherichia coli* O157:H7 corresponds to the viable but non-culturable state. *Res. Microbiol.* 159, 709–717.
- Boyle, M., Sichel, C., Fernández-Ibáñez, P., Arias-Quiroz, G.B., Iriarte-Puña, M., Mercado, A., Ubomba-Jaswa, E., McGuigan, K.G., 2008. Bactericidal effect of solar water disinfection under real sunlight conditions. *Appl. Environ. Microbiol.* 74, 2997–3001.
- Brözel, V.S., Cloete, T.E., 1991. Resistance of bacteria from cooling waters to bactericides. *J. Ind. Microbiol.* 8(4), 273–6.
- Castro-Alfárez, M., Polo-López, M.I., Fernández-Ibáñez, P., 2016. Intracellular mechanisms of solar water disinfection. *Sci. Rep.* 6, 38145.
- Chapman, H., Cartwright, T., Huston, R., O’Toole, J., 2008. Water quality and health risks from urban rainwater tanks. Cooperative Research Centre (CRC) for water quality and treatment research report no 42. Salisbury, Australia: CRC for water quality and treatment. ISBN 18766166787.

- Chen, N.T., Chang, C.W., 2010. Rapid quantification of viable *Legionella* in water and biofilm using ethidium monoazide coupled with real-time quantitative PCR. *J. Appl. Microbiol.* 109(2), 623–634.
- Cook, K.L., Bolster, C.H., 2007. Survival of *Campylobacter jejuni* and *Escherichia coli* in groundwater during prolonged starvation at low temperatures. *J. Appl. Microbiol.* 103, 573–583.
- Cuculić, V., Cukrov, N., Kwokal, Ž., Mlakar, M., 2009. Natural and anthropogenic sources of Hg, Cd, Pb, Cu and Zn in seawater and sediment of Mljet National Park, Croatia. *Estuar. Coast Shelf Sci.* 81(3), 311-320.
- Dawney, B., Pearce, J.M., 2012. Optimizing the solar water disinfection (SODIS) method by decreasing turbidity with NaCl. *J. Water. Sanit. Hyg. Dev.* 2(2), 87-94.
- De Kwaadsteniet, M., Dobrowsky, P.H., Van Deventer, A., Khan, W., Cloete, T.E., 2013. Domestic rainwater harvesting: microbial and chemical water quality and point-of-use treatment systems. *Water, Air, Soil Pollut.* 224(7).
- Delgado-Viscogliosi, P., Solognac, L., Delattre, J.M., 2009. Viability PCR, a culture-independent method for rapid and selective quantification of viable *Legionella pneumophila* cells in environmental water samples. *Appl. Environ. Microbiol.* 75(11), 3502-3512.
- Department of Water Affairs (DWA), 2009. Water for growth and development framework. http://www.dwa.gov.za/wfgd/documents/wfgd_frameworkv7.pdf (accessed 07.08.17).
- Department of Water Affairs (DWA), 2013. Department of Water Affairs Annual report 2012/2013. http://www.gov.za/sites/www.gov.za/files/DWA_AnnualReport_2012-13_FullDoc.pdf (accessed 20.04.17).
- Department of Water Affairs and Forestry (DWAf), 1996. South African Water Quality Guidelines 2nd Edition, Volume 1: Domestic Water Use. Pretoria: CSIR Environmental Services.
- Dobrowsky, P.H., Mannel, D., De Kwaadsteniet, M., Prozesky, H., Khan, W., Cloete, T.E., 2014a. Quality assessment and primary uses of harvested rainwater in Kleinmond, South Africa. *Water SA.* 40(3), 401–406.
- Dobrowsky, P.H., De Kwaadsteniet, M., Cloete, T.E., Khan, W., 2014b. Distribution of indigenous bacterial pathogens and potential pathogens associated with roof-harvested rainwater. *Appl. Environ. Microbiol.* 80(7), 2307–2316.
- Dobrowsky, P.H., Carstens, M., De Villiers, J., Cloete, T.E., Khan, W., 2015a. Efficiency of a closed-coupled solar pasteurization system in treating roof-harvested rainwater. *Sci. Total Environ.* 536, 206–214.

- Dobrowsky, P.H., Lombard, M., Cloete, W.J., Saayman, M., Cloete, T.E., Carstens, M., Khan, S., Khan, W., 2015b. Efficiency of microfiltration systems for the removal of bacterial and viral contaminants from surface and rainwater. *Water Air Soil Pollut.* 226(33), 1-14.
- Dobrowsky, P.H., Khan, S., Cloete, T.E., Khan, W., 2017. Microbial and physico-chemical characteristics associated with the incidence of *Legionella* spp. and *Acanthamoeba* spp. in rainwater harvested from different roofing materials. *Water Air Soil Pollut.* 228(85), 1-13.
- Dunn, O.J., Clark, V.A., 1974. *Applied statistics: analysis of variance and regression*, second ed. Wiley, New York.
- Dwidjosiswojo, Z., Richard, J., Moritz, M.M., Dopp, E., Flemming, H.C., Wingender, J., 2011. Influence of copper ions on the viability and cytotoxicity of *Pseudomonas aeruginosa* under conditions relevant to drinking water environments. *Int. J. Hyg. Environ. Health.* 214(6), 485–492.
- Evans, C.A., Coombes, P.J., Dunstan, R.H., 2006. Wind, rain and bacteria: the effect of weather on the microbial composition of roof-harvested rainwater. *Water Res.* 40(1), 37–44.
- Fields, B.S., Benson, R.F., Besser, R.E., 2002. *Legionella* and Legionnaires' Disease: 25 years of investigation. *Clin. Microbiol. Rev.* 15(3), 506–26.
- Fittipaldi, M., Nocker, A., Codony, F., 2012. Progress in understanding preferential detection of live cells using viability dyes in combination with DNA amplification. *J. Microbiol. Methods.* 91(2), 276–89.
- Gardner, T., Baisden, J., Millar, G., 2004. Rainwater first flush devices—are they effective. Presented at the Sustainable Water in the Urban Environment Conference. Australian Water Association, August 2004. Brisbane, Australia.
- Gikas, G.D., Tsihrintzis, V.A., 2012. Assessment of water quality of first-flush roof runoff and harvested rainwater. *J. Hydrol.* 466–467, 115-126.
- Gourmelon, M., Cillard, J., Pommepuy, M., 1994. Visible light damage to *Escherichia coli* in seawater: oxidative stress hypothesis. *J. Appl. Microbiol.* 77(1), 105-112.
- Harwood, V.J., Levine, A.D., Scott, T.M., Chivukula, V., Lukasik, J., Farrah, S.R., Rose, J.B., 2005. Validity of the indicator organism paradigm for pathogen reduction in reclaimed water and public health protection. *Appl. Environ. Microbiol.* 71(6), 3163–70.
- Herpers, B.L., De Jongh, B.M., Van Der Zwaluw, K., Van Hannen, E.J., 2003. Real-time PCR assay targets the 23S-5S spacer for direct detection and differentiation of *Legionella* spp. and *Legionella pneumophila*. *Clin. Microbiol. Rev.* 41(10), 4815–6.

- Hoerter, J.D., Arnold, A.A., Kuczynska, D.A., Shibuya, A., Ward, C.S., Sauer, M.G., Gizachew, A., Hotchkiss, T.M., Fleming, T.J., Johnson, S., 2005. Effects of sub-lethal UV-A irradiation on activity levels of oxidative defence enzymes and protein oxidation in *Escherichia coli*. *J. Photochem. Photobiol.* 81(3), 171-180.
- Huston, R., Chan, Y.C., Gardner, T., Shaw, G., Chapman, H., 2009. Characterisation of atmospheric deposition as a source of contaminants in urban rainwater tanks. *Water Res.* 43(6), 1630–1640.
- Huston, R., Chan, Y.C., Chapman, H., Gardner, T., Shaw, G., 2012. Source apportionment of heavy metals and ionic contaminants in rainwater tanks in a subtropical urban area in Australia. *Water Res.* 46(4), 1121–1132.
- Kapuscinski, R.B., Mitchell, R., 1981. Solar radiation induces sub-lethal injury in *Escherichia coli* in seawater. *Appl. Environ. Microbiol.* 41(3), 670-674.
- Kaushik, R., Balasubramanian, R., Armah, A., 2012. Influence of air quality on the composition of microbial pathogens in fresh rainwater. *Appl. Environ. Microbiol.* 78(8), 2813-2818.
- Lee, J.Y., Yang, J.S., Han, M., Choi, J., 2010. Comparison of the microbiological and chemical characterization of harvested rainwater and reservoir water as alternative water resources. *Sci. Total. Environ.* 408(4), 896–905.
- Lévesque, B., Pereg, D., Watkinson, E., Maguire, J.S., Bissonnette, L., Gingras, S., Dewailly, E., 2008. Assessment of microbiological quality of drinking water from household tanks in Bermuda. *Can. J. Microbiol.* 54(6), 495–500.
- Lyczak, J.B., Cannon, C.L., Pier, G.B., 2000. Establishment of *Pseudomonas aeruginosa* infection: lessons from a versatile opportunist. *Microb. Infect.* 2(9), 1051–1060.
- Maalej, S., Denis, M., Dukan, S., 2004. Temperature and growth-phase effects on *Aeromonas hydrophila* survival in natural seawater microcosms: role of protein synthesis and nucleic acid content on viable but temporarily non-culturable response. *Microbiology.* 150, 181–187.
- Malato Rodríguez, S.M., Gálvez, J.B., Rubio, M.M., Ibáñez, P.F., Padilla, D.A., Pereira, M.C., Mendes, J.F., De Oliveira, J.C., 2004. Engineering of solar photocatalytic collectors. *Solar Energy.* 77(5), 513-524.
- McGuigan, K.G., Méndez-Hermida, F., Castro-Hermida, J.A., Ares-Mazás, E., Kehoe, S.C., Boyle, M., Sichel, C., Fernández-Ibáñez, P., Meyer, B.P., Ramalingham, S., Meyer, E.A., 2006. Batch solar disinfection (SODIS) inactivates oocysts of *Cryptosporidium parvum* and cysts of *Giardia muris* in drinking water. *J. Appl. Microbiol.* 101, 453–463.

- McGuigan, K.G., Conroy, R.M., Mosler, H., Du Preez, M., Ubomba-Jaswa, E., Fernandez-Ibanez, P., 2012. Solar water disinfection (SODIS): a review from bench- top to roof-top. *J. Hazard .Mater.* 235, 29–46.
- Mena, K.D., Gerba, C.P., 2000. Risk assessment of *Pseudomonas aeruginosa* in water. *Rev Environ. Contam. T.* 201, 71–115.
- Muraca, P., Stout, J.E., Yu, V.L., 1987. Comparative assessment of chlorine, heat, ozone, and UV light for killing *Legionella pneumophila* within a model plumbing system. *Appl. Environ. Microbiol.* 53(2), 447-453.
- Mwenge Kahinda, J.M., Taigbenu, A.E., Boroto, J.R., Zere, T., 2007. Rainwater harvesting to improve domestic water supply and sanitation in rural South Africa. *Phys. Chem. Earth.* 32(15–18), 1050-1057.
- National Health and Medical Research Council, National Resource Management Ministerial Council (NHMRC & NRMCMC), 2011. Australian drinking water guidelines 6, Volume 1. National Water Quality Management Strategy. National Health and Medical Research Council, National Resource Management. Canberra, Australia.
- Navntoft, C., Ubomba-Jaswa, E., McGuigan, K.G., Fernandez-Ibáñez, P., 2008. Effectiveness of solar disinfection using batch reactors with non-imaging aluminium reflectors under real conditions: natural well water and solar light. *J. Photochem. Photobiol. B: Biol.* 93, 155–161.
- Oguma, K., Katayama, H., Ohgaki, S., 2004. Photoreactivation of *Legionella pneumophila* after inactivation by low-or medium-pressure ultraviolet lamp. *Water Res.* 38(11), 2757-2763.
- Oliver, J.D., 2010. Recent findings on the viable but non-culturable state in pathogenic bacteria. *FEMS Microbiol. Rev.* 34(4), 415–25.
- Owusu-Boateng, G., Gadogbe, M.K., 2015. Domestic rainwater harvesting in a water-stressed community and variation in rainwater quality from source to storage. *Consilience.* 14, 225-243.
- Pacey, A., Cullis, A., 1986. Rainwater harvesting: the collection of rainfall and runoff in rural areas. Intermediate Technology Publications, London, UK. ISBN 0946688222.
- Reyneke, B., Dobrowsky, P.H., Ndlovu, T., Khan, S., Khan, W., 2016. EMA-qPCR to monitor the efficiency of a closed-coupled solar pasteurization system in reducing *Legionella* contamination of roof-harvested rainwater. *Sci. Total. Environ.* 553, 662–670.
- Reyneke, B., Ndlovu, T., Khan, S., Khan, W., 2017. Comparison of EMA-, PMA- and DNase qPCR for the determination of microbial cell viability. *Appl. Microbiol. Biotechnol.* 101, 7371-7383.

Roosa, S., Wauven, C.V., Billon, G., Matthijs, S., Wattiez, R., Gillan, D.C., 2014. The *Pseudomonas* community in metal-contaminated sediments as revealed by quantitative PCR: a link with metal bioavailability. *Res. Microbiol.* 165, 647–56.

Savichtcheva, O., Okabe S., 2006. Alternative indicators of faecal pollution: relations with pathogens and conventional indicators, current methodologies for direct pathogen monitoring and future application perspectives. *Water Res.* 40(13), 2463–76.

Sazakli, E., Alexopoulos, A., Leotsinidis, M., 2007. Rainwater harvesting, quality assessment and utilization in Kefalonia Island, Greece. *Water Res.* 41(9), 2039–2047.

Sichel, C., Blanco, J., Malato, S., Fernandez-Ibáñez, P., 2007. Effects of experimental conditions on *E. coli* survival during solar photocatalytic water disinfection. *J. Photochem. Photobiol. A Chem.* 189(2), 239-246.

Sommer, B., Marino, A., Solarte, Y., Salas, M.L., Dierolf, C., Valiente, C., Mora, D., Rechsteiner, R., Setter, P., Wirojanagud, W., Ajarmeh, H., 1997. SODIS - an emerging water treatment process. *J. Water Supply Res. T.* 46(3), 127-137.

South African Bureau of Standards (SABS), 2015. South African National Standards (SANS) 241: Drinking water. Part 1: Microbiological, physical, aesthetic and chemical determinants. 2nd edition. Annexure 1. ISBN 978-0-626-29841-8.

Srivastava, S., Yadav, A., Seem, K., Mishra, S., Chaudhary, V., Nautiyal, C.S., 2008. Effect of high temperature on *Pseudomonas putida* NBRI0987 biofilm formation and expression of stress sigma factor RpoS. *Curr. Microbiol.* 56(5), 453-457.

Strauss, A., Dobrowsky, P.H., Ndlovu, T., Reyneke, B., Khan, W., 2016. Comparative analysis of solar pasteurization versus solar disinfection for the treatment of harvested rainwater. *BMC Microbiol.* 16(1), 289.

Thomas, V., McDonnell, G., Denyer, S.P., Maillard, J.Y., 2010. Free-living amoebae and their intracellular pathogenic microorganisms: risks for water quality. *FEMS Microbiol. Rev.* 34(3), 231-259.

Ubomba-Jaswa, E., Navntoft, C., Polo-Lopez, M.I., Fernandez-Ibáñez, P., McGuigan, K.G., 2009. Solar disinfection of drinking water (SODIS): an investigation of the effect of UV-A dose on inactivation efficiency. *Photochem. Photobiol. Sci.* 8, 587–595.

Ubomba-Jaswa, E., Fernández-Ibáñez, P., Navntoft, C., Polo-López, M.I., McGuigan, K.G., 2010. Investigating the microbial inactivation efficiency of a 25 L batch solar disinfection (SODIS) reactor

enhanced with a compound parabolic collector (CPC) for household use. *J. Chem. Technol. Biotechnol.* 85(8), 1028-1037.

United Nations (UN), 2015. The Millennium Development Goals Report 2015. http://www.un.org/millenniumgoals/2015_MDG_Report/pdf/MDG%202015%20rev%20%28July%201%29.pdf (accessed 30.07.17).

Vervaeren, H., Temmerman, R., Devos, L., Boon, N., Verstraete, W., 2006. Introduction of a boost of *Legionella pneumophila* into a stagnant-water model by heat treatment. *FEMS Microbiol. Ecol.* 58(3), 583–92.

Water Research Commission, 2014. Quality of harvested rainwater and application of point of use treatment systems. Report to the Water Research Commission, Project No. K5/2124 by Department of Microbiology, Stellenbosch University. Stellenbosch, South Africa.

Wilkes, G., Edge, T., Gannon, V., Jokinen, C., Lyautey, E., Medeiros, D., Neumann, N., Ruecker, N., Topp, E., Lapen, D.R., 2009. Seasonal relationships among indicator bacteria, pathogenic bacteria, *Cryptosporidium* oocysts, *Giardia* cysts, and hydrological indices for surface waters within an agricultural landscape. *Water Res.* 43(8), 2209–23.

World Health Organisation (WHO), 2007. *Legionella* and the prevention of legionellosis. World Health Organisation. Geneva, Switzerland. WHO Press:ISBN 92-4-156297-8.

World Health Organisation (WHO), 2011. Guidelines for drinking-water quality, fourth ed. World Health Organisation. Geneva, Switzerland: WHO Press. ISBN: 978-92-4- 154815-1.

Yaziz, M.I., Gunting, H., Sapari, N., Ghazali, A.W., 1989. Variations in rainwater quality from roof catchments. *Water Res.* 23(6), 761-765.

Chapter 3:

(Chapter 3 is a follow-up study of Chapter 2 and certain sections of the materials and methods have been repeated. Chapter 3 is compiled in the format of the Water Research journal and UK spelling is employed)

EMA-Amplicon-based taxonomic characterisation of the viable bacterial community present in untreated and SODIS treated roof-harvested rainwater

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Abstract

Illumina next generation sequencing is becoming the method of choice to study microbial communities, microbial diversity and community structures (microbial ecology). In the current study Illumina next generation sequencing was thus coupled with the viability dye ethidium monoazide bromide (EMA) to characterise the bacterial community present in roof-harvested rainwater pre- and post-treatment. The diversity and abundance of the viable bacterial community present in roof-harvested rainwater before (Tank 1) and after eight hrs of SODIS treatment (SODIS-CPC-1) was monitored. Taxonomic assignments were made using the SILVA and Ribosomal Database Project whereafter the alpha- and beta-diversity indices were calculated and used to investigate the effect of SODIS treatment on the viable bacterial population present in roof-harvested rainwater. Alpha-diversity indices, including species richness and Shannon diversity, were significantly ($p < 0.05$) lower in the SODIS-CPC-1 rainwater samples in comparison to Tank 1 rainwater samples, indicating a significant difference in the species richness between these samples. The Tank 1 rainwater samples were dominated by the families Nocardiaceae (16.5 %) and Pseudomonadaceae (8.9 %), while the SODIS-CPC-1 rainwater samples were dominated by Nocardiaceae (44.0 %) and Micrococcaceae (31.7 %). On the genus level, *Rhodococcus* (17.1 %) and *Pseudomonas* (9.2 %) dominated in the Tank 1 rainwater samples, while *Rhodococcus* (48.0 %) and *Arthrobacter* (35.2 %) were the most abundant in the SODIS-CPC-1 rainwater samples. Furthermore, beta-diversity analysis using the Bray-Curtis distance metric system, then resulted in two separate clusters for the Tank 1 and the SODIS-CPC-1 rainwater samples, indicating that there was a significant shift (PERMANOVA, $p < 0.05$) in the viable bacterial community after SODIS treatment. Signatures of potential opportunistic pathogenic bacteria were also observed in both Tank 1 and the SODIS-CPC rainwater samples with the genera *Pseudomonas* and *Clostridium XI* detected in all the untreated Tank 1 and SODIS treated rainwater samples. Based on the results obtained in the current study, quantitative microbial risk assessment studies should be performed on roof-harvested and SODIS treated rainwater to estimate the human risk exposure when utilising these water sources for potable purposes.

Keywords: Solar disinfection, alpha- and beta-diversity, species diversity, relative abundance, viable bacterial community shift

3.1. Introduction

Globally, rainwater harvesting plays an integral role in sustainable water management and it is predominantly utilised to supplement rapidly depleting water resources (De Kwaadsteniet et al. 2013). The public perceives rainwater as a pure water source and in many countries such as Australia and New Zealand, this water source is utilised as the major potable water supply (Ahmed et al. 2017; Dobrowsky et al. 2014a). However, research has indicated that rainwater may become contaminated when the rain droplets traverse the air or during the harvesting process (De Kwaadsteniet et al. 2013; Kaushik et al. 2012). Depending on the surrounding environment, atmospheric deposition (e.g. bioaerosols, chemical pollutants) may thus have a significant effect on the quality of rainwater (Abbasi & Abbasi, 2011; Williams et al. 2015). Furthermore, chemical and microbial pollutants which originate from dust (caused by wind and anthropogenic activity), animal faecal matter (such as bird faeces) and debris (organic material such as leaves) accumulate on the roof surface area and in the gutter system. This debris and waste material allows for the proliferation of a wide range of microbial pathogens and opportunistic pathogens and effectively washes into the rainwater harvesting tank during a rain event. Accordingly, virulent *Escherichia coli* (*E. coli*), *Legionella* spp., *Pseudomonas* spp., *Yersinia* spp., adenovirus and *Giardia* spp., amongst others, have been detected in stored rainwater (Ahmed et al. 2008; 2009; Dobrowsky et al. 2014b; 2014c).

As the presence of pathogens and opportunistic pathogens in harvested rainwater may pose a significant health risk to the end-users, it is recommended that this water source is treated prior to utilisation (Amin & Han, 2009; Dobrowsky et al. 2014a). While various water treatment technologies such as chlorination, filtration, solar pasteurization (SOPAS) and solar disinfection (SODIS) have been employed to reduce the level of particularly microbial contamination in rainwater, SODIS is considered more cost-effective and consistently yields good treatment efficiencies (McGuigan et al. 2012; Nieuwoudt & Mathews, 2005; Ubomba-Jaswa et al. 2010). Solar disinfection is also promoted by the World Health Organisation (WHO) as a water treatment method. Literature has indicated that the bactericidal effect of SODIS is based on the synergistic effect of direct ultraviolet [UV (UV-A and UV-B)] radiation and solar mild-heat to inactivate microorganisms (Amin et al. 2014). Ultraviolet-A radiation causes the generation of reactive oxygen species (ROS) which react with cellular constituents such as proteins, deoxyribonucleic acid (DNA), ribonucleic acid (RNA) and leads to an increased cell membrane permeability, followed by disruption of membrane potential gradients (Castro-Alferez et al. 2016). In contrast, UV-B radiation interferes directly with the genetic material of a cell by causing single nucleotide strand breaks and other nucleic acid modifications. Ultraviolet radiation is thus mutagenic and lethal, while heat leads to the lysis of the bacterial cell membrane, resulting in the inactivation of bacterial cells (Reed, 2004).

Nalwanga et al. (2016) assessed the quality of harvested rainwater in Southern Uganda and subsequently investigated the use of SODIS to treat harvested rainwater. Based on the indicator detection analysis (faecal enterococci and *E. coli*), the microbiological quality of the untreated

rainwater was compromised and not safe for drinking purposes. Solar disinfection was then employed to treat the harvested rainwater in 2 L polyethylene terephthalate (PET) bottles for six hrs by placing the PET bottles on a raised horizontal platform. Although SODIS resulted in a significant reduction in faecal enterococci and *E. coli* counts, *E. coli* were still detected by culturing assays in the SODIS treated harvested rainwater, albeit at values generally less than 10 organisms/100 mL (Nalwanga et al. 2016). Strauss et al. (2016) investigated the microbial quality of roof-harvested rainwater after SODIS treatment of six and eight hrs, respectively, using 2 L transparent (PET) bottles which were placed in a solar cooker. Results indicated that the indicator bacteria counts (*E. coli* and heterotrophic bacteria) were reduced to below the detection limit (< 1 CFU/100 mL) after six and eight hrs of treatment. The viability of *Legionella* spp. and *Pseudomonas* spp. was also assessed using ethidium monoazide bromide quantitative polymerase chain reaction (EMA-qPCR) analysis and while results indicated a significant reduction in both *Legionella* and *Pseudomonas* copy numbers, these opportunistic pathogens were still viable after SODIS treatment of eight hrs (Strauss et al. 2016). Limited information is however, available on the abundance and diversity of the overall microbial population present in roof-harvested rainwater pre- and post-treatment (Ahmed et al. 2017).

Next generation sequencing such as Illumina sequencing of the 16S ribosomal RNA (rRNA) gene, has been utilised to investigate the complexity of the whole bacterial community structure in sewage (Ye & Zhang, 2011), biosolids (Bibby et al. 2010), urban (surface run off) water (Ibekwe et al. 2013) and rainwater (Ahmed et al. 2017; Chidamba & Korsten, 2015). Information on the detailed community structure as well as the dominant bacterial taxa and the less common pathogens, which are generally present at low concentrations (< 1 % proportion of the sample), is obtained with a high taxonomic resolution (Ahmed et al. 2017; Sogin et al. 2006; Staley et al. 2015). Illumina sequencing is based on the detailed analysis of certain variable regions in the 16S rRNA prokaryotic gene, which has formed the foundation of most molecular-based assays used for bacterial identification. Although the sequencing of the whole 16S rRNA gene for accurate bacterial characterisation is required, numerous studies have reported on the accurate bacterial taxonomic characterisation using hypervariable regions (such as hypervariable region V3 and V4 or hypervariable region V5 and V6) of the 16S rRNA gene (Ahmed et al. 2017; Chidamba & Korsten, 2015; Claesson et al. 2009; Huse et al. 2008). Chidamba and Korsten (2015) used pyrosequencing to sequence the V3 and V4 hypervariable region of the 16S rRNA gene in order to assess the diversity of the microbial community present in seven rainwater and two river water samples. Results showed that there were significant similarities in the community structure between rainwater samples, which clearly differed from the community structure profiled for the river water samples. Furthermore, the authors detected low level signatures (few sequence reads, one in most cases) of potential pathogens (such as *Pseudomonas*, *Clostridium*, *Yersinia* and *Legionella*) in the rainwater which could pose potential health risks when used for potable purposes. The low level signature of the potential pathogens

could possibly be attributed to the low number of reads (10 956) which was obtained from the pyrosequencing platform.

In a recent study conducted by Ahmed et al. (2017), Illumina next generation sequencing was utilised to characterise the overall bacterial community present in rainwater and analyse the distribution of genera in 88 rainwater tanks located in the Brisbane and Currumbin regions of Australia. The authors were able to sequence and analyse a high number of reads (2 795 320) which further enabled them to detect and identify the dominant opportunistic pathogens such as *Clostridium*, *Mycobacterium*, *Legionella* and *Pseudomonas*, amongst others, present in the rainwater. It should however, be noted that a limitation of both the Chidamba and Korsten (2015) and the Ahmed et al. (2017) studies is that no information on the viability of these species was obtained as Illumina next generation sequencing does not elucidate the viability status of the bacteria.

Previous studies have used qPCR assays in conjunction with nucleic acid binding dyes such as EMA and propidium monoazide (PMA), to quantify the viable portion of a specific species present in a sample (Leifels et al. 2015; Mansi et al. 2014; Reyneke et al. 2016; Strauss et al. 2016). The nucleic acid binding dye binds to the DNA of cells (after photoactivation) with damaged and/or permeable membranes (non-viable cells) (Fittipaldi et al. 2012; Reyneke et al. 2016). This effectively prevents the PCR amplification of this DNA and leads to a strong signal reduction during qPCR as only the DNA from intact (viable) cells will be amplified. In a recent study conducted by Reyneke et al. (2017), various concentrations of EMA-, PMA- and DNase were compared and utilised in conjunction with qPCR assays for the determination of microbial cell viability. Based on the results obtained, it was concluded that EMA-qPCR assays (with an EMA concentration of 6 μM) were the most suitable for the identification of various opportunistic pathogens such as *Legionella pneumophila*, *Pseudomonas aeruginosa* and *Salmonella typhimurium*.

A comprehensive literature search indicated that nucleic acid binding dyes have not previously been used in combination with next generation sequencing techniques such as Illumina sequencing. As both qPCR and Illumina next generation sequencing are amplicon-based screening assays, rainwater samples in the current study were EMA pre-treated prior to analysis by Illumina next generation sequencing in order to screen for the overall viable bacterial community present in rainwater samples, pre-and post-treatment. As outlined in **Chapter two**, a solar disinfection system was connected to a compound parabolic collector (SODIS-CPC-1), which was further connected to a rainwater harvesting tank (Tank 1) for the treatment of roof-harvested rainwater. The primary aim of the current study was thus (i) to determine the diversity and abundance of the viable bacterial community present in the untreated rainwater (Tank 1) and the SODIS treated (for eight hrs) rainwater (SODIS-CPC-1), using Illumina next generation sequencing coupled with EMA, (ii) to determine whether the viable bacterial community differs after SODIS treatment (Tank 1 versus SODIS-CPC-1) and lastly, (iii) to detect and identify the primary viable pathogenic and opportunistic pathogenic genera persisting after SODIS treatment in roof-harvested rainwater. Detailed

information on the design and construction of the SODIS-CPC system is outlined in the materials and methods section of **Chapter two**.

3.2. Materials and methods

3.2.1. Sampling site

A rainwater harvesting tank was installed at the Welgevallen experimental farm, Stellenbosch University, South Africa (GPS co-ordinates: 33° 56' 36.19"S, 18° 52' 6.08"E) in June 2012 (**Figure 3.1**). The polyethylene rainwater tank (2 000 L) was connected to an asbestos roof (catchment area) with a Chrysotile (white asbestos) conveyance system. The tank was installed onto a metal stand to allow for the passive flow of the rainwater directly into the treatment system. The reactor of the SODIS-CPC system was placed in a Northern direction with an incline angle of 33° (with respect to the ground), for maximum solar irradiation exposure. Furthermore, the sampling site is situated on the periphery of Stellenbosch, next to a dairy farm where gravel roads pass the sampling site which is also surrounded by trees. However, no tree branches obstructed the catchment area. Moreover, various grazing crops (for sheep and cattle) and crops such as wheat and lucerne are stored in barn units located opposite the sampling site.



Figure 3.1: Rainwater harvesting systems located on Welgevallen experimental farm, Stellenbosch University, South Africa. The asbestos roof catchment area is connected to a rainwater harvesting tank (Tank 1) via the conveyance system. Harvested rainwater flows passively from Tank 1 into the SODIS-CPC-1 system.

3.2.2. Sample collection

The 10.6 L reactor (borosilicate glass cylinder) of the SODIS-CPC system was filled with harvested rainwater from the rainwater harvesting tank on the morning of a sampling session. The rainwater was then exposed to direct sunlight for eight hrs (Amin & Han, 2009; Strauss et al. 2016), after which a total of two samples were collected per sampling event. Samples collected directly from Tank 1 were designated as the untreated rainwater samples, while treated samples were collected from the SODIS-CPC-1 system. Samples were collected in 5 L sterile bottles and a total of seven sampling sessions were conducted from March to April 2017, resulting in 14 samples ($n = 14$) collected in total during the course of this study (**Appendix A, Table A2**). However, only representative samples ($n = 10$) were used in the current analysis based on the maximum direct normal irradiance (DNI), UV-A and UV-B radiation and the water temperature (after SODIS) of the samples (**Chapter two, Figure 2.6**). For the presentation of results in the figures, untreated samples collected during the respective sampling sessions one to five are designated as T1_1 (Tank 1 sampling session one), T1_2 (Tank 1 sampling session two), etc., while treated samples collected during the respective sampling sessions one to five are designated as S1_1 (SODIS-CPC-1 sampling session one), S1_2 (SODIS-CPC-1 sampling session two), etc.

Four control samples were included by combining 400 mL of each sample ($n = 5$) collected from Tank 1 resulting in a 2 L composite Tank 1 rainwater sample, while 400 mL of each sample ($n = 5$) collected from SODIS-CPC-1 was also combined resulting in a 2 L composite SODIS-CPC-1 rainwater sample. Each respective composite sample was then divided into two 1 L samples. One litre of the composite Tank 1 sample was not EMA-treated [control 1 – designated Tank 1 (C)], while the remaining 1 L was EMA pre-treated resulting in control 2 [designated Tank 1 (C-EMA)]. Similarly, 1 L of the composite SODIS-CPC-1 sample was not EMA-treated [control 3 – designated SODIS-CPC-1 (C)], while the remaining 1 L was EMA pre-treated resulting in control 4 [designated SODIS-CPC-1 (C-EMA)].

3.2.3. Rainwater concentration, EMA treatment, DNA extraction and quality control assessment

For each sampling session, 1 L of the untreated (Tank 1) and treated (SODIS-CPC-1), as well as the four control samples ($n = 14$) were concentrated by filtering as outlined in Dobrowsky et al. (2015). All the concentrated rainwater samples ($n = 12$), with the exception of controls Tank 1 (C) and SODIS-CPC-1 (C), were treated with 2.5 µg/mL EMA as previously described by Reyneke et al. (2017). Following the addition of EMA to the concentrated rainwater samples, the samples were incubated on ice for 10 min followed by a 15 min halogen light exposure (keeping the samples on ice to avoid over-heating during the photoactivation step). The EMA treated samples were washed with 1 mL NaCl (0.85 %) followed by centrifugation (16 000 × g for 5 min). Deoxyribonucleic acid was extracted using the Soil Microbe DNA MiniPrep™ Kit (Zymo Research, Irvine, USA) as per manufacturer's instructions by first re-suspending the obtained pellet in the lysis solution and

transferring the mixture to the ZR BashingBead™ Lysis Tubes. After DNA was extracted, quality control assessment was performed in order to determine the quality (purity), quantity and integrity of the 14 genomic DNA (gDNA) samples. The NanoDrop® ND-1000 (Nanodrop Technologies Inc., Wilmington, Delaware, USA) and Qubit™ fluorometer (Invitrogen Corporation, Carlsbad, CA, USA) (in conjunction with the Qubit™ dsDNA HS Assay Kit) were used to determine the UV absorbance ratios of 260 nm to 280 nm (A₂₆₀/A₂₈₀) and 230 nm (A₂₆₀/A₂₃₀) and the concentration of double-stranded DNA (dsDNA), respectively, of each sample in triplicate. In addition, the integrity of each sample was assessed by gel electrophoresis using a 1% agarose gel containing ethidium bromide (2% v/v) which was visualised using the Uvitec Alliance 2.7 chemiluminescent imager (Uvitec, Cambridge, UK).

3.2.4. 16S rRNA metagenomic sequencing

Deoxyribonucleic acid extracted from the 10 rainwater samples and four control samples were sent for 16S metagenomic sequencing library construction and sequencing at the Centre for Proteomic and Genomic Research (CPGR), Cape Town, South Africa, as previously described by Cason et al. (2017). Briefly, the sequencing library was prepared by amplifying a ~464 bp region located in the variable V3 and V4 region of the 16S rRNA prokaryotic gene using region of interest-specific primers (Klindworth et al. 2013) with overhang Illumina adapter sequences. The forward primer 5'-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCCTACGGGNGGCWGCAG-3' and the reverse primer 5'-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGACTACHVGGGTATCTAATCC-3' were used, where the underlined sequences refer to the 16S locus interest-specific sequence of the primer. The amplicons were purified from primer dimers and free primers using the AMPure XP clean-up kit (Beckman Coulter Genomics, Danvers, MA, USA) followed by an Index PCR using the Nextera XT Index kit (Illumina, San Diego, CA, USA) to attach dual indices and Illumina sequencing adapters to the original amplicon. A final amplicon purification step was performed using the AMPure XP clean-up kit (Beckman Coulter Genomics). Libraries were then normalised, pooled and sequenced on the MiSeq system using the MiSeq Reagent Kit v3 (Illumina).

3.2.5. Sequence data analysis

The open source bioinformatics program Mothur version 1.39.5 (Schloss et al. 2009) was used to process the sequence reads. The standard operating procedure described by Kozich et al. (2013) was followed with a few modifications (https://mothur.org/wiki/MiSeq_SOP). Contigs were created with the sequence paired-end reads and trimmed to 470 nucleotides. Sequences with homopolymers of 8 nucleotides, or ambiguous bases were removed. High-quality sequences were aligned against the SILVA version128 reference database (Pruesse et al. 2007). The sequences were further quality trimmed by using a 1% pre-cluster error and chimera removal was conducted using VSEARCH. Assignment of operational taxonomic units (OTUs) was performed at a 97 % identity using the optclust algorithm. Taxonomic assignments were made against the Ribosomal Database Project

(version 16) (Cole et al. 2008). The classification was performed with a bootstrap cut-off of 80% using the method described by Wang et al. (2007). The sequences from individual samples were rarefied to equal sample size based on the sample with fewest sequences which equalled 116 122.

3.2.6. Statistical analysis

Mothur version 1.39.5 was used to calculate sample coverage and the number of OTUs observed as well as alpha (α)-diversity indices including species richness, the Shannon diversity index (Shannon & Weaver, 1964) and the abundance-based coverage estimate (ACE) (Chao and Lee, 1992). Differences in bacterial community composition [beta (β)-diversity] were evaluated through permutational multivariate analysis of variance (PERMANOVA) and visualised using non-metric multidimensional scaling (NMDS) (Oksanen, 2017) which was performed using the computer software RStudio version 1.0.153 (R Development Core Team, 2017). Furthermore, relative abundances on family and genus level were also calculated using RStudio and were visualised using the computer software Microsoft Excel® version 15.31 (Microsoft Corporation, Redmond, WA, USA).

3.3. Results

3.3.1. Bacterial α -diversity analysis of control samples

Control samples (EMA-treated and corresponding EMA-untreated controls) were included in the Illumina next generation sequencing analysis to determine whether EMA treatment, prior to the sequencing analysis, reduced the species richness and the number of OTUs. This would indicate that the EMA treatment allowed for the characterisation of the viable population in the tank water and SODIS-treated samples. The sample coverage was calculated, which serves as an indication of the number of different species detected as a fraction of the cumulative number of species observed per study site, thus the total number of species detected with all methods combined per localisation are presented as a percentage (Westphal et al. 2008). The bacterial α -diversity was investigated which describes the diversity of species (based on the evenness and relative abundance) within a sample. Therefore, sample coverage (in percentage), the Shannon diversity index, species richness and abundance-based coverage estimates (ACE; statistical estimation of the total number of species present in a sample, based on the actual number of species detected) were used to describe the α -diversity of the respective control samples (**Table 3.1**).

Each control sample was analysed in a single reaction, therefore statistically significant conclusions regarding the differences between the control samples cannot be deduced. However, for all the control samples a mean sample coverage of 99.1 % (ranging from 98.9 % to 99.3 %) was obtained, indicating that sufficient sequence data was generated to obtain a nearly complete characterisation of the bacterial communities in the control samples.

Table 3.1: Alpha diversity indices parameters, including percentage sample coverage, Shannon diversity index, species richness and ACE, observed for control samples.

Control sample	% Coverage	*Species richness	Shannon	ACE
Control 1 [Tank 1 (C)]	98.9	1951	3.1	6316.5
Control 2 [Tank 1 (C-EMA)]	99.1	1723	3.1	4576.6
Control 3 [SODIS-CPC-1 (C)]	99.2	1205	1.0	6534.1
Control 4 [SODIS-CPC-1 (C-EMA)]	99.3	1006	0.9	6222.5

*The number of different species observed.

Furthermore, for the Tank 1 control samples (Control 1 and 2), a reduction in the species richness was observed in the EMA-treated control [Tank 1 (C-EMA): 1723] in comparison to the non-EMA treated control [Tank 1 (C): 1951] (**Table 3.1**). Similar results were obtained for the SODIS-CPC controls, where a reduction in species richness from 1205 to 1006, for Control 3 [SODIS-CPC-1 (C)] and Control 4 [SODIS-CPC-1 (C-EMA)], respectively, was obtained (**Table 3.1**). For the ACE analysis, a reduction in the EMA-treated control in comparison to the respective non-EMA-treated control samples was also observed. The ACE for Control 1 [Tank 1 (C)] was reduced from 6312.5 to 4576.6 in Control 2 [Tank 1 (C-EMA)], while the ACE for Control 3 [SODIS-CPC-1 (C)] was reduced from 6534.1 to 6222.5 in Control 4 [SODIS-CPC-1 (C-EMA)] (**Table 3.1**). In contrast, the Shannon diversity index for Control 1 (3.1) and Control 3 (1.0) was comparable to the Shannon diversity index obtained for Control 2 (3.1) and Control 4 (0.9), respectively (**Table 3.1**). The Shannon diversity index indicates the abundance and evenness of the species based on the species present in the sample, thus the abundance and evenness of the species present in Control 1 and 2 are the same, however the composition of species is different as the species richness indicates that the species in Control 1 are more diverse than Control 2.

3.3.2. Bacterial α -diversity analysis of untreated and SODIS treated rainwater samples

The amplicon sequencing analysis of the untreated and SODIS treated rainwater samples yielded a total of 1 179 075 and 1 196 969 sequences which were further clustered into a total of 5874 and 4020 OTUs for the Tank 1 ($n = 5$) and SODIS-CPC-1 ($n = 5$) rainwater samples at a similarity of 97%, respectively. Overall, the sequencing analysis resulted in a 99.1 ± 0.001 % and 99.3 ± 0.001 % mean sample coverage for the Tank 1 and SODIS-CPC-1 rainwater samples, respectively, suggesting a nearly complete coverage of the viable bacterial communities present in the rainwater samples (**Table 3.2**).

Various α -diversity indices were further used to investigate the species diversity and abundance within each sample. For the mean species richness, a significant difference ($p = 0.0033$) was observed for the Tank 1 rainwater samples (1865.0 ± 129.4) versus the SODIS-CPC-1 rainwater samples (1014.4 ± 216.7) (**Table 3.2**). Similarly, a significant difference ($p = 0.0107$) was observed

in the mean Shannon diversity indices for the Tank 1 rainwater samples (3.8 ± 0.9) versus the SODIS-CPC-1 rainwater samples (1.0 ± 0.6) (**Table 3.2**).

Table 3.2: Mean and standard deviation of α -diversity indices, including percentage sample coverage, Shannon diversity index, species richness and ACE, observed for Tank 1 (untreated) and SODIS-CPC (treated) rainwater samples. The significance (p value) for the respective indices between Tank 1 and SODIS-CPC-1 rainwater samples are also indicated.

Sample	% Coverage	Shannon	*Species richness	ACE
Tank 1	99.1 ± 0.001	3.8 ± 0.9	1865.0 ± 129.4	4997.7 ± 445.7
SODIS-CPC-1	99.3 ± 0.001	1.0 ± 0.6	1014.4 ± 216.7	5091.9 ± 1668.5
p value	0.0126	0.0107	0.0033	0.8812

*The number of different species observed.

In contrast, for the mean ACE, no significant difference ($p = 0.8812$) was observed for the Tank 1 rainwater samples (4997.7 ± 445.7) versus the SODIS-CPC-1 rainwater samples (5091.9 ± 1668.5) (**Table 3.2**). Rarefaction curves for the OTUs observed (species richness) for each sample collected from Tank 1 and SODIS-CPC-1 are depicted in **Figure 3.2** and indicates the dispersion of the number of different OTUs observed per sample.

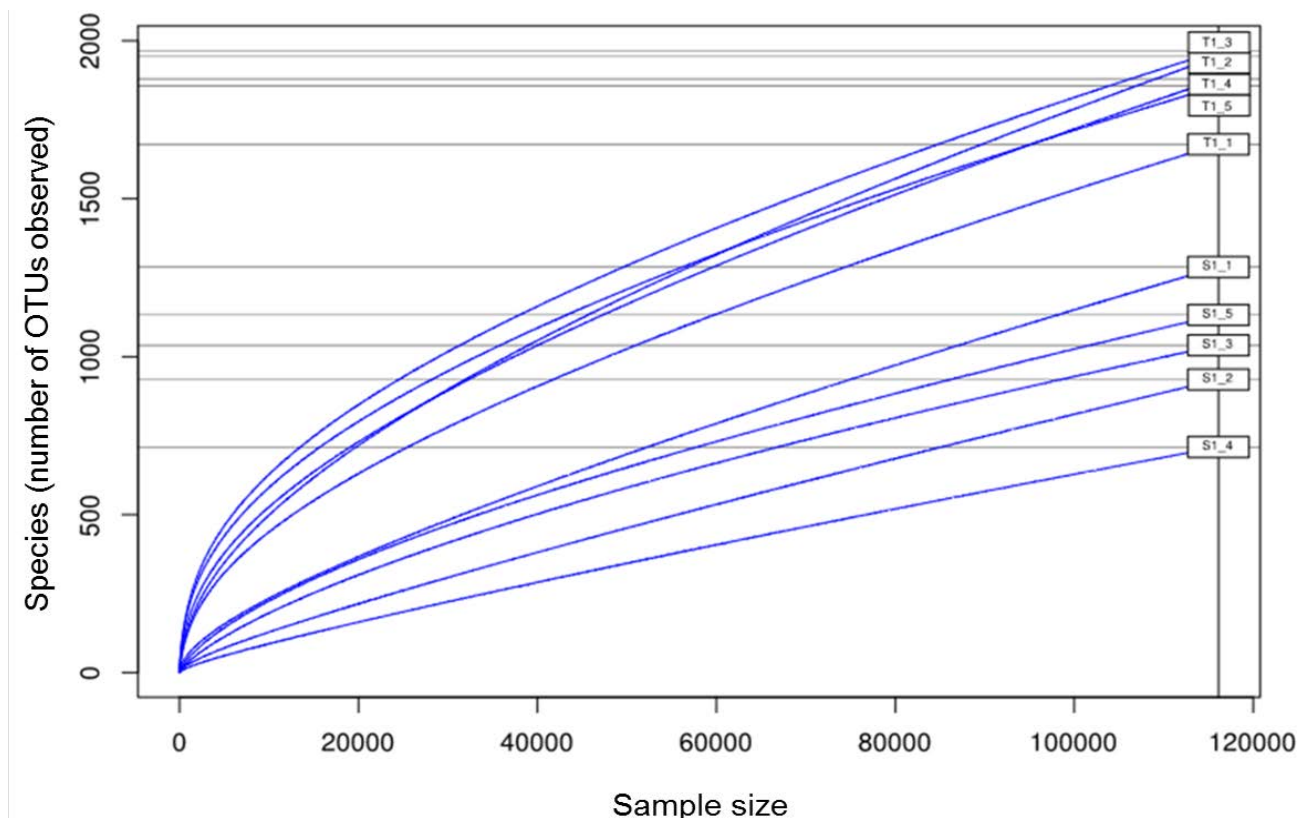


Figure 3.2: Rarefaction curves indicating the species richness (OTUs observed) for each sample collected from Tank 1 (T1_1, 2, 3, 4 and 5) and SODIS-CPC-1 (S1_1, 2, 3, 4 and 5).

3.3.3. Taxonomic diversity and characterisation of bacterial families and genera in rainwater samples

Taxonomic diversity analysis revealed that 312 233 and 660 200 of the sequences were classified into 133 and 77 unique families for the Tank 1 and SODIS-CPC-1 rainwater samples, respectively. Based on the relative abundance, the overall viable bacterial population in the Tank 1 rainwater samples was predominantly comprised of the families Nocardiaceae (16.5 %) followed by Pseudomonadaceae (8.9 %), Sphingomonadaceae (6.0 %), Planctomycetaceae (4.2 %), Chitinophagaceae (3.1 %) and Oxalobacteraceae (2.9 %) (**Figure 3.3**). Based on the relative abundance observed for the SODIS-CPC-1 rainwater samples, the viable bacterial population for these samples was predominantly comprised of the families Nocardiaceae (44.0 %) followed by Micrococcaceae (31.7 %), Oxalobacteraceae (5.0 %), Xanthomonadaceae (1.9 %), Rhizobiaceae (1.2 %) and Chitinophagaceae (0.9 %) (**Figure 3.3**).

On the genus level, 263 794 and 551 455 sequences were classified into 14 and 8 unique genera for the Tank 1 and SODIS-CPC-1 rainwater samples, respectively. Based on the relative abundance for the genera detected, the overall viable bacterial population in the Tank 1 rainwater samples was predominantly comprised of the genera *Rhodococcus* (17.1 %) followed by *Pseudomonas* (9.2 %), *Sphingobium* (4.6 %), *Undibacterium* (2.5 %), *Sediminibacterium* (1.4 %) and *Nevskia* (1.1 %). The overall viable bacterial population in the SODIS-CPC-1 rainwater samples were predominantly comprised of the genera *Rhodococcus* (48.0 %) followed by *Arthrobacter* (35.2 %), *Oxalicibacterium* (5.3 %), *Stenotrophomonas* (1.9 %), *Kaistia* (1.3 %) and *Sediminibacterium* (1.0 %) (**Figure 3.4**).

To subsequently investigate the β -diversity, which is the variation in species diversity between samples (Tank 1 vs. SODIS-CPC-1), the Bray-Curtis distance metric was used whereafter PERMANOVA was performed. This analysis provides information on the differences in composition of the viable bacterial populations between the untreated (Tank 1) and treated (SODIS-CPC-1) rainwater samples. Subsequently, a significant difference (PERMANOVA; $p < 0.05$) was observed between the β -diversity composition of the untreated (Tank 1) and SODIS treated (SODIS-CPC-1) rainwater samples as two distinct clusters were observed on the NMDS ordination plot (**Figure 3.5**). The Tank 1 rainwater samples also consisted of a greater number of low-abundance taxa.

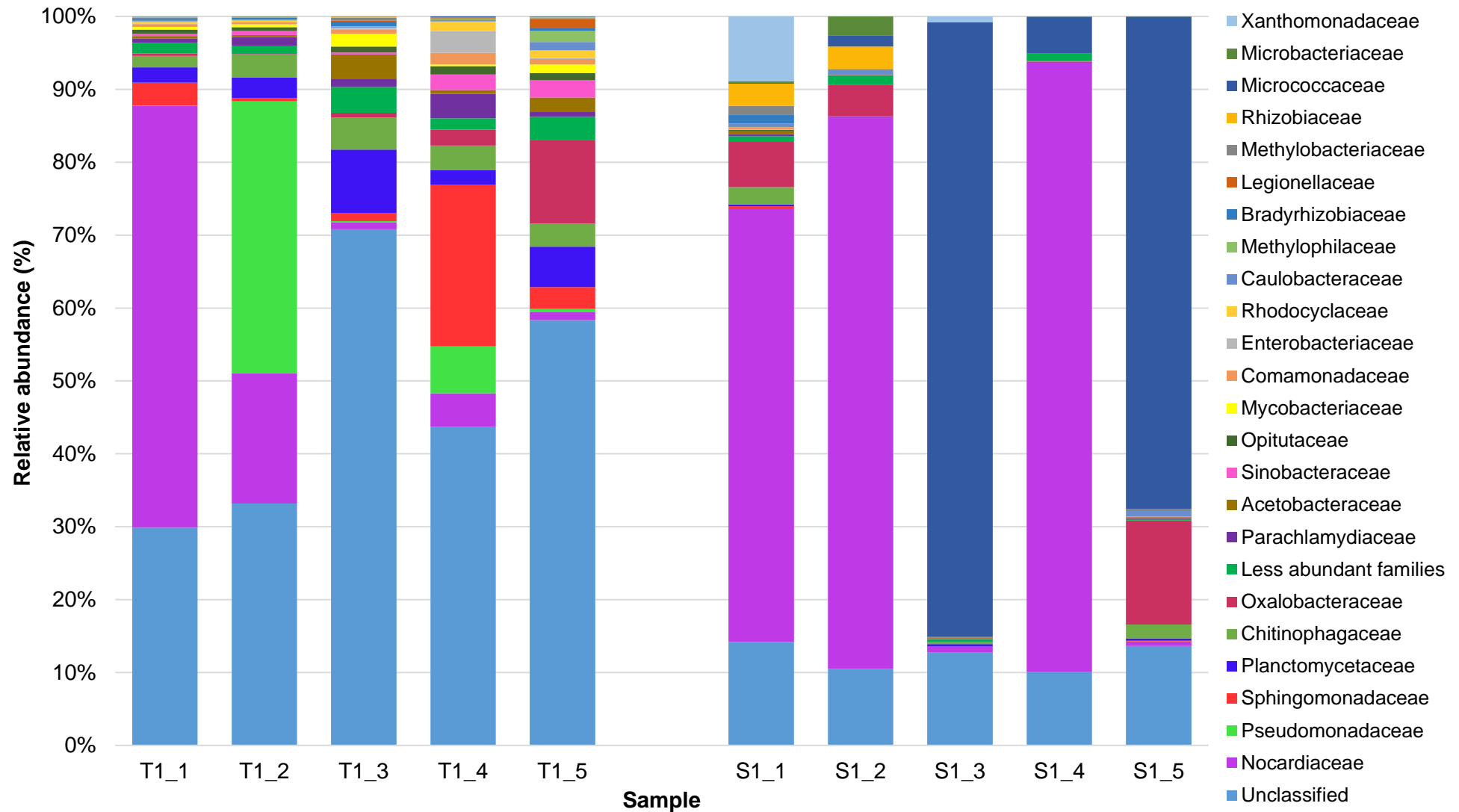


Figure 3.3: Relative abundance of the families which have a mean relative abundance of > 1 % in the before (Tank 1: T1_1, 2, 3, 4 and 5) and after SODIS treated (SODIS-CPC-1: S1_1, 2, 3, 4 and 5) rainwater samples collected during the five sampling sessions. Sequences with a homology $\geq 80\%$ were used.

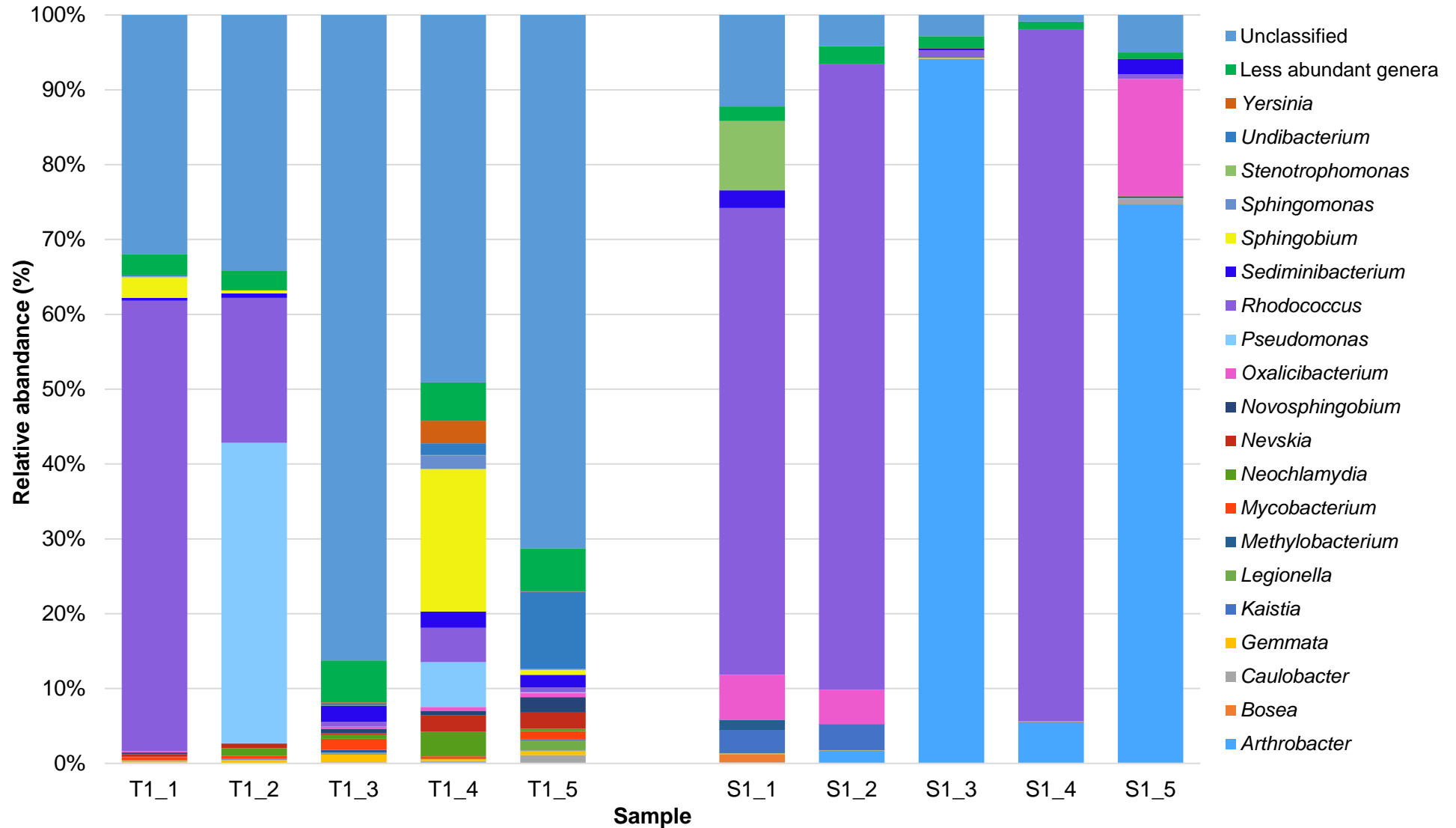


Figure 3.4: Relative abundance of the genera with a mean relative abundance of > 1 % in the before (Tank 1: T1_1, 2, 3, 4 and 5) and after SODIS treated (SODIS-CPC-1: S1_1, 2, 3, 4 and 5) rainwater samples collected during the five sampling sessions. Sequences with a homology ≥ 80% were used.

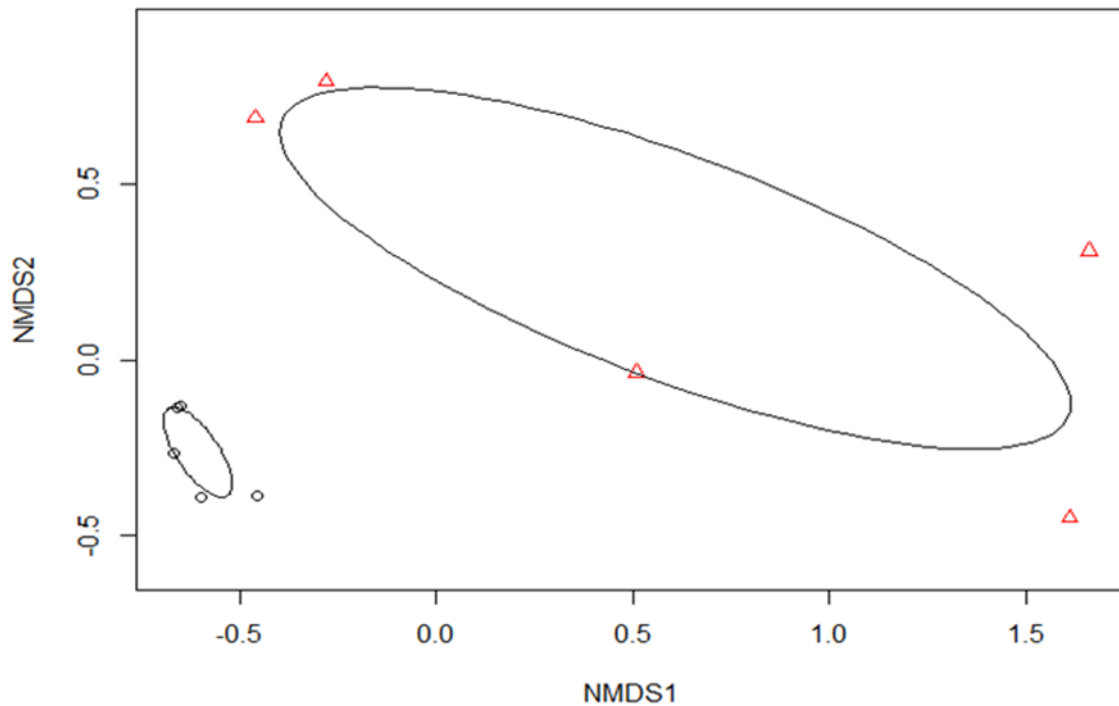


Figure 3.5: Non-metric multidimensional scaling ordination plot showing the relative differences in OTU community composition. The Bray-Curtis distance metric was used to quantify the similarity between the observed patterns. Ellipses indicate a 95 % confidence interval for replicates. Two distinct clusters are observed (PERMANOVA, $p < 0.05$) where Tank 1 rainwater samples are represented by red triangles, while SODIS-CPC-1 rainwater samples are represented by black circles.

3.3.4. Signatures of potential pathogenic and opportunistic pathogenic genera present in rainwater samples

In order to determine whether opportunistic pathogens and pathogenic genera were present in the Tank 1 and SODIS-CDC-1 samples, taxonomic assignments were made using the SILVA and RDP databases. Fourteen potential pathogenic or opportunistic pathogenic genera were identified which occurred in either the untreated (Tank 1) or the SODIS treated (SODIS-CPC-1) rainwater samples or in both sample groups (**Table 3.3**). All the Tank 1 rainwater samples (100 %) were positive for the presence of *Legionella* spp., *Mycobacterium* spp., *Pseudomonas* spp., *Yersinia* spp., *Clostridium III* and *Clostridium senso strictu* (**Table 3.3**). Similarly, *Pseudomonas* spp. and *Clostridium senso strictu* were detected in all the SODIS-CPC-1 rainwater samples (100 %; **Table 3.3**). After SODIS treatment the frequency of detection for the remaining dominant genera which were present in all the Tank 1 samples i.e. *Clostridium III*, *Legionella* spp., *Mycobacterium* spp. and *Yersinia* spp., however, decreased to 20 %, 60 %, 60 % and 60 %, respectively (**Table 3.3**). In addition, *Acinetobacter* spp. and *Aeromonas* spp. were detected at 80 % and 60 % in both the Tank 1 and SODIS-CPC-1 rainwater samples, respectively (**Table 3.3**). In contrast, *Clostridium XI* (60 %) and *Brevibacterium* spp. (20 %) were detected in the Tank 1 rainwater samples, however these genera were not detected after SODIS treatment in the SODIS-CPC-1 rainwater samples (**Table 3.3**).

Similarly, *Staphylococcus* spp. (40 %), *Campylobacter* spp. (20 %) and *Burkholderia* spp. (20 %) were only present in the SODIS-CPC-1 samples, however these genera were not detected in the Tank 1 rainwater samples (**Table 3.3**). *Corynebacterium* spp. were also detected in 60 % of the Tank 1 water samples, while the frequency of detection decreased to 20 % in the SODIS-CPC-1 rainwater samples (**Table 3.3**).

Table 3.3: Percentage rainwater samples (Tank1 and SODIS-CPC-1) that tested positive for certain potentially pathogens and opportunistic pathogens.

Genera	Tank 1 (<i>n</i> = 5)	SODIS-CPC-1 (<i>n</i> = 5)
<i>Acinetobacter</i>	80	80
<i>Aeromonas</i>	60	60
<i>Brevibacterium</i>	20	ND
<i>Burkholderia</i>	ND	20
<i>Campylobacter</i>	ND	20
<i>Clostridium III</i>	100	20
<i>Clostridium sensu stricto</i>	100	100
<i>Clostridium XI</i>	60	ND
<i>Corynebacterium</i>	60	20
<i>Legionella</i>	100	60
<i>Mycobacterium</i>	100	60
<i>Pseudomonas</i>	100	100
<i>Staphylococcus</i>	ND	40
<i>Yersinia</i>	100	60

ND – not detected

3.4. Discussion

Based on an extensive literature search, this is the first study where a viability dye, namely EMA, was utilised in conjunction with the next generation sequencing platform, Illumina, to monitor the overall viable bacterial community present in rainwater pre- and post-treatment. The concept of EMA-qPCR, which refers to the quantification of a specific viable organism in a mixed-culture (see **section 1.4**), has previously been extensively investigated and its reliability has been verified (Mansi, 2014; Leifels et al. 2015; Reyneke et al. 2017). Four control samples were however, included in the current study to confirm that the use of EMA in combination with the Illumina platform primarily detected the viable community present in the samples. Non-EMA and EMA pre-treatment control samples were thus included in the current study. It was hypothesised that a higher α -diversity, which refers to the mean species diversity and species richness in a sample, would be observed in the non-EMA control samples as the DNA of the viable as well as the non-viable portion of the bacterial community would be amplified. Accordingly, the species richness calculated for the EMA treated control samples, Control 2 [Tank 1 (C-EMA)] and Control 4 [SODIS-CPC-1 (C-EMA)], was lower than the species richness observed for the non-EMA treated control samples, Control 1 [Tank 1 (C)] and Control 3 [SODIS-CPC-1 (C)]. Similarly, the ACE calculated for Tank 1 (C-EMA) and SODIS-

CPC-1 (C-EMA) was lower than the values calculated for the non-EMA controls, Tank 1 (C) and SODIS-CPC-1 (C), which further supported the hypothesis.

The Shannon diversity index for the Tank 1 EMA (C-EMA) and non-EMA (C) controls were however, the same, while the Shannon diversity index was slightly lower in the EMA treated SODIS-CPC-1 (C-EMA; 0.9) sample in comparison to the non-EMA treated SODIS-CPC-1 (C; 1.0) sample. The Shannon diversity index indicates the abundance and evenness of the species in a sample which implies that the abundance and evenness of the species present in the non-EMA and EMA untreated Tank controls as well as the non-EMA and EMA SODIS treated controls were similar. However, based on the species richness values, the species present in the non-EMA pre-treated samples [Tank 1 (C) and SODIS-CPC-1 (C)] were more diverse than the species present in the EMA pre-treated control samples [Tank 1 (C-EMA) and SODIS-CPC-1 (C-EMA)]. It is postulated that the DNA of both the viable and non-viable bacterial populations was amplified in the non-EMA pre-treated samples, which may have contributed to the increased species diversity present. In contrast, based on the principle of EMA which excludes the DNA of cells with damaged and/or permeable membranes (non-viable cells) (Fittipaldi et al. 2012; Reneyke et al. 2016), the DNA of only the viable bacterial population was amplified in the Tank 1 (C-EMA) and SODIS-CPC-1 (C-EMA) controls. These findings correlate to a study conducted by Chang et al. (2010) where the use of EMA ensured the amplification of only the viable portion of the population. However, due to the lack of biological replicates for the control samples, the significance (p value) of these observations (for the control samples) could not be determined.

For the EMA-Illumina analysis of the untreated ($n = 5$) and SODIS-CPC treated ($n = 5$) rainwater samples, the mean percentage sample coverage obtained for both consortiums was similar (Tank 1: 99.1 % and SODIS-CPC-1: 99.3 %). These results were also comparable to results obtained in a recent study conducted by Ahmed et al. (2017), where the authors used Illumina next generation sequencing to characterise the bacterial community present in 88 rainwater tanks at two different sites (mean coverage of 96 % recorded for both sites). The comprehensive mean sample coverage values obtained in the current study indicates that an expansive analysis of the bacterial communities present in the samples was detected. The high mean percentage sample coverage further correlates to the high number of OTUs observed in the current study (37 763 OTUs) as well as in the Ahmed et al. (2017) study where 46 523 and 38 114 OTUs were observed for the Brisbane and Currumbin sites, respectively. In comparison Chidamba and Korsten (2015) used pyrosequencing to investigate seven roof-harvested rainwater samples and two river water samples, with only 1 092 OTUs observed in total (sample coverage not reported). Furthermore, while the rarefaction curves (**Figure 3.2**) for the current study did not indicate a full saturation of the working population, a sharp initial incline was observed for both the untreated tank water samples as well as the SODIS-CPC samples whereafter the beginning of a plateau was detected. This suggests that the most abundant taxa of the viable bacterial community were recorded (Chidamba & Korsten, 2015).

In order to investigate and characterise the diversity and abundance of the viable bacterial community present in the harvested rainwater before and after SODIS treatment, various α - and β -diversity indices were calculated. Results for the α -diversity indices (species richness, ACE and the Shannon diversity index) indicated that the mean species richness in the Tank 1 rainwater samples was significantly higher ($p = 0.0033$) than the mean species richness observed for the SODIS-CPC-1 rainwater samples. Thus, a greater number of diverse species were present in the Tank 1 rainwater samples. Similarly, the mean Shannon diversity index obtained for the Tank 1 rainwater samples was significantly higher ($p = 0.0126$) in comparison to the mean Shannon diversity index observed for the SODIS-CPC-1 rainwater samples, suggesting a greater abundance and evenness of the viable bacterial community present in the Tank 1 rainwater samples. In addition, the lower mean Shannon diversity index recorded in the SODIS-CPC-1 rainwater samples (1.0 ± 0.6) also suggests that a lower species diversity was present in these samples, in comparison to the Tank 1 rainwater samples (3.8 ± 0.9). The mean ACE values for Tank 1 and SODIS-CPC-1 rainwater samples did not differ significantly ($p > 0.05$), however, as mentioned the ACE is a statistical estimation of the total number of species present in a sample, based on the actual number of species detected.

In order to investigate the β -diversity, the whole community structure (based on OTUs), including the OTUs classified to family and genus level, between the Tank 1 and the SODIS-CPC-1 rainwater samples were compared. Taxonomic diversity analysis resulted in the identification of 133 viable bacterial families present in the Tank 1 rainwater samples, which then decreased to 77 viable bacterial families after SODIS treatment. Similarly, on the genus level, 14 viable bacterial genera were detected in the Tank 1 samples which then decreased to eight viable bacterial genera in the SODIS treated samples. It should be noted that for the Tank 1 rainwater samples, a number of OTUs (mean of 47.2 %) could not be classified into any known bacterial family, however this number significantly decreased ($p = 0.010$) in the SODIS-CPC-1 rainwater samples and a mean of only 12.2 % of the number of OTUs could not be classified. Similarly, on the genus level, 54.6 % of the OTUs could not be classified into any known genera (Tank 1 samples) which then significantly decreased ($p = 0.0033$) to 5.0 % in the SODIS-CPC-1 rainwater samples. In addition, an increased percentage of low abundance families (2.2 %) and genera (4.3 %) were observed in the Tank 1 rainwater samples which contributed to the higher species diversity (α -diversity indices) recorded in these samples. In comparison, 0.7 % and 1.5 % low abundance families and genera, respectively, were recorded after SODIS treatment. It is hypothesised that the low abundance and unclassified species exhibited a decreased competitive advantage in the sample environment in comparison to the high relative abundance species identified in the untreated and SODIS-CPC treated rainwater samples (Koch, 2001; Roller & Schmidt, 2015).

Based on the results obtained in the current study, a clear community shift (families and genera) was observed between the untreated rainwater and the SODIS treated rainwater samples (**Figure 3.3 and 3.4**). For example, while the family Nocardiaceae dominated in both the Tank 1 and SODIS-

CPC-1 rainwater samples with mean relative abundance percentages of 16.5 % and 44.0 % recorded, respectively; overall the viable bacterial population for these samples was predominantly comprised of diverse families. For the Tank 1 rainwater samples, Pseudomonadaceae (8.9 %) and Sphingomonadaceae (6.0 %) were two of the dominant families identified, while the family Micrococcaceae (31.7 %) exhibited a high relative abundance in the SODIS-CPC-1 rainwater samples. Similarly, while the *Rhodococcus* genus dominated in both the Tank 1 (17.1 %) and SODIS-CPC-1 (48 %) rainwater samples, *Pseudomonas* (9.2 %) and *Arthrobacter* (35.2 %) were the second most abundant genera present in the untreated Tank 1 samples and the SODIS treated rainwater samples, respectively (**Figure 3.4**).

The dominance of the Nocardiaceae family in both sample groups is however, not surprising as genera associated with this family inhabits a wide range of aquatic and terrestrial environments (Niyomvong et al. 2012). In addition, they are saprophytes which live on decaying organic matter such as leaves, debris, etc. (Goodfellow, 2014). Moreover, while there are no overhanging tree branches over the catchment area, the sampling site is surrounded by trees (**Figure 3.1**) and gravel roads run adjacent to the sampling site. Leaves, decaying organic matter and dust are thus present on the catchment area and in the gutter and could have washed into the rainwater harvesting tank during a rain event.

The family Nocardiaceae consists of eight phylogenetically related genera which include *Prescottella*, *Gordonia*, *Nocardia* and *Rhodococcus*, amongst others (Goodfellow, 2014). The prevalence of the family Nocardiaceae then correlates to the taxonomic analysis of the genus classification, where *Rhodococcus* spp. was the dominant genus present in the Tank 1 rainwater samples (17.1 %) as well as in the SODIS-CPC-1 rainwater samples (48 %). In addition, it is well known from literature that *Rhodococcus* spp. are classified as extremophiles and are resistant to environmental threats such as UV and osmotic stress (Urbano et al. 2013). This is possibly due to its large bacterial chromosome (9.7 Mb) (McLeod et al. 2006) and the presence of three large linear plasmids (Van der Geize & Dijkhuizen, 2004). This genus further possesses high-fidelity DNA repair systems which render them genetically stable (Santos et al. 2007).

The second most abundant family in the Tank 1 rainwater samples was Pseudomonadaceae (8.9 %), however this family was the 17th most abundant family in SODIS-CPC-1 rainwater samples with a relative abundance of 0.01 %. The genus *Pseudomonas*, which falls under the Pseudomonadaceae family, was then also the second most abundant genus in the Tank 1 rainwater samples (9.2 %), however this genus only comprised 0.01 % of the genera detected in the SODIS-CPC-1 rainwater samples. Ahmed et al. (2017) established a significant positive correlation between the presence of *Pseudomonas* and bird faeces and attributed the presence of this genus in untreated rainwater to bird faecal matter contamination. Research has also indicated that *Pseudomonas* are able to persist during unfavourable environmental conditions such as high temperature and UV radiation, through the overexpression of the sigma factor *algT* (Srivastava et al. 2008; Santos et al.

2013). Moreover, *Pseudomonas* spp. are able to form associations with protozoa as a survival mechanism, where they exist as intracellular parasites (Thomas et al. 2010).

Sphingomonadaceae (6.0 %) was the third most abundant family present in the Tank 1 rainwater samples, however this family only accounted for 0.01 % of the total community detected in SODIS-CPC-1 rainwater samples. This correlates to the results observed on genus level where *Sphingobium* contributed to 4.6 % of the overall viable bacterial genera observed in Tank 1 rainwater samples. However, this genus had a relative abundance of 0.002 % in SODIS-CPC-1 rainwater samples. Chidamba and Korsten (2015) reported that the Sphingomonadaceae family accounted for 7.4 % of the total bacterial community sequenced in their rainwater and river water samples. *Sphingobium* are generally isolated from soil and Singh and Lal (2009) isolated *Sphingobium* species from a hexachlorocyclohexane (an insecticide used for agricultural purposes on farms) contaminated soil environment. This genus could possibly be found on the catchment area due to agricultural activities on the farm, where the sampling site was located.

The second most abundant family in the SODIS-CPC-1 rainwater samples was reported as Micrococcaceae (31.7%). The genus *Arthrobacter*, which forms part of the Micrococcaceae family, then accounted for 35.2 % of the overall viable bacterial genera in the SODIS-CPC-1 rainwater samples. *Arthrobacter* is also commonly found in soil environments and Wang and Xie (2012) isolated an *Arthrobacter* strain from agricultural soil in China, which was able to utilise atrazine (a herbicide used for the prevention of pre- and post-emergence of broadleaf weeds in crops) as its sole nitrogen source. Kuhlman et al. (2005) isolated various bacteria, such as *Arthrobacter*, *Cellulomona*, *Curtobacterium* and *Geodermatophilus*, from rock varnish in the Whipple mountains, USA and stated that these bacteria are radiation-resistant as they were resistant to UV-C exposure. This was corroborated by Osman et al. (2008) who reported that *Arthrobacter* spp. exhibited elevated resistance to UV radiation. Similarly, Tempest and Moseley (1982) stated that *Arthrobacter radiotolerans* is UV-immutable as this bacterium is able to precisely repair UV-induced DNA damage.

Despite *Nocardiaceae* and *Rhodococcus* being the most abundant family and genus, respectively, in the before and after SODIS treatment samples, a definite community shift in the viable bacterial population occurred after SODIS treatment as overall different families and genera were identified in the Tank 1 and SODIS-CPC-1 rainwater samples. These variations in community structure were further supported by results obtained from the Bray-Curtis distance metric analysis as two separate clusters were observed for the Tank 1 and SODIS-CPC-1 rainwater samples (**Figure 3.5**). There was also a significant difference (PERMANOVA; $p < 0.05$) between the community structures, which subsequently indicates that there was a significant shift in the viable bacterial community after SODIS treatment. These results correlate to the lower species richness and Shannon diversity indices obtained for the rainwater samples collected after SODIS treatment (SODIS-CPC-1).

While next generation sequencing has primarily been used as a tool to study microbial communities (microbial ecology) (Ahmed. et al. 2017; Chidamba & Korsten, 2015; Zhang et al. 2017), it has also

been used for the detection of pathogenic species present in biosolids (Bibby et al. 2010), raw sewage (Ye & Zhang, 2011) and in urban waters (Ibekwe et al. 2013). Thus, the SILVA and RDP [as recommended by Liu et al. (2008)] taxonomic classification databases were used to identify possible signatures of opportunistic pathogens and pathogens present at genus level in the Tank 1 and SODIS treated rainwater samples. It should be noted that not all species in the respective genera identified are considered pathogenic. For example, there are 202 known species of the *Pseudomonas* genus and research has indicated that only certain species cause disease and are associated with human infection, including *P. aeruginosa*, *P. fluorescens*, *P. putida*, *P. stutzeri*, *P. maltophilia* and *P. putrefaciens* (Bonares et al. 2016; Thomas et al. 2013).

Fourteen different opportunistic pathogenic genera were identified in the Tank 1 and/or SODIS-CPC-1 rainwater samples. Chidamba and Korsten (2015) detected various genera in roof-harvested rainwater samples which are known to contain pathogenic species, including *Acinetobacter*, *Clostridium*, *Chromobacterium*, *Legionella*, *Pseudomonas*, *Serratia*, *Tatlockia* and *Yersinia* using pyrosequencing. Of these genera, *Acinetobacter*, *Clostridium*, *Legionella*, *Pseudomonas* and *Yersinia* were also detected in the current study using Illumina next generation sequencing, which is an ultra-high throughput sequencing technique with a high sequencing resolution (more reads hence a better sequencing depth) (Caporaso et al. 2012). Research has previously indicated the presence of numerous opportunistic pathogenic species in rainwater including *L. pneumophila*, *P. aeruginosa*, *Aeromonas hydrophila*, *Staphylococcus aureus*, *Campylobacter jejuni*, amongst others (Ahmed et al. 2008; Dobrowsky et al. 2014b; Kaushik et al. 2012), using molecular detection methods such as PCR and qPCR. In addition, using EMA-qPCR, Reyneke et al. (2016) indicated the presence of viable *Legionella* spp. in roof-harvested rainwater (mean concentration of 2.3×10^6 copies/mL) and after solar pasteurization treatment at a temperature of 95 °C (mean concentration of 1.4×10^4 copies/mL). Similarly, Strauss et al. (2016) detected the presence of viable *Legionella* spp. and *Pseudomonas* spp. using EMA-qPCR, in roof-harvested rainwater and after eight hrs of SODIS treatment (solar cooker with 2 L PET bottles as reactors) at a maximum temperature of 89 °C.

In the current study, *Pseudomonas* and *Clostridium sensu stricto* were detected in all (100 %) the untreated rainwater samples and all (100 %) the SODIS treated rainwater samples. As previously indicated, *Pseudomonas* was one of the dominant genera detected in the Tank 1 rainwater samples and while present in all the SODIS-CPC-1 samples, a low relative abundance of this genus was detected. A study conducted in Sweden by Bagge et al. (2010) investigated the prevalence of *Clostridium*, which is well-known to persist in the gut of warm-blooded mammals, in the faecal matter of dairy farm cattle and reported that *C. butyricum* [which falls under the *Clostridium sensu stricto* group (Lawson & Rainey, 2016)] was isolated from cattle faeces. A dairy farm is located directly adjacent to the sampling site used in the current study and it is thus hypothesised that the dispersion of cattle faeces due to anthropogenic activity (vehicles that drive past etc.) could have contributed

to the presence of *Clostridium* on the catchment area which then washed into the tank during a rain event. Previous studies have also indicated that *Clostridium* are resistant to treatment by chemicals, moist heat and UV radiation, through the production of α/β -type small, acid-soluble proteins such as DacB and Spm (Raju et al. 2007; Paredes-Sabja et al. 2008).

Clostridium III, *Legionella*, *Mycobacterium* and *Yersinia* were detected in all the untreated rainwater samples with *Legionella*, *Mycobacterium* and *Yersinia* persisting in at least three (60 %) of the SODIS treated rainwater samples and *Clostridium III* persisting in one (20 %) of the SODIS treated rainwater samples. As previously indicated by Reyneke et al. (2016) and Strauss et al. (2016), viable *Legionella* spp. were detected using EMA-qPCR in SOPAS and SODIS treated rainwater. The persistence of this organism is mainly attributed to their ability to survive as intracellular parasites in protozoan species such as *Acanthamoeba* spp. (Dobrowsky et al. 2017; Fields et al. 2002) as well as their association with biofilms (Murga et al. 2001). In addition, research has shown that this genus contains heat shock proteins which make *Legionella* spp. more thermo-tolerant at increased temperatures ($> 50^{\circ}\text{C}$) (Fields et al. 2002)

Mycobacterium occur mostly in water environments as well as in food sources where members of this genus can cause serious diseases in humans such as tuberculosis and leprosy which is caused by *M. tuberculosis* and *M. leprae*, respectively (Glickman & Jacobs, 2001). Bohrerova and Linden (2006) investigated the effect of UV radiation, as a drinking water treatment method, on *Mycobacterium terrae* (*M. terrae*). The authors then reported that UV radiation resulted in a 2-log reduction of *M. terrae* and further stated that *M. terrae* was more resistant to UV treatment than many other bacteria (not stating which bacteria) (Bohrerova & Linden 2006). A study conducted by Wells et al. (2006) assessed the validity of various detection methods [such as TaqMan-based PCR assays, serologic enzyme-linked immunosorbent assays (ELISA) and culture assays] for the detection of *Mycobacterium avium* (*M. avium*) subsp. *paratuberculosis* in uninfected and infected dairy cattle. Faecal, milk and blood samples were screened for *M. avium* subsp. *paratuberculosis* whereafter the faecal samples of 23 % ($n = 1\ 808$) of the dairy cattle tested positive for this organism. In addition, Raizman et al. (2004) investigated the distribution of *M. avium* subsp. *paratuberculosis* on infected and uninfected dairy farms in Minnesota, USA by screening faecal as well as environmental samples from various sites on the dairy farm. Results then indicated that *M. avium* subsp. *paratuberculosis* was present in cattle faecal samples, in addition it was detected in cow alleyways, manure storage, calving areas, water runoff and post weaned calve areas (Raizman et al. 2004).

Acinetobacter spp., *Aeromonas* spp., *Brevibacterium* spp. and *Clostridium XI* were detected in 80 %, 60 %, 20 % and 60 %, respectively, of the untreated Tank 1 samples. *Brevibacterium* spp. and *Clostridium XI* were not detected in any of the SODIS-CPC-1 rainwater samples, while *Acinetobacter* spp. and *Aeromonas* spp. persisted in 80 % and 60 % of the SODIS treated rainwater samples, respectively. *Brevibacterium* is commonly found in food sources, on the human skin and in soil

environments where it survives at a pH range of 5.5 to 9.0 (Fischer, 2017). Santos et al. (2013) investigated the effect of UV radiation on various bacteria, including *Brevibacterium*, *Acinetobacter*, *Pseudomonas*, *Staphylococcus* and *Micrococcus*, amongst others, and concluded that *Brevibacterium* was quite sensitive to UV radiation (more sensitive to UV-A radiation in comparison to the other bacteria tested). Dodson et al. (1994) and Shick and Dunlap (2002) suggested that the lack of protective pigmentation and specialised DNA repair systems in *Brevibacterium* also contributes to the UV sensitivity of this genus.

Acinetobacter spp. are known to cause infection, such as bacteraemia, endocarditis and urinary tract infections in debilitated patients in hospital environments, however, they are also widely distributed in soil and water environments (Doughari et al. 2011). Hörtnagl et al. (2011) investigated the effect of UV radiation on the growth efficiency of various bacteria, including *Acinetobacter*, *Sphingomonas* and *Acidovorax*, amongst others. It was shown that the growth efficiency in *Acinetobacter lwoffii* increased significantly during UV radiation. In addition, Zenoff et al. (2006) reported that *Acinetobacter johnsonii* are able to endure as well as rapidly recover after UV radiation. It was then concluded that *Acinetobacter* spp. are highly tolerant to UV radiation (Hörtnagl et al. 2011).

Furthermore, *Burkholderia*, *Campylobacter* and *Staphylococcus* were not detected in the untreated rainwater samples in the current study, possibly as the relative abundance of these genera were too low (and a higher sequencing depth is required for their detection), however, they were detected in at least one of the SODIS treated rainwater samples. *Burkholderia* is an opportunistic pathogen which is associated with nosocomial infections in patients suffering from chronic granulomatous disease and cystic fibrosis (LiPuma, 1998; Woods et al. 2006). This genus is also able to survive in harsh, competitive environments where it is widely distributed in various ecological niches including, soil, fresh water, marine water, animals and humans (Stoyanova et al. 2007). *Campylobacter* is a well-known zoonotic pathogen which causes acute gastroenteritis in humans, especially in children below the age of four (Zendehbad et al. 2013). Abdollahpour et al. (2015) investigated the possibility of wild-bird faeces as a source of *Campylobacter*. *Campylobacter* was then isolated from dry (5.7 %) and wet (23.9 %) faecal samples of wild-birds (e.g. house sparrow, pigeons, pied wagtail etc.) in Iran. The authors concluded that wild-birds may serve as a reservoir for *Campylobacter* spp. which possibly poses a health risk to humans (Abdollahpour et al. 2015). Haughton et al. (2012) examined the susceptibility of *Campylobacter* spp. to UV radiation in order to investigate the use of UV radiation as a method to decontaminate liquids, raw chicken and contact surfaces which are used during the processing of raw chicken. Different *Campylobacter coli* and *C. jejuni* strains (including *C. jejuni* 323 BC, 1135 DF, 1136 DF, 1146 DF, amongst others) were exposed to UV radiation in a liquid medium (initial concentration of 10^7 CFU/mL) for two min at a distance of three, 12 and 23 cm, whereafter results indicated that *C. jejuni* 323 BC was reduced by less than 1-log, while, no significant reduction was observed for *C. jejuni* 1136 DF even after 10 min at a distance of 23 cm. The authors further

concluded that *C. jejuni* 323 BC and *C. jejuni* 1136 DF were the least UV susceptible isolates in comparison to all other strains tested (Haughton et al. 2012).

Members of the genus *Staphylococcus* such as *S. aureus* are opportunistic pathogens known to produce toxins and are able to cause infections resulting in acute and chronic diseases (Kadariya et al. 2014). *Staphylococcus* spp. are also associated with nosocomial and community acquired infections as this genus is widely distributed in soil and are inhabitants of animals and humans (Kadariya et al. 2014). Gómez et al. (2014) conducted a study in Spain where they screened for *S. aureus* in the faecal samples of small mammals such as mice, brown rats, white-toothed shrews etc. After the successful isolation and characterisation of *S. aureus* strains, the presence of the *mecC* gene (allows for the resistance against penicillin-like antibiotics) was also detected. The authors then concluded that small mammals serve as a reservoir for *S. aureus* containing the *mecC* gene which poses a risk to human health (Gómez et al. 2014).

The 14 genera (including the different groups of *Clostridium*) identified in the current study were also detected by Ahmed et al. (2017). These authors however, detected 37 different opportunistic pathogenic genera using Illumina next generation sequencing. However, while both studies (the current study and Ahmed et al. 2017) utilised Illumina next generation sequencing, Ahmed et al. (2017) did not use the pre-treatment of a viability dye prior to DNA extractions, thus the authors sequenced and detected both the viable and non-viable bacterial community. Furthermore, Ahmed et al. (2017) sampled 88 rainwater harvesting tanks in two different geographical urban areas (Brisbane and Currumbin). The presence of television aerials and overhanging trees were also present at various catchment sites in the Brisbane and Currumbin areas, in comparison to the current study where the sampling site was situated on the periphery of Stellenbosch, South Africa and no television aerials or overhanging trees obstructed the study site.

Ahmed et al. (2017) further suggested that the presence of these potential opportunistic pathogenic genera could be attributed to various environmental factors (farming, anthropogenic activity, industries, etc.) as well as to the period of dry days preceding a rain event. Moreover, signatures of pathogenic genera such as *Mycobacterium*, *Clostridium*, *Leptospira*, *Campylobacter*, *Acinetobacter*, *Aeromonas* and *Enterobacter* (Craven et al. 2000; Dhama et al. 2011; Abdollahpour et al. 2015, Dahiru & Enabulele, 2015; Allocati et al. 2016) have previously been detected in bird and bat faecal samples. It is thus hypothesised that the presence of these potential opportunistic pathogenic genera in the treated and SODIS treated rainwater in the current study may be ascribed to bird faecal matter [*Pseudomonas* (Ahmed et al. 2017) and *Campylobacter* (Abdollahpour et al. 2015)] and soil and dust on the catchment area [*Acinetobacter* (Doughari et al. 2011) and *Brevibacterium* (Fischer, 2017)]. *Clostridium* and *Mycobacterium* have also previously been associated with cattle faecal matter (Wells et al. 2006; Bagge et al. 2010).

3.5. Conclusions

Based on an extensive literature search, to date high throughput next generation sequencing has only been used twice to characterise the bacterial community present in harvested rainwater (Chidamba & Korsten, 2015; Ahmed. et al. 2017). However, in the current study the diversity and abundance of the viable bacterial community present in rainwater before and after SODIS treatment (eight hrs) was monitored using EMA in combination with Illumina next generation sequencing. Using this technique, it was shown that the treatment of roof-harvested rainwater by solar radiation (UV light) and heat decreased the bacterial species diversity significantly after SODIS and resulted in a community shift in the viable bacterial population. Furthermore, various potential opportunistic pathogenic bacteria were identified in roof-harvested rainwater after SODIS treatment for eight hrs. From literature, numerous of the bacterial genera identified have been detected in bird faecal matter, dirt and debris (Abdollahpour et al. 2015, Allocati et al. 2016, Craven et al. 2000; Dahiru & Enabulele, 2015; Dhama et al. 2011) and Ahmed et al. (2017) correlated the presence of dust, organic matter and bird faeces on the rainwater catchment site to the deterioration of the microbial water quality of roof-harvested rainwater.

Ishii et al. (2013) suggested that next generation sequencing be used to determine the abundance and diversity of pathogenic bacteria in water samples, whereafter quantitative analysis should be performed in order to quantify specific pathogenic species present in the water samples. As the taxonomic data obtained in the current study was based on the V3 to V4 hypervariable region of the 16S rRNA prokaryotic gene, further investigation is needed to interpret the relative abundance of a specific species present in the rainwater samples as information on only the dominant families and genera was obtained. Thus, further research should focus on the use of EMA-qPCR to screen for the specific potential opportunistic pathogenic genera detected in the current study to quantitatively investigate the abundance of these organisms in rainwater and identify the primary species present. Once the absolute species numbers are determined, quantitative microbial risk assessment studies should be performed in order to determine the risk these bacteria pose to human health. An educated decision on the primary uses of the SODIS treated roof-harvested rainwater can then be formulated.

3.6. References

- Abbasi, T., Abbasi, S.A., 2011. Sources of pollution in rooftop rainwater harvesting systems and their control. *Crit. Rev. Environ. Sci. Technol.* 41(23), 2097–2167.
- Abdollahpour, N., Zendeabad, B., Alipour, A., Khayat-zadeh, J., 2015. Wild-bird feces as a source of *Campylobacter jejuni* in children's playgrounds in Iran. *Food Control.* 50, 378–381.
- Ahmed, W., Huygens, F., Goonetilleke, A., Gardner, T., 2008. Real-time PCR detection of pathogenic microorganisms in roof-harvested rainwater in Southeast Queensland, Australia. *Appl. Environ. Microbiol.* 74(17), 5490–5496.

- Ahmed, W., Sawant, S., Huygens, F., Goonetilleke, A., Gardner, T., 2009. Prevalence and occurrence of zoonotic bacterial pathogens in surface waters determined by quantitative PCR. *Water Res.* 43(19), 4918–4928.
- Ahmed, W., Staley, C., Hamilton, K.A., Beale, D.J., Sadowsky, M.J., Toze, S., Haas, C.N., 2017. Amplicon-based taxonomic characterization of bacteria in urban and peri-urban roof-harvested rainwater stored in tanks. *Sci. Total. Environ.* 576, 326-334.
- Allocati, N., Petrucci, A.G., Giovanni, P.D., Masulli, M., Llio, C.D., Laurenzi, V.D., 2016. Batman disease transmission: zoonotic pathogens from wildlife reservoirs to human populations. *Cell Death Dis.* 2, 16048.
- Amin, M.T., Han, M.Y., 2009. Roof-harvested rainwater for potable purposes: application of solar collector disinfection (SOCO-DIS). *Water Res.* 43(20), 5225–5235.
- Amin, M.T., Nawaz, M., Amin, M.N. & Han, M. 2014. Solar disinfection of *Pseudomonas aeruginosa* in harvested rainwater: A step towards potability of rainwater. *PLoS One.* 9(3):1–10.
- Bagge, E., Persson, M., Johansson, K.E., 2010. Diversity of spore-forming bacteria in cattle manure, slaughterhouse waste and samples from biogas plants. *J. Appl. Microbiol.* 109(5), 1549-1565
- Bibby, K., Viau, E., Peccia, J., 2010. Pyrosequencing of the 16S rRNA gene to reveal bacterial pathogen diversity in biosolids. *Water Res.* 44, 4252–4260.
- Bohrerova, Z., Linden, K.G., 2006. Assessment of DNA damage and repair in *Mycobacterium terrae* after exposure to UV irradiation. *J. Appl. Microbiol.* 101(5), 995-1001.
- Bonares, M.J., Vaisman, A., Sharkawy, A., 2016. Prosthetic vascular graft infection and prosthetic joint infection caused by *Pseudomonas stutzeri*. *IDCases*, 6, 106-108.
- Caporaso, J.G., Lauber, C.L., Walters, W.A., Berg-Lyons, D., Huntley, J., Fierer, N., Owens, S.M., Betley, J., Fraser, L., Bauer, M., Gormley, N., 2012. Ultra-high-throughput microbial community analysis on the Illumina HiSeq and MiSeq platforms. *ISME J.* 6(8), 1621-1624.
- Cason, E.D., Williams, P.J., Ojo, E., Castillo, J., DeFlaun, M.F., van Heerden, E., 2017. Hexavalent chromium bioreduction and chemical precipitation of sulphate as a treatment of site-specific fly ash leachates. *World J. Microbiol. Biotechnol.* 33(88), 1-9.
- Castro-Alfárez, M., Polo-López, M.I., Fernández-Ibáñez, P., 2016. Intracellular mechanisms of solar water disinfection. *Sci. Rep.* 6, 38145.
- Chang, B., Taguri, T., Sugiyama, K., Amemura-Maekawa, J., Kura, F., Watanabe, H., 2010. Comparison of ethidium monoazide and propidium monoazide for theselective detection of viable

Legionella cells. Jpn. J. Infect. Dis. 63, 119–123.

Chao, A., Lee, S.M., 1992. Estimating the number of classes via sample coverage. J. Am. Stat. Assoc. 87(417), 210-217.

Chidamba, L., Korsten, L., 2015. Pyrosequencing analysis of roof-harvested rainwater and river water used for domestic purposes in Luthengele village in the Eastern Cape province of South Africa. Environ. Monit. Assess. 187(2), 41.

Claesson, M.J., O'Sullivan, O., Wang, Q., Nikkilä, J., Marchesi, J.R., Smidt, H., de Vos, W.M., Ross, R.P., O'Toole, P.W., 2009. Comparative analysis of pyrosequencing and a phylogenetic microarray for exploring microbial community structures in the human distal intestine. PloS One, 4(8), p.e6669.

Cole, J.R., Wang, Q., Cardenas, E., Fish, J., Chai, B., Farris, R.J., Kulam-Syed-Mohideen, A.S., McGarrell, D.M., Marsh, T., Garrity, G.M., Tiedje, J.M., 2008. The Ribosomal Database Project: improved alignments and new tools for rRNA analysis. Nucleic Acids Res. 37(suppl_1), D141-D145.

Craven, S.E., Stern, N.J., Line, E., Bailey, J.S., Cox, N.A., Fedorka-Cray, P., 2000. Determination of the incidence of *Salmonella* spp., *Campylobacter jejuni*, and *Clostridium perfringens* in wild birds near broiler chicken houses by sampling intestinal droppings. Avian Dis. 44, 715–720.

Dahiru, M., Enabulele, O.I., 2015. *Acinetobacter baumannii* in birds feces: a public health threat to vegetables and irrigation farmers. Adv. Microbiol. 5, 693–698.

De Kwaadsteniet, M., Dobrowsky, P.H., Van Deventer, A., Khan, W., Cloete, T.E., 2013. Domestic rainwater harvesting: microbial and chemical water quality and point-of-use treatment systems. Water, Air, Soil Pollut. 224(7).

Dhama, K., Mahendran, M., Tiwari, R., Dayal Singh, S., Kumar, D., Singh, S., Sawant, P.M., 2011. Tuberculosis in birds: Insights into the *Mycobacterium avium* infections. Vet. Med. Int. 2011, 1-14.

Dobrowsky, P.H., Mannel, D., De Kwaadsteniet, M., Prozesky, H., Khan, W., Cloete, T.E., 2014a. Quality assessment and primary uses of harvested rainwater in Kleinmond, South Africa. Water SA. 40(3), 401–406.

Dobrowsky, P.H., De Kwaadsteniet, M., Cloete, T.E., Khan, W., 2014b. Distribution of indigenous bacterial pathogens and potential pathogens associated with roof-harvested rainwater. Appl. Environ. Microbiol. 80(7), 2307–2316.

Dobrowsky, P.H., Van Deventer, A., De Kwaadsteniet, M., Ndlovu, T., Khan, S., Cloete, T.E., Khan, W., 2014c. Prevalence of virulence genes associated with pathogenic *Escherichia coli* strains

isolated from domestically harvested rainwater during low-and high-rainfall periods. *Appl. Environ. Microbiol.* 80(5), 1633-1638.

Dobrowsky, P.H., Lombard, M., Cloete, W.J., Saayman, M., Cloete, T.E., Carstens, M., Khan, S., Khan, W., 2015. Efficiency of microfiltration systems for the removal of bacterial and viral contaminants from surface and rainwater. *Water Air Soil Pollut.* 226(33), 1-14.

Dobrowsky, P.H., Khan, S., Khan, W. 2017. Resistance of *Legionella* and *Acanthamoeba mauritaniensis* to heat treatment as determined by relative and quantitative polymerase chain reactions. *Environ. Res.* 158, 82-93.

Dodson, M.L., Michaels, M.L., Lloyd, R.S., 1994. Unified catalytic mechanism for DNA glycosylases. *J. Biol. Chem.* 269(52), 32709-32712.

Doughari, H.J., Ndakidemi, P.A., Human, I.S. and Benade, S., 2011. The ecology, biology and pathogenesis of *Acinetobacter* spp.: an overview. *Microbes Environ.* 26(2), 101-112.

Fields, B.S., Benson, R.F. Besser, R.E. 2002. *Legionella* and Legionnaires' disease: 25 years of investigation. *Clin. Microbiol. Rev.* 15(3), 506-526.

Fischer, J., 2017. Habitat. *Brevibacterium linens*. Available: http://bioweb.uwlax.edu/bio203/s2012/fischer_jaco/habitat.htm [2017, November 28].

Fittipaldi, M., Nocker, A., Codony, F., 2012. Progress in understanding preferential detection of live cells using viability dyes in combination with DNA amplification. *J. Microbiol. Methods.* 91(2):276-289.

Glickman, M.S., Jacobs, W.R., 2001. Microbial pathogenesis of *Mycobacterium tuberculosis*: dawn of a discipline. *Cell.* 104(4), 477-485.

Gómez, P., González-Barrio, D., Benito, D., García, J.T., Viñuela, J., Zarazaga, M., Ruiz-Fons, F., Torres, C., 2014. Detection of methicillin-resistant *Staphylococcus aureus* (MRSA) carrying the *mecC* gene in wild small mammals in Spain. *J. Antimicrob. Chemother.* 69(8), 2061-2064.

Goodfellow, M., 2014. The family Nocardaceae. *The Prokaryotes: Actinobacteria*, 4th Ed. Rosenberg, E., DeLong, E.F., Lory, S., Stacebrandt, E., Thompson, F., pp. 595-650. Springer, UK.

Haughton, P.N., Grau, E.G., Lyng, J., Cronin, D., Fanning, S., Whyte, P., 2012. Susceptibility of *Campylobacter* to high intensity near ultraviolet/visible 395±5nm light and its effectiveness for the decontamination of raw chicken and contact surfaces. *Int. J. Food Microbiol.* 159(3), 267-273.

Hörtnagl, P., Pérez, M.T., Sommaruga, R., 2011. Contrasting effects of ultraviolet radiation on the growth efficiency of freshwater bacteria. *Aquatic Ecol.* 45(1), 125-136.

- Huse, S.M., Dethlefsen, L., Huber, J.A., Welch, D.M., Relman, D.A., Sogin, M.L., 2008. Exploring microbial diversity and taxonomy using SSU rRNA hypervariable tag sequencing. *PLoS Genetics*, 4(11), p.e1000255.
- Ibekwe, A.M., Leddy, M., Murinda, S.E., 2013. Potential human pathogenic bacteria in a mixed urban watershed as revealed by pyrosequencing. *PLoS One*. 8, e79490.
- Ishii, S., Segawa, T. & Okabe, S. 2013. Simultaneous quantification of multiple food and waterborne pathogens by use of microfluidic quantitative PCR. *Appl. Environ. Microbiol.* 79(9), 2891-2898.
- Kadariya, J., Smith, T.C., Thapaliya, D., 2014. *Staphylococcus aureus* and staphylococcal food-borne disease: an ongoing challenge in public health. *BioMed Res. Int.* 2014, 827965.
- Kaushik, R., Balasubramanian, R., De La Cruz, A.A., 2012. Influence of air quality on the composition of microbial pathogens in fresh rainwater. *Appl. Environ. Microbiol.* 78, 2813-2818.
- Klindworth, A., Pruesse, E., Schweer, T., Peplies, J., Quast, C., Horn, M. and Glöckner, F.O., 2013. Evaluation of general 16S ribosomal RNA gene PCR primers for classical and next-generation sequencing-based diversity studies. *Nucleic Acids Res.* 41(1), e1.
- Koch, A.L., 2001. Oligotrophs versus copiotrophs. *Bioessays*, 23(7), 657-661.
- Kozich, J.J., Westcott, S.L., Baxter, N.T., Highlander, S.K., Schloss, P.D., 2013. Development of a dual-index sequencing strategy and curation pipeline for analyzing amplicon sequence data on the MiSeq Illumina sequencing platform. *Appl. Environ. Microbiol.* 79(17), 5112-5120.
- Kuhlman, K.R., Allenbach, L.B., Ball, C.L., Fusco, W.G., La Duc, M.T., Kuhlman, G.M., Anderson, R.C., Stuecker, T., Erickson, I.K., Benardini, J., Crawford, R.L., 2005. Enumeration, isolation, and characterization of ultraviolet (UV-C) resistant bacteria from rock varnish in the Whipple Mountains, California. *Icarus*. 174(2), 585-595.
- Lawson, P.A., Rainey, F.A., 2016. Proposal to restrict the genus *Clostridium* Prazmowski to *Clostridium butyricum* and related species. *Int. J. Syst. Evol. Microbiol.* 66(2), 1009-1016.
- Leifels, M., Jurzik, L., Wilhelm, M., Hamza, I.A., 2015. Use of ethidium monoazide and propidium monoazide to determine viral infectivity upon inactivation by heat, UV-exposure and chlorine. *Int. J. Hyg. Environ. Health.* 218(8), 686-693.
- LiPuma, J.J., 1998. *Burkholderia cepacia*: management issues and new insights. *Clin. Chest Med.* 19(3), 473-486.
- Liu, Z., DeSantis, T.Z., Andersen, G.L., Knight, R., 2008. Accurate taxonomy assignments from 16S rRNA sequences produced by highly parallel pyrosequencers. *Nucleic Acids Res.* 36, e120.

- Mansi, A., Amori, I., Marchesi, I., Marcelloni, A.M., Proietto, A.R., Ferranti, G., Magini, V., Valeriani, F., Borella, P., 2014. *Legionella* spp. survival after different disinfection procedures: Comparison between conventional culture, qPCR and EMA–qPCR. *Microchem. J.* 112, 65-69.
- McGuigan, K.G., Conroy, R.M., Mosler, H., Du Preez, M., Ubomba-Jaswa, E., Fernandez-Ibanez, P. 2012. Solar water disinfection (SODIS): a review from bench- top to roof-top. *J. Hazard .Mater.* 235, 29–46.
- McLeod, M.P., Warren, R.L., Hsiao, W.W., Araki, N., Myhre, M., Fernandes, C., Miyazawa, D., Wong, W., Lillquist, A.L., Wang, D., Dosanjh, M., 2006. The complete genome of *Rhodococcus* sp. RHA1 provides insights into a catabolic powerhouse. *Proc. Natl. Acad. Sci. U.S.A.* 103(42), 15582-15587.
- Murga, R., Forster, T.S., Brown, E., Pruckler, J.M., Fields, B.S., Donlan, R.M. 2001. Role of biofilms in the survival of *Legionella pneumophila* in a model potable-water system. *Microbiology.* 147(11), 3121-3126.
- Nalwanga, R., Muyanja, C.K., McGuigan, K.G., Quilty, B., 2016. A study of the bacteriological quality of roof-harvested rainwater and an evaluation of SODIS as a suitable treatment technology in rural sub-Saharan Africa. In Press: *J. Environ. Chem. Eng.* <http://dx.doi.org/10.1016/j.jece.2016.12.008>.
- Nieuwoudt, M.N., Mathews, E.H., 2005. A mobile solar water heater for rural housing in Southern Africa. *Build. Environ.* 40(9), 1217–1234.
- Niyomvong, N., Pathom-aree, W., Thamchaipenet, A., Duangmal, K., 2012. Actinomycetes from tropical limestone caves. *Chiang Mai J. Sci.* 39(3), 373-388.
- Oksanen, J., Blanchet, F.G., Kindt, R., Legendre, P., Minchin, P.R., O'Hara, R.B., Simpson, G.L., Solymos, P., Henry, M., Stevens, H., Szoecs, E., Wagner, H., 2017. *Vegan: Community Ecology Package*. R package version 2.3-0. Available: <http://CRAN.R-project.org/package=vegan>.
- Osman, S., Peeters, Z., La Duc, M.T., Mancinelli, R., Ehrenfreund, P., Venkateswaran, K., 2008. Effect of shadowing on survival of bacteria under conditions simulating the Martian atmosphere and UV radiation. *Appl. Environ. Microbiol.* 74(4), 959-970.
- Paredes-Sabja, D., Sarker, N., Setlow, B., Setlow, P., Sarker, M.R., 2008. Roles of DacB and Spm proteins in *Clostridium perfringens* spore resistance to moist heat, chemicals, and UV radiation. *Appl. Environ. Microbiol.* 74(12), 3730-3738.
- Pruesse, E., Quast, C., Knittel, K., Fuchs, B.M., Ludwig, W., Peplies, J., Glöckner, F.O., 2007. SILVA: a comprehensive online resource for quality checked and aligned ribosomal RNA sequence data compatible with ARB. *Nucleic Acids Res.* 35(21), 7188-7196.

R Development Core Team, 2017. R: A Language and Environment for Statistical Computing. Vienna, Austria: the R Foundation for Statistical Computing.

Raizman, E.A., Wells, S.J., Godden, S.M., Bey, R.F., Oakes, M.J., Bentley, D.C., Olsen, K.E., 2004. The distribution of *Mycobacterium avium* ssp. *paratuberculosis* in the environment surrounding Minnesota dairy farms. J. Dairy Sci. 87(9), 2959-2966.

Raju, D., Setlow, P., Sarker, M.R., 2007. Antisense-RNA-mediated decreased synthesis of small, acid-soluble spore proteins leads to decreased resistance of *Clostridium perfringens* spores to moist heat and UV radiation. Appl. Environ. Microbiol. 73(7), 2048-2053.

Reed, R.H. 2004. The inactivation of microbes by sunlight: solar disinfection as a water treatment process. Adv. Appl. Microbiol. 54, 333-365.

Reyneke, B., Dobrowsky, P.H., Ndlovu, T., Khan, S., Khan, W. 2016. EMA-qPCR to monitor the efficiency of a closed-coupled solar pasteurization system in reducing *Legionella* contamination of roof-harvested rainwater. Science of the Total Environment. 553:662–670.

Reyneke, B., Ndlovu, T., Khan, S., Khan, W., 2017. Comparison of EMA-, PMA-and DNase qPCR for the determination of microbial cell viability. Appl. Microbiol. Biotechnol. 101(19), 7371–7383.

Roller, B.R., Schmidt, T.M., 2015. The physiology and ecological implications of efficient growth. ISME J. 9(7), 1481-1487.

Santos, S.C., Alviano, D.S., Alviano, C.S., Goulart, F.R., de Pádula, M., Leitão, Á.C., Martins, O.B., Ribeiro, C.M., Sasaki, M.Y., Matta, C.P., Bevilaqua, J., 2007. Comparative studies of phenotypic and genetic characteristics between two desulfurizing isolates of *Rhodococcus erythropolis* and the well-characterized *R. erythropolis* strain IGTS8. J. Ind. Microbiol. Biotechnol. 34(6), 423-431.

Santos, A.L., Oliveira, V., Baptista, I., Henriques, I., Gomes, N.C., Almeida, A., Correia, A., Cunha, Â., 2013. Wavelength dependence of biological damage induced by UV radiation on bacteria. Arch. Microbiol. 195(1), 63-74.

Schloss, P.D., Westcott, S.L., Ryabin, T., Hall, J.R., Hartmann, M., Hollister, E.B., Lesniewski, R.A., Oakley, B.B., Parks, D.H., Robinson, C.J., Sahl, J.W., 2009. Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. Appl. Environ. Microbiol. 75(23), 7537-7541.

Shick, J.M., Dunlap, W.C., 2002. Mycosporine-like amino acids and related gadusols: biosynthesis, accumulation, and UV-protective functions in aquatic organisms. Annu. Rev. Physiol. 64(1), 223-262.

- Shannon, C.E., Weaver, W., 1964. *The Mathematical Theory of Communication*, pp. 1 - 132. The University of Illinois Press: Urbana, Illinois, USA.
- Singh, A., Lal, R., 2009. *Sphingobium ummariense* sp. nov., a hexachlorocyclohexane (HCH)-degrading bacterium, isolated from HCH-contaminated soil. *Int. J. Syst. Evol. Microbiol.* 59(1), 162-166.
- Sogin, M.L., Morrison, H.G., Huber, J.A., Welch, D.M., Huse, S.M., Neal, P.R., Arrieta, J.M., Herndl, G.J., 2006. Microbial diversity in the deep sea and the underexplored "rare biosphere". *Proc. Natl. Acad. Sci.* 103(32), 12115-12120.
- Srivastava, S., Yadav, A., Seem, K., Mishra, S., Chaudhary, V., Nautiyal, C.S., 2008. Effect of high temperature on *Pseudomonas putida* NBRI0987 biofilm formation and expression of stress sigma factor RpoS. *Curr. Microbiol.* 56(5), 453-457.
- Staley, C., Gould, T.J., Wang, P., Phillips, J., Cotner, J.B., Sadowsky, M.J., 2015. Species sorting and seasonal dynamics primarily shape bacterial communities in the upper Mississippi River. *Sci. Total Environ.* 505, 435-445.
- Stoyanova, M., Pavlina, I., Moncheva, P., Bogatzevska, N., 2007. Biodiversity and incidence of *Burkholderia* species. *Biotechnol. Biotechnol. Equip.* 21(3), 306-310.
- Strauss, A., Dobrowsky, P.H., Ndlovu, T., Reyneke, B., Khan, W., 2016. Comparative analysis of solar pasteurization versus solar disinfection for the treatment of harvested rainwater. *BMC Microbiol.* 16(1), 289.
- Tempest, P.R., Moseley, B.E., 1982. Lack of ultraviolet mutagenesis in radiation-resistant bacteria. *Mutation Research Letters.* 104(4-5), 275-280.
- Thomas, B.S., Okamoto, K., Bankowski, M.J., Seto, T.B., 2013. A lethal case of *Pseudomonas putida* bacteremia due to soft tissue infection. *Infect. Dis. Clin. Pract. (Baltim. Md.).* 21(3), 147-213.
- Thomas, V., McDonnell, G., Denyer, S.P., Maillard, J.Y., 2010. Free-living amoebae and their intracellular pathogenic microorganisms: risks for water quality. *FEMS Microbiol. Rev.* 34(3), 231-259.
- Ubomba-Jaswa, E., Fernández-Ibáñez, P., Navntoft, C., Polo-López, M.I., McGuigan, K.G. 2010. Investigating the microbial inactivation efficiency of a 25 L batch solar disinfection (SODIS) reactor enhanced with a compound parabolic collector (CPC) for household use. *J. Chem. Technol. Biotechnol.* 85(8), 1028-1037.

- Urbano, S.B., Albarracín, V.H., Ordoñez, O.F., Farías, M.E., Alvarez, H.M., 2013. Lipid storage in high-altitude Andean Lakes extremophiles and its mobilization under stress conditions in *Rhodococcus* sp. A5, a UV-resistant actinobacterium. *Extremophiles*, 17(2), 217-227.
- Van der Geize, R., Dijkhuizen, L., 2004. Harnessing the catabolic diversity of rhodococci for environmental and biotechnological applications. *Curr. Opin. Microbiol.* 7(3), 255-261.
- Wang, Q., Garrity, G.M., Tiedje, J.M., Cole, J.R., 2007. Naive Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. *Appl. Environ. Microbiol.* 73(16), 5261-5267.
- Wang, Q., Xie, S., 2012. Isolation and characterization of a high-efficiency soil atrazine-degrading *Arthrobacter* sp. strain. *Int. Biodeterior. Biodegradation.* 71, 61-66.
- Wells, S.J., Collins, M.T., Faaberg, K.S., Wees, C., Tavoranpanich, S., Petrini, K.R., Collins, J.E., Cernicchiaro, N., Whitlock, R.H., 2006. Evaluation of a rapid fecal PCR test for detection of *Mycobacterium avium* subsp. *paratuberculosis* in dairy cattle. *Clin. Vaccine Immunol.* 13(10), 1125-1130.
- Westphal, C., Bommarco, R., Carré, G., Lamborn, E., Morison, N., Petanidou, T., Potts, S.G., Roberts, S.P., Szentgyörgyi, H., Tscheulin, T., Vaissière, B.E., 2008. Measuring bee diversity in different European habitats and biogeographical regions. *Ecol. Monograph.* 78(4), 653-671.
- Williams, M.T., Ndlovu, T., Dobrowsky, P.H., Waso, M., Khan, W., 2015. Quality profile of fresh rainwater versus rainwater harvested from various catchment materials. Unpublished Honours Thesis, Stellenbosch University.
- Woods, Donald E., Pamela A. Sokol., 2006. "The genus Burkholderia." In *The Prokaryotes*, pp. 848-860. Springer New York.
- Ye, L., Zhang, T., 2011. Pathogenic bacteria in sewage treatment plants as revealed by 454 pyrosequencing. *Environ. Sci. Technol.* 45, 7173–7179.
- Zhang, Y., Oh, S., Liu, W.T., 2017. Impact of drinking water treatment and distribution on the microbiome continuum: an ecological disturbance's perspective. *Environ. Microbiol.* 19(8), 3163–3174.
- Zenoff, V.F., Sineriz, F., Farías, M.E., 2006. Diverse responses to UV-B radiation and repair mechanisms of bacteria isolated from high-altitude aquatic environments. *Appl. Environ. Microbiol.* 72(12), 7857-7863.
- Zendehbad, B., Arian, A.A., Alipour, A. 2013. Identification and antimicrobial resistance of

Campylobacter species isolated from poultry meat in Khorasan province, Iran. Food Control. 32, 724-727.

Chapter 4:

General Conclusions and Recommendations

(UK spelling is employed)

4.1. General Conclusions and Recommendations

South Africa is a water scarce country with the Western Cape province currently experiencing severe drought. Water rationing and restrictions have thus been implemented to curtail excessive potable water use. Domestic rainwater harvesting (DRWH) has been earmarked by the South African government as an alternative water resource, as rainwater harvesting technologies have the potential to supply water to rural and peri-urban areas where standard water infrastructure technologies cannot be implemented (Amin & Han, 2009; Huston et al. 2012; De Kwaadsteniet et al. 2013). However, the quality of roof-harvested rainwater may deteriorate during the harvesting process due to the presence of atmospheric pollution and contaminants (such as organic matter, dust and animal faeces) on the catchment area and in the gutter piping (Helmreich & Horn, 2009; Abbasi & Abbasi, 2011). Accordingly, numerous studies have reported that particularly the microbial quality of roof-harvested rainwater does not adhere to drinking water standards (Ahmed et al. 2010; 2012; De Kwaadsteniet et al. 2013; Dobrowsky et al. 2014a; 2014b). It is therefore essential to implement treatment technologies to reduce the level of microbial contamination in stored rainwater. Preliminary treatment of rainwater can be achieved by diverting the first flush of harvested rain, which is thought to contain higher levels of microbial and chemical pollutants (Yaziz et al. 1989). However, primary treatment methods are still required to inactivate specifically pathogenic microorganisms. Solar disinfection (SODIS) is considered one of the most efficient and cost-effective methods for the treatment of rainwater as it relies on a free renewable energy source, the sun.

The primary aim of **Chapter two** was thus to design and construct a SODIS system, fitted with a compound parabolic collector (CPC) (SODIS-CPC), which was (i) cost-effective, (ii) robust in nature, (iii) required minimum maintenance and (iv) exhibited an increased treatment efficiency. In order to achieve this aim, material sourcing was conducted to construct the most efficient and cost-effective SODIS-CPC system. Stainless steel (grade 304) was used for the profile and frame construction, while a highly reflective stainless steel [grade 430 bright annealed (BA)] sheet was used for the CPC as these materials are commercially available in South Africa, robust, corrosion resistant and cost-effective. Moreover, the lower the wavelength of an electromagnetic wave [such as ultra-violet (UV) light], the more energy is emitted [National Aeronautics and Space Administration (NASA), 2017], which implies that lower wavelengths exhibit higher microbial inactivation efficiencies (Beck et al. 2017). Borosilicate glass was selected for the reactor material as results have shown that this material exhibits the best UV transmittance (optical lower limit cut off at ~ 273 nm), which implies that higher microbial inactivation efficiencies should be obtained (as it transmits UV-A and UV-B wavelengths) in comparison to other materials such as poly(methyl methacrylate) (PMMA) and polyethylene terephthalate (PET).

The cost per SODIS-CPC system was calculated as R 6 451.18 (**Appendix A**). As indicated in **Appendix A**, assuming the operational sustainability of a system is 10 years, 1 L of SODIS treated water would cost approximately R 0.17 using the SODIS-CPC system designed in this study.

Moreover, once the system is installed on-site, minimal operational costs are involved and the price per litre of treated water would remain the same. While water treated by this system is estimated to be more costly than water treated in a standard PET bottle (R 0.014 L⁻¹) as reported by Sobsey et al. (2008), at least six PET bottles are required to treat 10.6 L of water. Furthermore, research has indicated that the ageing of PET bottles leads to a reduction in UV transmittance, resulting in a decrease in microbial inactivation efficiency photoproducts (Wegelin et al. 2001). Thus, PET bottles have a shorter life span than a borosilicate glass reactor and have to be frequently replaced. Furthermore, since the frame and CPC are both constructed from stainless steel, the system is robust and limited maintenance is required. However, depending on the location of the system, dust and debris may need to be removed (washed off) from the CPC to maintain optimum reflective capabilities. The extension poles will also have to be adjusted as the seasons change to ensure that the reactor is perpendicular to the sun. In line with the aims of the current study, a (i) cost-effective, (ii) robust and (iii) low maintenance SODIS-CPC system was thus designed. Optimisation of the construction of the system may include the use of multiple reactors in series per system (as this system contains only one reactor – treatment volume of 10.6 L) in order to increase the treatment volume to adhere to a household potable water demand of 25 L of water per person per day [United Nations (UN), 2010].

After construction was completed (**section 2.2.2, Chapter two**), two SODIS-CPC systems were connected to two separate 2 000 L rainwater harvesting tanks installed on Welgevallen Experimental farm, which were mounted onto metal stands to ensure the passive flow of harvested rainwater from the tanks into the SODIS-CPC systems. One rainwater harvesting tank was utilised without pre-treatment (Tank 1), while the second SODIS system was installed next to a rainwater harvesting tank connected to a first flush diverter [Tank 2 (FF)]. In order to ensure that the system exhibits an increased treatment efficiency, various physicochemical properties as well as the chemical and microbial quality of the untreated and SODIS-CPC treated rainwater were investigated. Results obtained for the turbidity analysis indicated that there was no significant difference ($p < 0.05$) in turbidity between samples collected from Tank 1 (no first flush diverter) and Tank 2 (FF) where pre-filtration was employed. Similarly, no significant differences ($p < 0.05$) were recorded between SODIS-CPC-1 and SODIS-CPC-2 (FF) as well as Tank 1 versus SODIS-CPC-1 and Tank 2 (FF) versus SODIS-CPC-2 (FF). Turbidity levels of roof-harvested rainwater recorded in the current study were within the aesthetic turbidity value [≤ 5 Nephelometric Turbidity Units (NTU)] stipulated by South African National Standards (SANS) 241 [South African Bureau of Standards (SABS), 2015] for drinking water. Research has indicated that an operational water turbidity value of ≤ 1 is required, as higher turbidity values may negatively affect the efficiency of SODIS treatment and filtration systems (clogging of filters) (SABS, 2015). Contradictory evidence was however, presented by Meera and Ahammed (2008) who reported that increased SODIS inactivation efficiencies were obtained for water samples where moderate turbidity (≤ 38 NTU) values were recorded. Research conducted by Ubomba-Jaswa et al. (2010) validated this theory as these authors stated that

suspended particles present in water samples with higher turbidity, scatters and absorbs light (enhanced transmission of UV radiation in a water medium), resulting in an increased water radiative emissivity which effectively leads to higher water temperatures and an increased disinfection efficiency. In principle, the authors hypothesised that for SODIS treatment, increased turbidity was directly proportional to increased temperature. This theory could possibly elucidate the increased temperatures (45 °C to 59 °C) recorded in samples collected from the SODIS-CPC-1 system as, while not significant, higher NTU values were generally recorded in these samples. In comparison, lower temperatures (39 °C to 53 °C) and NTU values were generally recorded in the pre-filtered samples collected from the SODIS-CPC-2 (FF) system. However, it is typically accepted that water with increased turbidity does not adhere to the stipulated physicochemical parameters of drinking water (SABS, 2015). Moreover, it is assumed that these water sources also contain higher levels of chemical and microbial contaminants.

Chemical analysis revealed that all cation and anion concentrations of untreated and SODIS-CPC treated rainwater samples were within the respective drinking water guidelines [Department of Water Affairs and Forestry (DWAF), 1996; National Health and Medical Research Council & Natural Resource Management Ministerial Council (NHMRC & NRMMC, 2011; SABS, 2015; World Health Organisation (WHO), 2011]. Based on the results obtained it was further hypothesised that the levels of chemical pollutants were reduced using the pre-filtration first flush diverter as the anion (except for F and NO₂) and cation (except for Al, K, Mg, Na and P) concentrations were significantly lower in Tank 2 (FF) and SODIS-CPC-2 (FF) rainwater samples in comparison to Tank 1 and SODIS-CPC-1 rainwater samples. It is further concluded that no leaching of metal ions occurred from the SODIS-CPC system components as there was no significant differences ($p > 0.05$) (except for cation: Zn, Hg and Pb and anion: F and NO₂) of metal cation and anion concentrations observed when comparing untreated and treated rainwater samples.

For the microbiological analysis, results indicated that *Escherichia coli* (*E. coli*) counts were effectively reduced to below the detection limit after SODIS treatment in both SODIS-CPC-1 and SODIS-CPC-2 (FF) rainwater samples, while the HPC counts were reduced after SODIS treatment to within the drinking water guideline of 1×10^4 CFU/100 mL (DWAF, 1996). Total coliforms were also reduced to below the detection limit during sampling sessions 1 to 4 for both SODIS-CPC systems, however total coliforms were still detected after SODIS treatment during sampling sessions 5 to 7. The ambient solar radiation was the lowest during these three sampling sessions (5 to 7) with temperatures below 52 °C recorded in the treated samples, suggesting that a UV-A irradiation above 20.3 W/m² and a temperature higher than 52 °C is required to inactivate total coliforms. These results correspond to a study conducted by Strauss et al. (2016) where rainwater in PET bottles were exposed to UV for six and eight hrs using a solar cooker and it was observed that after eight hrs of SODIS treatment all indicator organisms (*E. coli* and HPC) were reduced to below the detection limit (< 1 CFU/100 mL) at temperatures > 71 °C. In addition, Nalwanga et al. (2016) investigated the

microbiological quality of harvested rainwater before and after SODIS treatment in a rural area in Southern Uganda. The research group then found that there was microbial contamination (presence of *E. coli* and enterococci) in the majority of the rainwater samples, however after SODIS treatment (six hrs under sunny conditions and two days under cloudy conditions), the *E. coli* and enterococci counts decreased significantly (Nalwanga et al. 2016).

Ethidium monoazide bromide quantitative polymerase chain reaction (EMA-qPCR) analysis then indicated that a reduction in viable *Legionella* and *Pseudomonas* copy numbers was observed after SODIS treatment. Moreover, the overall mean concentration of viable *Legionella* and *Pseudomonas* copy numbers were lower in Tank 2 (FF) in comparison to the Tank 1 rainwater samples, indicating that pre-filtration using a first flush diverter reduces the microbial load including *Legionella* and *Pseudomonas* spp. copy numbers. These findings correlate to research where it was indicated that the greatest load of bacterial contamination is present in the first flush and subsequently the ensuing rainwater is less polluted (Fewtrell & Kay, 2007). Interestingly, based on the results obtained in the current study, solar exposure or ambient UV radiation yielded an increased disinfection efficiency in viable *Legionella* copy numbers. This correlates to a study conducted by Muraca et al. (1987) where it was indicated that *Legionella* was reduced from 10^7 CFU/mL to 10^2 CFU/mL within 20 minutes when irradiated with UV light. In contrast, in the current study results obtained for *Pseudomonas* spp. EMA-qPCR analysis indicated that *Pseudomonas* are susceptible to high temperatures as well as to the effect of high solar radiation. Amin et al. 2014 recorded similar results where the research group used SODIS (eight to nine hrs) for the inactivation of *Pseudomonas aeruginosa* in PET bottles. Culture based analysis indicated that *P. aeruginosa* were only completely inactivated under sunny conditions where a water temperature > 55 °C and solar irradiance levels above 775 W/m² was reached (Amin et al. 2014).

Future research should thus focus on quantitative microbial risk assessment studies to determine the health risks associated with the consumption of harvested rainwater where viable opportunistic pathogens such as *Legionella* and *Pseudomonas* are detected. Research should also focus on a combination of treatment technologies such as the addition of a photocatalytic material (such as titanium dioxide) to the SODIS system to possibly enhance the microbial inactivation efficiency (McCullagh et al. 2007). Titanium dioxide is considered the most suitable photocatalyst due to the lack of toxicity and chemical and photochemical stability (Byrne et al. 2010). The application of biological control agents (viruses, phage particles and phage proteins) should also be investigated in combination with SODIS to effectively inactivate opportunistic pathogens, which persist after heat and UV treatment. Lammertyn et al. (2008) showed that bacteriophages are able to infect members of *Legionella*, while phage particles and proteins have previously been used in human medicine as well as in areas of food safety and wastewater treatment (Petty et al. 2007). Although the SODIS-CPC systems used in the current study improved the quality of harvested rainwater, based on the total coliform counts and the presence of viable opportunistic pathogens (*Legionella* and

Pseudomonas spp.) after SODIS treatment, it is recommended that the treated rainwater is utilised as a supplementary water source for domestic purposes. Moreover, it is recommended that a first flush diverter is installed as part of a standard rainwater harvesting system (Fewtrell & Kay, 2007). The first flush diverter should however, be cleaned and drained regularly to prevent the breeding of mosquitoes and other insects and remove microbial and chemical contaminants present in the first flush downpipe.

Numerous studies have indicated that a poor correlation exists between indicator organisms and opportunistic pathogenic genera in water sources (Harwood et al. 2005; Savichtcheva & Okabe, 2006; Wilkes et al. 2009; Ahmed et al. 2010; Dobrowsky et al. 2014b). This was verified in the current study where the *E. coli* and total coliform (sampling sessions one to four) counts were reduced to below the detection limit after SODIS treatment (for eight hrs) (< 1 CFU/100 mL) and HPC were within the drinking water guideline of 1×10^4 CFU/100 mL (DWAF, 1996), while *Legionella* and *Pseudomonas* spp. were still viable at a mean concentration of 1.43×10^6 and 1.15×10^5 gene copies/mL, respectively. Studies usually also only focus on identifying groups of organisms and subsequently, limited information is available on the abundance and diversity of the overall viable bacterial community present in roof-harvested rainwater pre- and post-treatment. The primary aim of **Chapter three** was thus (i) to determine the diversity and abundance of the viable bacterial community present in the untreated rainwater (Tank 1) and the SODIS treated (for eight hrs) rainwater (SODIS-CPC-1), using the SODIS-CPC system which was constructed in **Chapter two**, by performing Illumina next generation sequencing coupled with EMA, (ii) to determine whether the viable bacterial community differs after SODIS treatment (Tank 1 vs. SODIS-CPC-1) and lastly, (iii) to detect and identify the primary viable pathogenic and opportunistic pathogenic genera persisting after SODIS treatment in roof-harvested rainwater.

Using the SILVA and Ribosomal Database Project (RDP) taxonomic classification databases (Liu et al. 2008), sequences were clustered into 37 763 operational taxonomic units (OTUs) to determine the diversity and abundance of the viable bacterial community. The sequences obtained for the Tank 1 rainwater samples could be grouped into 133 unique families which then decreased to 77 unique families in the SODIS treated rainwater samples. Similarly, 14 unique genera (which had a relative abundance of > 1 %) were observed in the Tank 1 rainwater samples, which decreased to eight unique genera (which had a relative abundance of > 1 %) in the SODIS-CPC-1 rainwater samples. Although the most abundant taxa between the Tank 1 and SODIS-CPC-1 rainwater samples were the same, for example Nocardiaceae was the most abundant family in the Tank 1 as well as in the SODIS-CPC-1 rainwater samples, there was a significant shift observed in the overall viable bacterial community persisting after SODIS treatment as the relative abundance of the taxa between Tank 1 and SODIS-CPC-1 rainwater samples differed significantly. For example, Pseudomonadaceae (second highest relative abundance) and Sphingomonadaceae (third highest relative abundance) accounted for 8.9 % and 6.0 % of the total families detected in Tank 1 rainwater

samples, respectively, however these two families only accounted for 0.01 % and 0.09 % of the total families detected in the SODIS-CPC-1 rainwater samples, respectively. Micrococcaceae (31.7 %) and Oxalobacteraceae (5.0 %) were then the second and third most relative abundant families, respectively, detected in the SODIS-CPC-1 rainwater samples. Thus, SODIS treatment of eight hrs using the SODIS-CPC system designed in the current study had a significant effect on the viable bacterial community.

These findings were further supported by the α -diversity and β -diversity indices. For the α -diversity indices, there was a significant decrease ($p = 0.0033$) in the species richness in the SODIS-CPC-1 rainwater samples after SODIS treatment in comparison to the Tank 1 rainwater samples. In addition, the Shannon diversity index of the Tank 1 rainwater samples significantly decreased ($p = 0.0107$) after SODIS treatment and indicated that there was an intensive disturbance in the viable community structure. A similar result was recorded by Zhang et al. (2017), where a decrease in the Shannon diversity index of a bacterial community in groundwater (using Illumina next generation sequencing) was reported after a water treatment process. It was also further indicated that the lowered Shannon diversity index obtained for the SODIS-CPC-1 rainwater samples was due to a lower species richness and that a smaller group of bacteria, specifically *Rhodococcus* and *Anthrobacter* spp., dominated the SODIS-CPC-1 rainwater samples and survived SODIS treatment. When investigating the β -diversity index, two separate clusters were observed indicating a community shift. Thus, SODIS treatment had a significant effect on the β -diversity and significantly ($p < 0.05$) altered the viable bacterial community.

Rainwater harvesting has been employed in numerous countries, including South Africa, as an alternative water source (Malema et al. 2016), however studies have detected the presence of numerous pathogens and opportunistic pathogens such as *Mycobacterium* (Albrechtsen, 2002), *Aeromonas* and *Yersinia* (Dobrowsky et al. 2014b), *Legionella* (Ahmed et al. 2010) and *Pseudomonas* (Strauss et al. 2016), amongst others, in stored rainwater. This correlates to results obtained in the current study for untreated roof-harvested rainwater, however, signatures of potentially pathogenic genera were still observed after SODIS treatment. For example, *Pseudomonas* and *Clostridium sensu stricto* were detected in all the SODIS-CPC-1 samples while *Mycobacterium*, *Legionella*, *Aeromonas* and *Yersinia*, amongst others, were detected in at least 60 % ($n = 5$) of the SODIS-CPC-1 rainwater samples. The presence of these potential pathogenic organisms can be linked to various contaminants (Ahmed et al. 2012; Sánchez et al. 2015; Waso et al. 2016). For example, possible sources of potential pathogenic organisms such as *Pseudomonas* (Ahmed et al. 2017) and *Campylobacter* (Abdollahpour et al. 2015) can be attributed to bird faecal matter on the catchment area (roof), while the presence of *Acinetobacter* (Doughari et al. 2011) and *Brevibacterium* (Fischer, 2017) can be attributed to the dispersion of soil which occurs during anthropogenic activity (such as vehicles driving on the gravel roads which pass the catchment area). In addition, the presence of *Clostridium* (Bagge et al. 2010) and *Mycobacterium* (Wells et al. 2006)

may be ascribed to cattle faecal matter, as a result of anthropogenic activity and wind dispersion. Following a rain event, all these contaminants are washed into the collection tank which subsequently compromises the microbial quality of rainwater. Resultantly, waterborne disease outbreaks have been associated with the consumption of harvested rainwater (Simmons et al. 2001; 2008; Franklin et al. 2009).

While the combination of EMA with Illumina next generation sequencing was a proof of concept study to investigate the viable whole community profile of rainwater pre-and post-treatment, it is highly recommended that molecular-based detection methods rather than culture based assays are routinely employed for the assessment of microbial water quality as it provides a complete analysis of the entire bacterial community including the viable but non-culturable portion of the population. In addition, molecular-based assays are considerably more sensitive and yield a higher output of data. While these assays are more costly, next generation sequencing tools such as Illumina have also become the techniques of choice to study microbial community biodiversity and structure (Chidamba & Korsten, 2015; Postma et al. 2016; Ahmed et al. 2017). A general limitation of Illumina next generation sequencing however, is that it fails to accurately identify pathogenic organisms to species level based on the 16S rRNA prokaryote gene, as a greater sequencing depth is required. In addition, as Illumina next generation sequencing only measures the relative abundance of OTUs, it is suggested by Ishii et al. (2013) and Ahmed et al. (2017) that Illumina next generation sequencing must be used as a broad screening tool for pathogenic genera after which qPCR methods should be employed for the accurate detection and quantification of a specific pathogenic species.

4.2. References

- Abbasi, T. & Abbasi, S.A., 2011. Sources of pollution in rooftop rainwater harvesting systems and their control. *Critical Reviews in Environmental Science and Technology*. 41(23), 2097–2167.
- Abdollahpour, N., Zendeabad, B., Alipour, A., Khayat-zadeh, J., 2015. Wild-bird feces as a source of *Campylobacter jejuni* in children's playgrounds in Iran. *Food Control*. 50, 378–381.
- Ahmed, W., Goonetilleke, A., Gardner, T., 2010. Implications of faecal indicator bacteria for the microbiological assessment of roof-harvested rainwater quality in Southeast Queensland, Australia. *Canadian Journal of Microbiology*. 56(6), 471–479.
- Ahmed, W., Hodgers, L., Sidhu, J.P.S., Toze, S., 2012. Fecal indicators and zoonotic pathogens in household drinking water taps fed from rainwater tanks in Southeast Queensland, Australia. *Applied and Environmental Microbiology*. 78(1), 219–226.
- Ahmed, W., Staley, C., Hamilton, K.A., Beale, D.J., Sadowsky, M.J., Toze, S., Haas, C.N., 2017. Amplicon-based taxonomic characterization of bacteria in urban and peri-urban roof-harvested rainwater stored in tanks. *Science of the Total Environment*. 576, 326-334.

- Albrechtsen, H.J., 2002. Microbiological investigations of rainwater and greywater collected for toilet flushing. *Water Science and Technology*. 46(6–7), 311–316.
- Amin, M.T., Han, M.Y., 2009. Roof-harvested rainwater for potable purposes: application of solar collector disinfection (SOCO-DIS). *Water Research*. 43(20), 5225–5235.
- Amin, M.T., Nawaz, M., Amin, M.N., Han, M., 2014. Solar disinfection of *Pseudomonas aeruginosa* in harvested rainwater: a step towards potability of rainwater. *PloS One*. 9(3), 1–10.
- Bagge, E., Persson, M., Johansson, K.E., 2010. Diversity of spore-forming bacteria in cattle manure, slaughterhouse waste and samples from biogas plants. *Journal of Applied Microbiology*. 109(5), 1549-1565
- Beck, S.E., Ryu, H., Boczek, L.A., Cashdollar, J.L., Jeanis, K.M., Rosenblum, J.S., Lawal, O.R., Linden, K.G., 2017. Evaluating UV-C LED disinfection performance and investigating potential dual-wavelength synergy. *Water Research*. 109, 207-216.
- Byrne, J.A., Fernandez-Ibañez, P.A., Dunlop, P.S., Alrousan, D.M., Hamilton, J.W., Abdel-Mottaleb, M.S., 2010. Photocatalytic enhancement for solar disinfection of water: a review. *International Journal of Photoenergy*. 2011, 798051.
- Chidamba, L., Korsten, L., 2015. Pyrosequencing analysis of roof-harvested rainwater and river water used for domestic purposes in Luthengele village in the Eastern Cape province of South Africa. *Environmental Monitoring and Assessment*. 187(41), <https://doi.org/10.1007/s10661-014-4237-0>.
- De Kwaadsteniet, M., Dobrowsky, P.H., Van Deventer, A., Khan, W., Cloete, T.E., 2013. Domestic rainwater harvesting: microbial and chemical water quality and point-of-use treatment systems. *Water, Air, and Soil Pollution*. 224(7), 1-19.
- Department of Water Affairs and Forestry (DWAF), 1996. *South African Water Quality Guidelines 2nd Edition, Volume 1: Domestic Water Use*. Pretoria: CSIR Environmental Services.
- Dobrowsky, P.H., Mannel, D., De Kwaadsteniet, M., Prozesky, H., Khan, W., Cloete, T.E., 2014a. Quality assessment and primary uses of harvested rainwater in Kleinmond, South Africa. *Water SA*. 40(3), 401–406.
- Dobrowsky, P.H., De Kwaadsteniet, M., Cloete, T.E., Khan, W., 2014b. Distribution of indigenous bacterial pathogens and potential pathogens associated with roof-harvested rainwater. *Applied and Environmental Microbiology*. 80(7), 2307–2316.
- Doughari, H.J., Ndakidemi, P.A., Human, I.S., Benade, S., 2011. The ecology, biology and pathogenesis of *Acinetobacter* spp.: an overview. *Microbes and Environments*. 26(2), 101-112.

- Fewtrell, L., Kay, D., 2007. Microbial quality of rainwater supplies in developed countries: a review. *Urban Water Journal*, 4(4), 253-260.
- Fischer, J., 2017. Habitat. *Brevibacterium linens*. Available: http://bioweb.uwlax.edu/bio203/s2012/fischer_jaco/habitat.htm (Accessed 28.11.17).
- Franklin, L.J., Fieding, J.E., Gregory, J., Gullan, L., Lightfoot, D., Poznanski, S.Y., Vally, H., 2009. An outbreak of *Salmonella typhimurium* 9 at a school camp linked to contamination of rainwater tanks. *Epidemiology and Infection*. 137(3), 434-440.
- Harwood, V.J., Levine, A.D., Scott, T.M., Chivukula, V., Lukasik, J., Farrah, S.R., Rose, J.B., 2005. Validity of the indicator organism paradigm for pathogen reduction in reclaimed water and public health protection. *Applied and Environmental Microbiology*. 71(6), 3163–70.
- Helmreich, B., Horn, H., 2009. Opportunities in rainwater harvesting. *Desalination*. 248(1–3), 118–124.
- Huston, R., Chan, Y.C., Chapman, H., Gardner, T., Shaw, G., 2012. Source apportionment of heavy metals and ionic contaminants in rainwater tanks in a subtropical urban area in Australia. *Water Research*. 46(4), 1121–1132.
- Ishii, S., Segawa, T., Okabe, S. 2013. Simultaneous quantification of multiple food and waterborne pathogens by use of microfluidic quantitative PCR. *Applied and Environmental Microbiology*. 79(9), 2891-2898.
- Lammertyn, E., Voorde, J.V., Meyen, E., Maes, L., Mast, J., Anné, J., 2008. Evidence for the presence of *Legionella* bacteriophages in environmental water samples. *Microbial Ecology*. 56(1), 191-197.
- Liu, Z., DeSantis, T.Z., Andersen, G.L., Knight, R., 2008. Accurate taxonomy assignments from 16S rRNA sequences produced by highly parallel pyrosequencers. *Nucleic Acids Research*. 36, e120.
- Malema, S., Abia, L.K.A., Mwenge Kahinda, J., Ubomba-Jaswa, E., 2016. Gaining a better understanding of the factors that influence the quality of harvested rainwater in South Africa – a review. WISA 2016 Biennial conference. 16 - 19 May 2016. Durban, South Africa.
- McCullagh, C., Robertson, J.M., Bahnemann, D.W., Robertson, P.K., 2007. The application of TiO₂ photocatalysis for disinfection of water contaminated with pathogenic micro-organisms: a review. *Research on Chemical Intermediates*. 33(3), 359-375.
- Meera, V., Ahammed, M.M., 2008. Solar disinfection for household treatment of roof-harvested rainwater. *Water Science & Technology - Water Supply*. 8, 153–160.

Muraca, P., Stout, J.E., Yu, V.L., 1987. Comparative assessment of chlorine, heat, ozone, and UV light for killing *Legionella pneumophila* within a model plumbing system. *Applied and Environmental Microbiology*. 53(2), 447-453.

Nalwanga, R., Muyanja, C.K., McGuigan, K.G., Quilty, B., 2016. A study of the bacteriological quality of roof-harvested rainwater and an evaluation of SODIS as a suitable treatment technology in rural sub-Saharan Africa. *Journal of Environmental Chemical Engineering*. In Press. <http://dx.doi.org/10.1016/j.jece.2016.12.008>

National Aeronautics and Space Administration (NASA), 2017. Frequency, wavelength & wnergy Activity. https://heasarc.gsfc.nasa.gov/docs/xte/learning_center/universe/ener_act.html (Accessed 30.07.17).

National Health and Medical Research Council & Natural Resource Management Ministerial Council (NHMRC & NRMCMC), 2011. Australian Drinking water guidelines 6, volume 1. National Water Quality Management Strategy. National Health and Medical Research Council and National Resource Management Ministerial Council, Commonwealth of Australia, Canberra.

Petty, N.K., Evans, T.J., Fineran, P.C., Salmond, G.P., 2007. Biotechnological exploitation of bacteriophage research. *Trends in Biotechnology*. 25(1), 7-15.

Postma, A., Slabbert, E., Postma, F., Jacobs, K., 2016. Soil bacterial communities associated with natural and commercial *Cyclopi*a spp. *FEMS Microbiology Ecology*. 92(3), 1-10.

Sánchez, A.S., Cohim, E., Kalid, R.A., 2015. A review on physicochemical and microbiological contamination of roof-harvested rainwater in urban areas. *Sustainability of Water Quality and Ecology*. 6, 119–137.

Savichtcheva, O., Okabe S., 2006. Alternative indicators of faecal pollution: relations with pathogens and conventional indicators, current methodologies for direct pathogen monitoring and future application perspectives. *Water Research*. 40(13), 2463–76.

Simmons, G., Hope, V., Lewis, G., Whitmore, J., Gao, W., 2001. Contamination of potable roof-collected rainwater in Auckland, New Zealand. *Water Research*. 35(6), 1518–1524.

Simmons, G., Jury, S., Thornley, C., Harte, D., Mohiuddin, J., Taylor, M., 2008. A Legionnaires' disease outbreak: a water blaster and roof-collected rainwater systems. *Water Research*. 42(6–7), 1449–1458.

Sobsey, M.D., Stauber, C.E., Casanova, L.M., Brown, J.M., Elliott, M.A., 2008. Point of use household drinking water filtration: a practical, effective solution for providing sustained access to

safe drinking water in the developing world. *Environmental Science & Technology*. 42(12), 4261-4267.

South African Bureau of Standards (SABS), 2015. South African National Standards (SANS) 241, Drinking water. Part 1: Microbiological, physical, aesthetic and chemical determinants. 2nd edition. Annexure 1. South African Bureau of Standards, Pretoria, South Africa. ISBN 978-0-626-29841-8.

Strauss, A., Dobrowsky, P.H., Ndlovu, T., Reyneke, B., Khan, W., 2016. Comparative analysis of solar pasteurization versus solar disinfection for the treatment of harvested rainwater. *BMC Microbiology*. 16(1), 289.

Ubomba-Jaswa, E., Fernández-Ibáñez, P., Navntoft, C., Polo-López, M.I., McGuigan, K.G., 2010. Investigating the microbial inactivation efficiency of a 25 L batch solar disinfection (SODIS) reactor enhanced with a compound parabolic collector (CPC) for household use. *Journal of Chemical Technology and Biotechnology*. 85(8), 1028-1037.

United Nations (UN), 2010. The human right to water and sanitation. Available: http://www.un.org/waterforlifedecade/pdf/human_right_to_water_and_sanitation_media_brief.pdf (Accessed 13.07.17).

Waso, M., Ndlovu, T., Dobrowsky, P.H., Khan, S., Khan, W., 2016. Presence of microbial and chemical source tracking markers in roof-harvested rainwater and catchment systems for the detection of faecal contamination. *Environmental Science and Pollution Research*. 23(17), 16987-17001.

Wegelin, M., Canonica, S., Alder, C., Marazuela, D., Suter, M.F., Bucheli, T.D., Haefliger, O.P., Zenobi, R., McGuigan, K.G., Kelly, M.T., Ibrahim, P., 2001. Does sunlight change the material and content of polyethylene terephthalate (PET) bottles? *Journal of Water Supply: Research and Technology-AQUA*, 50(3), 125-135.

Wells, S.J., Collins, M.T., Faaberg, K.S., Wees, C., Tavorpanich, S., Petrini, K.R., Collins, J.E., Cernicchiaro, N., Whitlock, R.H., 2006. Evaluation of a rapid fecal PCR test for detection of *Mycobacterium avium* subsp. *paratuberculosis* in dairy cattle. *Clinical and Vaccine Immunology*. 13(10), 1125-1130.

Wilkes, G., Edge, T., Gannon, V., Jokinen, C., Lyautey, E., Medeiros, D., Neumann, N., Ruecker, N., Topp, E., Lapen, D.R., 2009. Seasonal relationships among indicator bacteria, pathogenic bacteria, *Cryptosporidium* oocysts, *Giardia* cysts, and hydrological indices for surface waters within an agricultural landscape. *Water Research*. 43(8), 2209–23.

World Health Organisation (WHO), 2011. Guidelines for drinking-water quality. 4th edition. World Health Organisation. Geneva, Switzerland, WHO Press. ISBN, 978-92-4- 154815-1.

Yaziz, M.I., Gunting, H., Sapari, N., Ghazali, A.W., 1989. Variations in rainwater quality from roof catchments. *Water Research*. 23(6), 761-765.

Zhang, Y., Oh, S., Liu, W.T., 2017. Impact of drinking water treatment and distribution on the microbiome continuum: an ecological disturbance's perspective. *Environmental Microbiology*. 19(8), 3163-3174.

Appendix A:

**Additional information on the
SODIS-CPC system and first flush diverter**

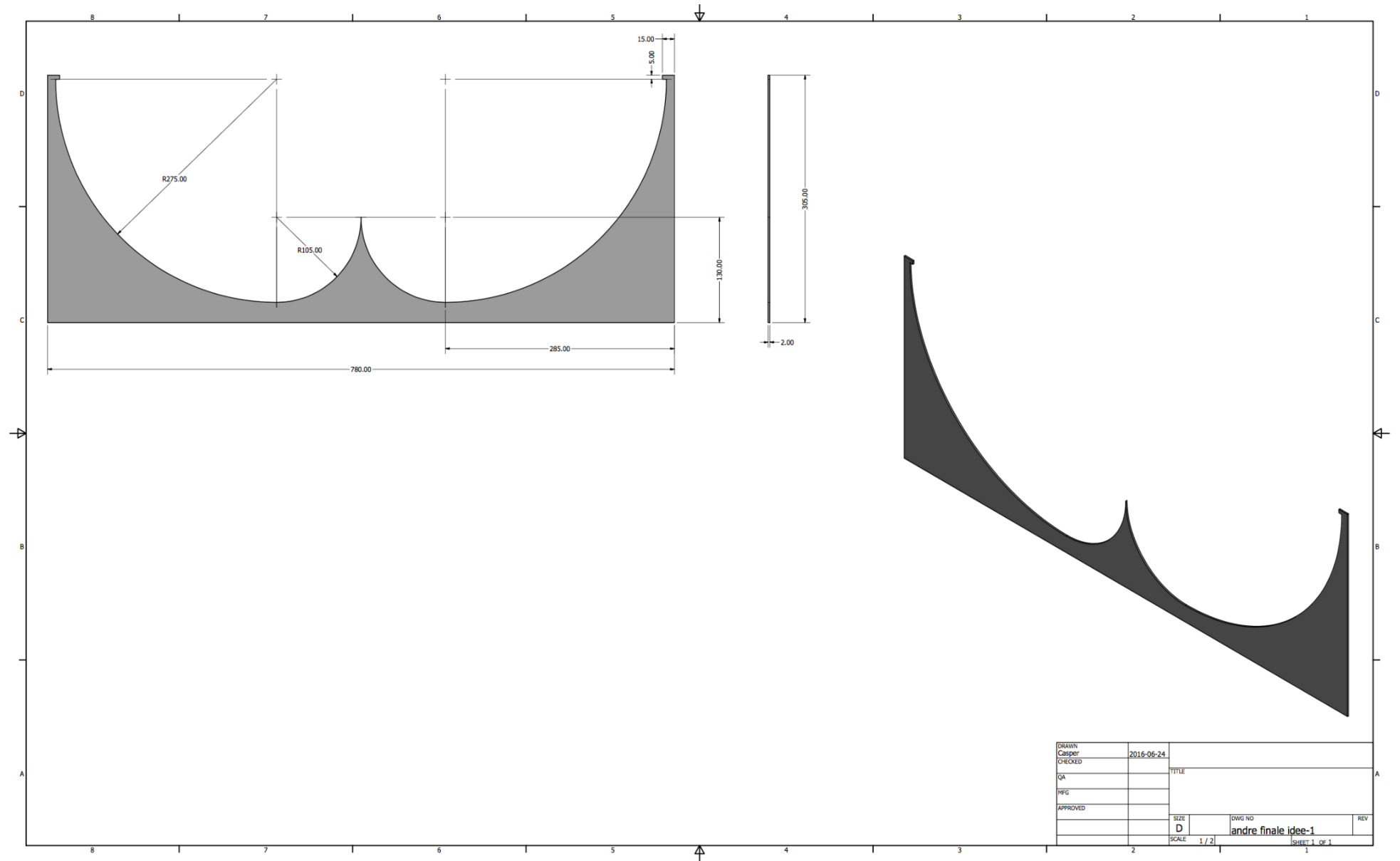


Figure A1: Schematic diagram of the arch profile in a 2-D and 3-D figure.

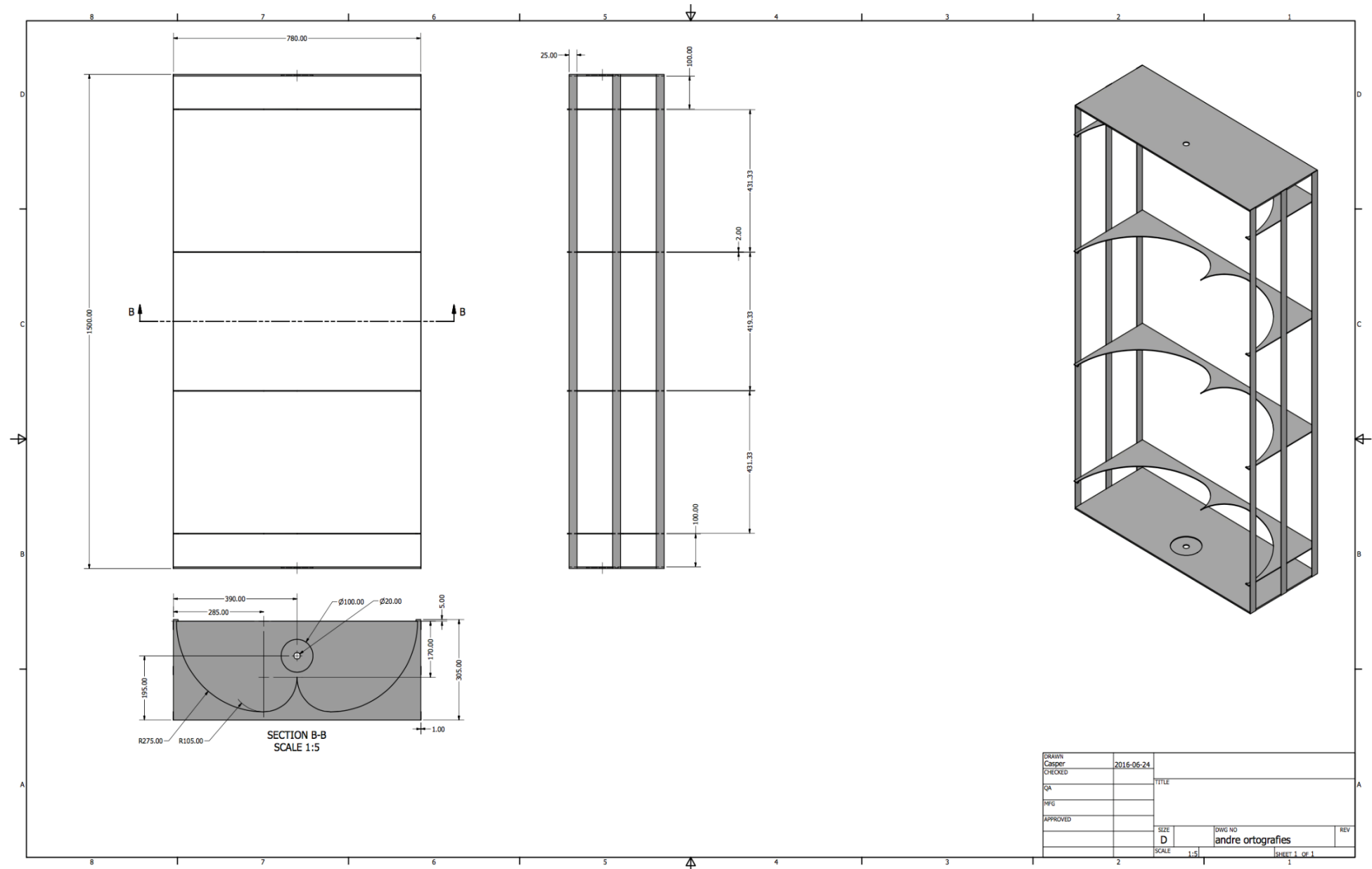


Figure A2: Schematic diagram of the SODIS-CPC frame from the top and side view, as well as in a 3-D figure.

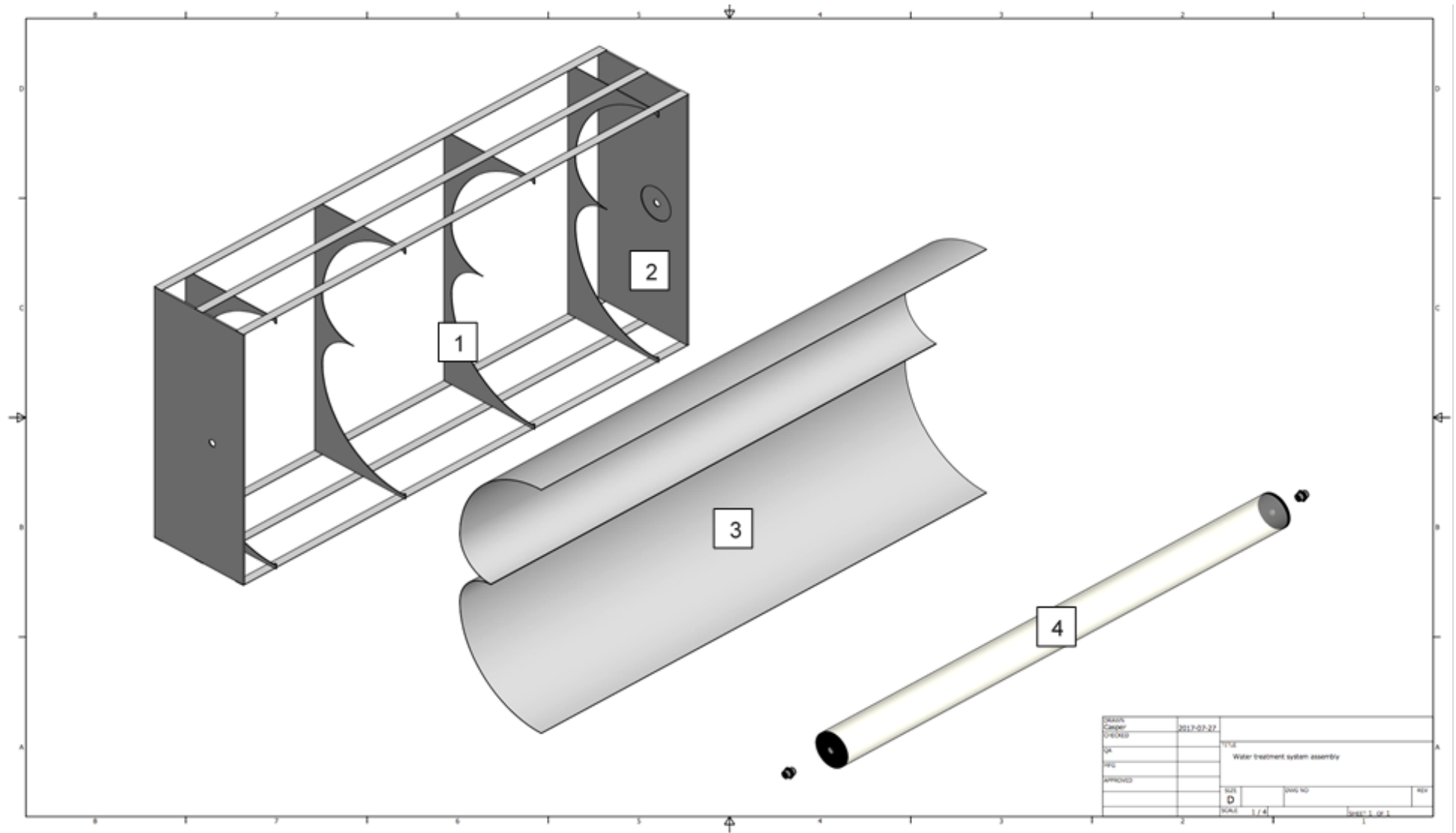


Figure A3: The four (1) arch profiles aligned with the two (2) end plates to form the CPC frame. (3) A stainless steel [grade 430 bright annealed (BA)] sheet which was bent and superimposed onto the end plates to fit the curves of the arch profiles. (4) The borosilicate glass tube was then positioned in the centre of the CPC, where the two arches connect, axially along the focus of the CPC reflector.

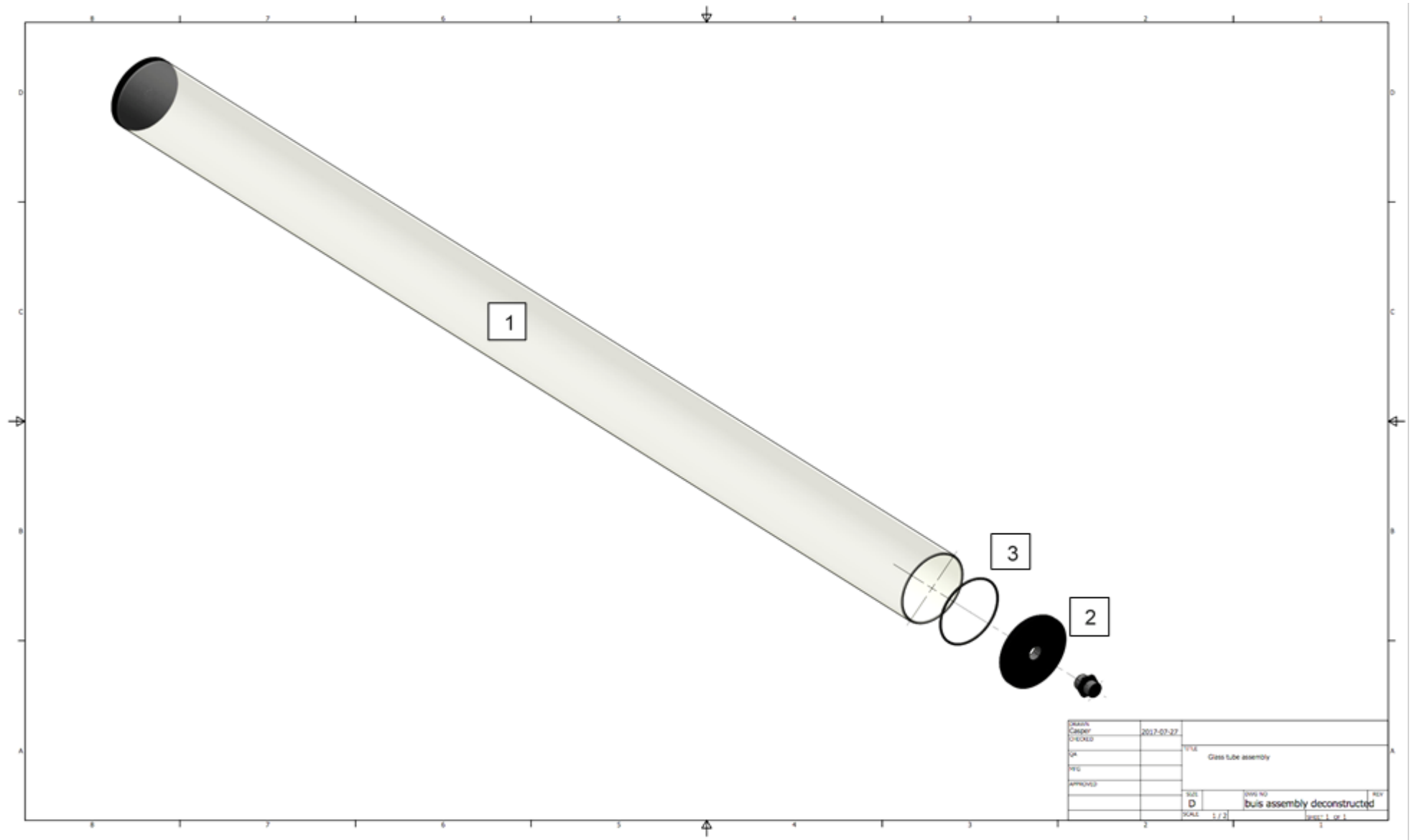


Figure A4: (1) PVC socket which screw into the (2) polymethyl methacrylate plug containing a (3) rubber O-ring. The plug as a whole then seals the ends of the (4) borosilicate glass reactor



Figure A5: First flush diverter which is connected to the conveyance system between the catchment area and the rainwater harvesting tank (Tank 2).

Table A1: A total cost analysis for the construction of one SODIS-CPC system.

Item	Quantity	Price
Frame (1500 mm x 25 mm x 2 mm flat bars, stainless steel grade 304)	1	R 1 109.42
Arch profile (305 mm x 780 mm x 2 mm, stainless steel grade 304)	4	R 840.36
End plates (305 mm x 780 mm x 2 mm, stainless steel grade 304)	2	R 928.94
Highly reflective 0.5 mm thick stainless steel [grade 430 bright annealed (BA)] sheet (596 mm x 1 500mm)	2	R 357.60
Roll of highly reflective sheet	2	R 349.41
Borosilicate glass cylinder (2.5 mm x 1500 mm)	1	R 1 478.58
Polymethyl methacrylate plug	2	R 25.00
O-ring	2	R 2.00
PVC socket screw	2	R 2.40
PVC ball valve tap	2	R 36.80
Polypropylene sheet (1500 mm x 780 mm)	1	R 120.00
Extension pole	4	R 408.42
Total (excluding VAT)		R 5 658.93
VAT		R 792.25
Total (including VAT)		R 6 451.18

Table A2: Nomenclature of the 28 samples collected during the study period.

Sampling event	Tank 1	SODIS-CPC-1	Tank 2 (FF)	SODIS-CPC-2 (FF)
1	Tank 1 #1	SODIS-CPC-1 #1	Tank 2 (FF) #1	SODIS-CPC-2 (FF) #1
2	Tank 1 #2	SODIS-CPC-1 #2	Tank 2 (FF) #2	SODIS-CPC-2 (FF) #2
3	Tank 1 #3	SODIS-CPC-1 #3	Tank 2 (FF) #3	SODIS-CPC-2 (FF) #3
4	Tank 1 #4	SODIS-CPC-1 #4	Tank 2 (FF) #4	SODIS-CPC-2 (FF) #4
5	Tank 1 #5	SODIS-CPC-1 #5	Tank 2 (FF) #5	SODIS-CPC-2 (FF) #5
6	Tank 1 #6	SODIS-CPC-1 #6	Tank 2 (FF) #6	SODIS-CPC-2 (FF) #6
7	Tank 1 #7	SODIS-CPC-1 #7	Tank 2 (FF) #7	SODIS-CPC-2 (FF) #7