QUALITY OF HARVESTED RAINWATER and Application of Point of Use Treatment Systems

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Report
to the Water Research Commission

by

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This report emanates from the Water Research Commission project K5/2124, entitled: Point of use disinfections systems designed for domestic rainwater harvesting (DRWH) tanks for improved water quality in rural communities. Also available is *Domestic Rainwater Harvesting: Survey of Perceptions of Users in Kleinmond* (**Report No. 2124/2/14**)

EXECUTIVE SUMMARY

Background

The quality of the essential commodity water is being compromised by contaminants originating from, for example, anthropogenic sources, industrial activities and agriculture. Water scarcity due to severe drought in many regions of the world also represents a significant challenge to the availability of this resource. Domestic rainwater harvesting (DRWH), which involves the collection and storage of water from rooftops and diverse surfaces, is successfully implemented worldwide as a sustainable water supplement. Available literature on the chemical and microbial quality of DRWH, with a particular focus on the sources of microbial pollution and the major pathogens associated with the water source was reviewed. Incidences of disease that have been linked to the consumption and utilisation of harvested rainwater are also discussed. In addition, various procedures and methods used for the disinfection and treatment of harvested rainwater, such as, the implementation of filter systems (activated carbon, slow sand filtration, etc.), heat treatment and chlorination, amongst others, are also presented.

A survey of the quality of water collected in DRWH in the Kleinmond housing scheme

Rainwater samples were collected from domestic rainwater harvesting tanks (DRWH) in a sustainable housing development in Kleinmond, South Africa. Water samples were collected on eight occasions from 29 tanks during the period of March to August 2012. The chemical and microbial parameters were compared to drinking water standards stipulated by the South African, 2005 and Australian Quality Guidelines, 2011. The rainwater quality was within all the chemical standards (cations, anions, metal ions, pH and temperature) analysed for potable water, with the concentration of organic matter (COD) ranging between 4 mg L^{-1} to 9.5 mg L^{-1}. However, the total coliform, *Escherichia coli*, enterococci, heterotrophic bacteria and faecal coliform counts exceeded the stipulated guidelines in numerous rainwater samples. During this study, no *Legionella, Pseudomonas* or *Campylobacter* species were detected through culturing methods. The identification of microorganisms, isolated from the respective culture media, during the sampling period, was determined by amplifying the 16S rRNA gene through PCR and subsequent sequencing. Opportunistic pathogens and human pathogenic species associated with the genera, *Aeromonas, Klebsiella, Enterobacter, Salmonella, Yersinia*, amongst many others, were isolated and identified. Furthermore, of the 92 *E. coli* strains isolated from ten DRWH tanks, 6% were presumptively positively identified as *E. coli* 0157:H7 using 16S rRNA sequencing. The microbial analysis results thus indicate that the harvested rainwater was not fit for potable use without treatment.

Filter assessment – the efficiency of filtration systems in removing chemical and microbial contaminants from rainwater

The aim of this study was to evaluate the efficiency of four household point-of-use treatment systems, namely, activated carbon, PVA nanofibre column, slow sand filtration and an activated carbon/PVA nanofibre column, for the treatment of harvested rainwater. Three polyethylene DRWH tanks (2000 L) were installed at the Welgevallen Experimental farm, Stellenbosch University, South Africa. The various treatment systems were then intermittently connected to the various DRWH tanks during the high rainfall period (June to October 2013). Parameters used to monitor the four filtration systems included, amongst others, metal ion, cation and anion analysis as well as heterotrophic bacteria, *E. coli* and total coliform enumeration. Chemical analyses indicated that while numerous cation and anion concentrations were within drinking water guidelines in the unfiltered and filtered rainwater, the concentrations of isolated cations, such as aluminium, antimony, manganese and iron, increased after filtration through the respective filtration systems. Results for slow sand filtration and activated carbon filters indicated that the biological layer that had developed on the

filtration media had not matured and for this reason chemical and microbial parameters were not reduced to within drinking water guidelines. A polyvinyl (alcohol) (PVA) nanofibre membrane without activated carbon in a column filtration system was analysed and results indicated that this system was also not effective in reducing the microbial numbers to within drinking water guidelines. Lastly, by utilising a PVA nanofibre membrane with activated carbon in a column filtration system, one litre of potable water was produced as all heterotrophic bacteria, *E. coli* and total coliform counts were reduced to zero and were within drinking water guidelines. However, PCR assays indicated that *Klebsiella* spp. *Legionella* spp., *Pseudomonas* spp. and *Yersinia* spp. were not removed by the activated carbon/PVA nanofibre column.

Solar pasteurization system – the efficiency of solar water pasteurization system in disinfecting water from domestic rainwater harvesting tanks

The first phase of the study was aimed at pasteurizing rainwater samples in laboratory scale experiments. Analysis of results showed that the thermal death time of the heterotrophic bacteria in harvested rainwater was 30 minutes at a treatment temperature of 72˚C. In addition, the majority of the phycrophiles and thermophiles isolated from heat treated rainwater samples belonged to the *Bacillaceae* family. The aim of the second phase of the study was then to monitor the efficiency of a solar pasteurization system in reducing the microbiological load in harvested rainwater and to determine the change in chemical contaminant concentrations after rainwater had undergone pasteurization. A solar pasteurization system was connected to one of the rainwater harvesting tanks installed on the Welgevallen Experimental farm and unpasteurized as well as pasteurized rainwater samples were collected for chemical and microbial analysis. The temperature ranges of the pasteurized rainwater samples were 55 to 57˚C, 64 to 66˚C, 72 to 74˚C, 78 to 81˚C and 90 to 91˚C. Indicator bacteria including, heterotrophic bacteria, *E. coli* and total coliforms where reduced to zero at pasteurization temperatures of 72˚C and above. However, with the use of molecular techniques microorganism such as; *Yersinia* spp., *Legionella* spp., and *Pseudomonas* spp., were detected in rainwater samples pasteurized at temperatures greater than 72˚C. All cations were within the drinking water guidelines according, with the exception of iron (55˚C, 65˚C, 78˚C, 91˚C) aluminium (78˚C), lead (55˚C, 65˚C, 91˚C) and nickel (55˚C, 65˚C, 78˚C) which were detected in the pasteurized rainwater samples and were above the respective guidelines. It is hypothesized that these elements could have leached from the stainless steel storage tanks of the pasteurization system and it is therefore recommended that the storage tank of the pasteurization system be manufactured from an alternative material, such as a high grade polymeric material, which is able to withstand the high temperatures yet will not negatively influence the quality of harvested rainwater.

List of products:

Published/accepted articles

1. DE KWAADSTENIET M, DOBROWSKY PH, VAN DEVENTER A, KHAN W and CLOETE TE (2013) Domestic rainwater harvesting: Microbial and chemical water quality and point-of-use treatment systems. Water, Air and Soil Pollution **224 (7)** 1629.
2. DOBROWSKY PH, VAN DEVENTER A, DE KWAADSTENIET M, NDLOVU T, KHAN S, CLOETE TE and KHAN W (2014). Prevalence of virulence genes associated with pathogenic *Escherichia coli* strains isolated from domestically harvested rainwater during low and high rainfall periods. Applied and Environmental Microbiology, **80 (5)** 1633-1638.
3. DOBROWSKY PH, DE KWAADSTENIET M, CLOETE TE and KHAN W (2014). Distribution of indigenous bacterial and potential pathogens associated with roof-harvested rainwater. Applied and Environmental Microbiology, **80 (7)**

Articles under review

1. DOBROWSKY PH, MANNEL D, DE KWAADSTENIET M, PROZESKY H, KHAN W and CLOETE TE (2014). Microbial and chemical quality assessment and perception and primary uses of domestic harvested rainwater in Kleinmond, South Africa. Water SA (3008)

Articles in preparation

1. DOBROWSKY PH, DE KWAADSTENIET M, CLOETE TE and KHAN W (2014). Efficiency of a solar pasteurization system for the treatment of roof harvested rainwater. In preparation to be submitted to Water Research.

International Conferences

1. KHAN W, DOBROWSKY P, DE KWAADSTENIET M and CLOETE TE (2013) Domestic rainwater harvesting: Primary chemical and microbial contaminants. Invited Speaker at the Pan Africa Chemistry Network (PACN) Congress on Sustainability in Africa, 3-5 December 2013 at the African Hall, United Nations in Addis Ababa, Ethiopia.

National Conferences (Oral presentations)

1. DOBROWSKY PH, MANNEL D, PROZESKY H, DE KWAADSTENIET M, KHAN W and CLOETE, TE (2013) Microbial and chemical quality assessment and perception and primary uses of domestic harvested rainwater in Kleinmond, South Africa. 4th Municipal Water Quality Conference, Sun City, South Africa, 7 – 10 July 2013.

National Conferences (Poster presentations)

1. DOBROWSKY PH, DE KWAADSTENIET M, CLOETE W, KHAN W and CLOETE, TE (2013) Optimisation of a nanofibre and activated carbon filtration system for the treatment of harvested rainwater. 18th Biennial Conference of the South African Society of Microbiology, Bela Bela, South Africa, 24-27 November 2013.
2. DOBROWSKY PH, DE KWAADSTENIET M, CLOETE TE and KHAN W (2013) The isolation and identification of pathogenic bacteria from domestic rainwater harvesting tanks. 18th Biennial Conference of the South African Society of Microbiology, Bela Bela, South Africa, 24-27 November 2013.

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CONTENTS

LIST OF FIGURES

LIST OF TABLES

ACRONYMS & ABBREVIATIONS

ADWG	Australian Drinking Water Guidelines
BAC	Biological Activated Carbon
BF	Bucket Filter
BSF	Biosand Filter
CAF	Central Analytical Facility (Stellenbosch University)
CCA	ChromoCult® Coliform Agar
cDNA	Complementary Deoxyribonucleic Acid
CFU	Colony Forming Units
CPA	Chlorophenoxy Acetic Acid
COD	Chemical Oxygen Demand
CSIR	Council for Science and Industrial Research
DNA	Deoxyribunucleic acid
DOC	Dissolved Organic Solid
DRWH	Domestic Rainwater Harvesting
DST	Department of Science and Technology
DWA	Department of Water Affairs
DWAF	Department of Water Affairs and Forestry
EAEC	Enteroaggregative *Escherichia coli*
EAF	EPEC Adherence Factor
EHEC	Enterohaermorrhagic *Escherichia coli*
EIEC	Enteroinvasive *Escherichia coli*
ENT	Enterococci
EPEC	Enteropathogenic *Escherichia coli*
ETEC	Enterotoxigenic *Escherichia coli*
FC	Faecal Coliform
GAC	Granular Activated Carbon
GVPC	Glycine, Vancomycin, Polymyxin B and Cycloheximide
HPC	Heterotrophic Plate Count
HRW	Harvested Rainwater
HUS	Haemolytic-Uraemic Syndrome
ICP-AES	Inductively Coupled Plasma Atomic Emission Spectrometry
IMViC	Indole, Methyl red, Voges-Proskauer and Citrate Test
LT	Labile Enterotoxin
MDG	Millennium Development Goal
MF	Membrane Filtration
NA	Nutrient Agar
NGO	Non-Governmental Organisation
PCA	Plate Count Agar

PCR	Polymerase Chain Reaction
PE	Phycoerythrin
PI	Propidium Iodide
PVA	Polyvinyl (alcohol)
R2A	Reasoner´s 2A
RDP	Reconstruction and Development Programme
RHRW	Roof Harvested Rainwater
RMA	Repeated measures ANOVA
RWH	Rainwater Harvesting
SANS	South African National Standards
SAWS	South African Weather Services
SIM	Sulfide-Indole-Motility
SLT	Shiga-like toxins
SABS	South African Bureau of Standards
SOCO-DIS	Solar Collector Disinfection
SODIS	Solar Disinfection
SOPAS	Solar Pasteurization
SS	*Salmonella Shigella* (Agar)
ST	Stable Toxin
TC	Total Coliform
TDT	Thermal Death Time
TNTC	Too Numerous To Count
TOC	Total Organic Carbon
UN	United Nations
UNICEF	United Nations Children's Fund
UV	Ultraviolet
WHO	World Health Organisation

CHAPTER 1: MICROBIAL AND CHEMICAL WATER QUALITY AND POINT OF USE TREATMENT SYSTEMS

1.1 INTRODUCTION

Approximately 780 million people worldwide, with the majority in developing countries, do not have access to a potable water source (UNICEF and WHO, 2012). The economic, social and environmental impacts of poor water supplies and sanitation services are well documented (Mara, 2003; Moore et al., 2003; Montgomery and Elimelech, 2007; Johnson et al., 2008). Furthermore, the elimination of the burden of disease, closely related to the availability of adequate, safe and affordable water supplies (Theron and Cloete, 2002; Ashbolt, 2004; Eshelby, 2007), have a direct and positive impact on the economy of an individual, a household and the community. Rainwater harvesting (RWH) is an alternative technology that could assist in the provision of water directly to the household for drinking and domestic purposes. The World Health Organisation (WHO) identifies RWH as an alternative improved water source along with protected dug wells, boreholes and standpipes. Millions of people are currently using RWH for drinking water purposes and an almost two-fold increase has been observed for both rural and urban users (Table 1.1) (UNICEF and WHO, 2012). Rainwater harvesting could also provide water for small-scale home based productive activities such as vegetable gardening, which could make a positive contribution towards food security for the people from lower social economic groups (Mwenge Kahinda et al., 2010).

Table 1.1: Global users of different drinking water sources (population) (UNICEF and WHO, 2012).

Facility type	Urban (millions)		Rural (millions)		Total (millions)	
	1990	**2010**	**1990**	**2010**	**1990**	**2010**
Piped on premises	1820	2763	538	973	2358	3737
Public taps	120	205	168	260	288	465
Boreholes	138	255	878	996	1016	1251
Rainwater	**6**	**13**	**41**	**76**	**47**	**89**
Dug wells	111	151	843	656	954	807
Springs	15	33	235	221	250	254
Trucks and carts with drums	24	42	20	43	44	85
Surface water	17	11	313	175	331	187

Rainwater harvesting refers to the collection, concentration and storage of rainwater runoff for the use in domestic and agricultural activities (Gould, 1999). One millimetre of rainwater collected per one square meter of collection surface equals one litre of water (FAO, 1985). Rainwater harvesting is classified into three groups based on the type of catchment surface used. *In situ* RWH systems utilise part of the target area as the catchment area whereas external RWH systems utilise an uncultivated area. In addition, domestic RWH (DRWH) systems utilise rooftops, courtyards or treatment systems for the collection of water into RWH tanks for domestic purposes (Mwenge Kahinda et al., 2008; Helmreich and Horn, 2009). In addition, the storage facility (above or underground tanks) and target area (domestic use, agricultural, garden watering and small scale activities) are also considered two major components of a RWH system (Mwenge Kahinda et al., 2008). Domestic rainwater harvesting (DRWH) has the potential to improve water availability in rural communities in Southern Africa, with 55 000 households utilising a rainwater tank on site, as their main source for drinking in 2010 in South Africa. However this number only represents 0.4% of the total number of households (Figure 1.1) (Statistics South Africa, 2010).

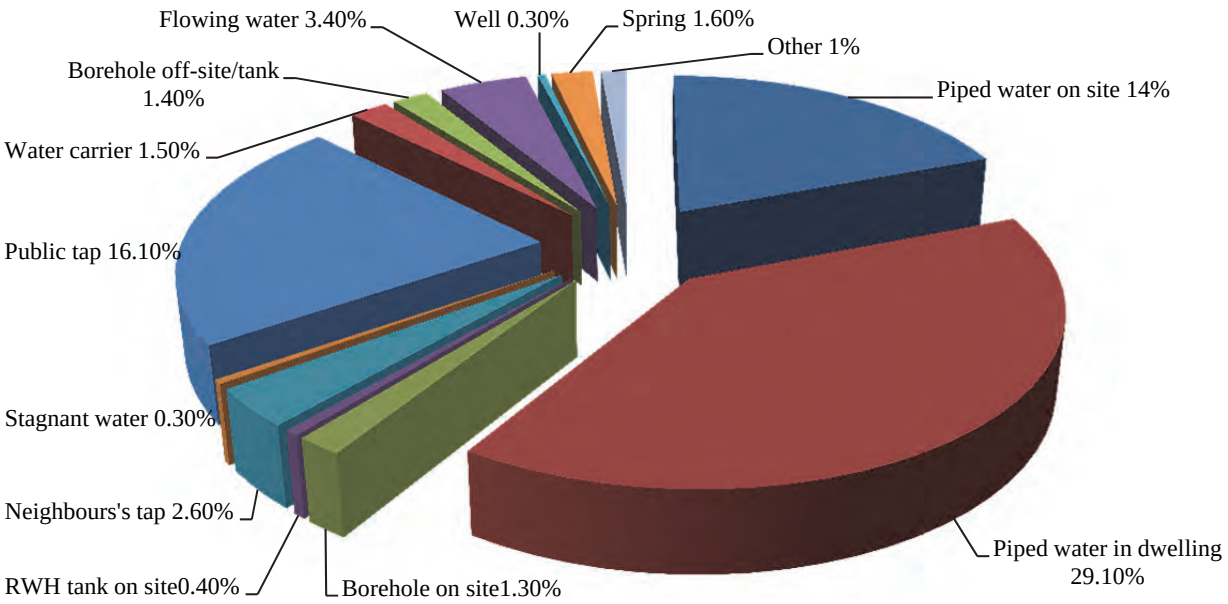

Figure 1.1: Comparison of the main sources of drinking water in South African households (Statistics South Africa, 2010)

Worldwide RWH is being promoted by governmental organisations as an alternative water resource (Lee et al., 2010b; Australian Government, 2011; Rowe, 2011). A similar tendency is being observed in South Africa with the government at national and local level promoting RWH through small scale pilot projects. For example, the South African Department of Water and Environmental Affairs (DWEA) initiated a RWH pilot project that provided financial assistance for the construction of 64 underground tanks. These tanks were distributed in 26 villages in the following four provinces; Eastern Cape, Limpopo, KwaZulu-Natal and Free State. A set of guidelines for the construction and the use and maintenance of underground tanks were also compiled during this programme (De Lange, 2006). In the 2010/2011 term DWAF provided 5280 RWH tanks to rural communities as part of their rural development programme (Mabudafhasi, 2011). Moreover, the Department of Science and Technology (DST) commissioned the Council of Science and Industrial Research (CSIR) to investigate technologies that will improve the sustainability and quality of low-income subsidised housing. Rainwater harvesting was one of the technologies evaluated in a demonstration house. Four hundred and ten pilot-scale houses were constructed in Kleinmond, Western Cape, with each house provided with a RWH tank. The quality of the roof-harvested water collected in the tanks is currently being evaluated (CSIR, 2011).

The eThekwini municipality, in Kwazulu-Natal, also installed 500 rainwater tanks in the Inanda informal settlement. Two tanks were installed at a school and the other 498 tanks were installed at housing sites (Naidoo, 2005). Another interesting fact is that in 2010 the Nelson Mandela Bay Municipality changed their by-laws to promote the use of DRWH tanks. This move was supported by the South African Department of Water Affairs (DWA). This measure was taken to reduce water usage after the drought in 2010 affected the Algoa Water Supply System (DWA, 2010).

Non-governmental organisations (NGOs) that promote the use of rainwater harvesting in South Africa include the Mvula Trust, the Water for Food Movement, the Rainman Landcare Foundation, the World Vision, the International Water Management Institute and the Association for Water and Rural Development. Although NGOs understand the social factors that play a role in the implementation of an innovative water technology, such as RWH, their projects are usually conducted on a smaller scale (Mwenge Kahinda and Taigbenu, 2011).

Hurdles that need to be overcome for DRWH to be widely used include an investigation into the possible health risks associated with DRWH. Microbial and chemical contaminants have been detected in DRWH tanks, and if this water is used for potable purposes, it could produce adverse health effects. People with a compromised immune system, the elderly and the young are especially susceptible to water-borne diseases (Obi et al., 2006). Domestic rainwater harvesting tanks could also serve as a breeding space for mosquitos. Mosquitos are the vectors for various diseases including malaria. Certain regions in South Africa are affected by malaria and in these regions special precautions must be undertaken to prevent the breeding of mosquitos in DRWH tanks (Vasudevan et al., 2000; Mandal et al., 2011). There is also a lack of research on the role that social factors play in the acceptance and use of DRWH. Other limitations include financial constraints for people in rural communities due to unaffordable initial investment, lack of clear legal legislation on the use of DRWH in South Africa (Mwenge Kahinda et al., 2005), skills shortage for proper implementation, maintenance and risk management of DRWH tanks and physical constraints such as dependency on the rainfall season and lack of space for DRWH tanks in informal settlements (Mwenge Kahinda et al., 2007).

1.1.1 Quality of Water Collected By Domestic Rainwater Harvesting

Microbial and chemical contaminants in DRWH tanks can originate from: i) raindrops that traverse through polluted air; ii) the catchment areas, iii) and the storage tanks (Figure 1.2). This review focuses only on studies that investigated the microbial and chemical quality of water collected from DRWH tank systems to include all three possible contamination sources. A review by Abbasi and Abbasi (2011) included studies that also investigated the microbial and chemical quality of roof runoff water and rainwater.

Factors that influence the quality of harvested rainwater include: i) roof geometry (size, exposure, inclination); ii) roof material (chemical characteristics, roughness, surface coating, age, weatherability); iii) rainfall event (wind speed, intensity, pollutant concentration); iv) other meteorological factors (seasons, weather characteristics, antecedent dry period); v) concentration of substances in the atmosphere (transport, emission, half-life, phase distribution); vi) location of the roof (proximity of pollution sources) and vii) maintenance history of the roof (Abbasi and Abbasi, 2011).

1.1.1.1 *Chemical Quality of Water Collected By Domestic Rainwater Harvesting*

Research on the occurrence of chemical contaminants in rainwater has received less attention than studies investigating for the presence of microbial contaminants (Table 1.2). This may be due to the fact that the presence of chemical contaminants does not pose an immediate health risk unlike microbial pathogens, which cause disease. However, a few studies have detected lead concentrations in rainwater above the drinking water guidelines as recommended by the respective country's water authorities (Simmons et al., 2001; Huston et al., 2012). Lead could have serious health effects if present in drinking water, especially among young children, due to possible developmental neurological effects (Goyer, 1993). The low levels of fluoride in water may also be detrimental when rainwater is used as the only water source (Sazakli et al., 2007). Fluoride present in water reduces dental decay and therefore fluoride supplementation is recommended where rainwater is used as the primary and sole drinking water source (Satur et al., 2010).

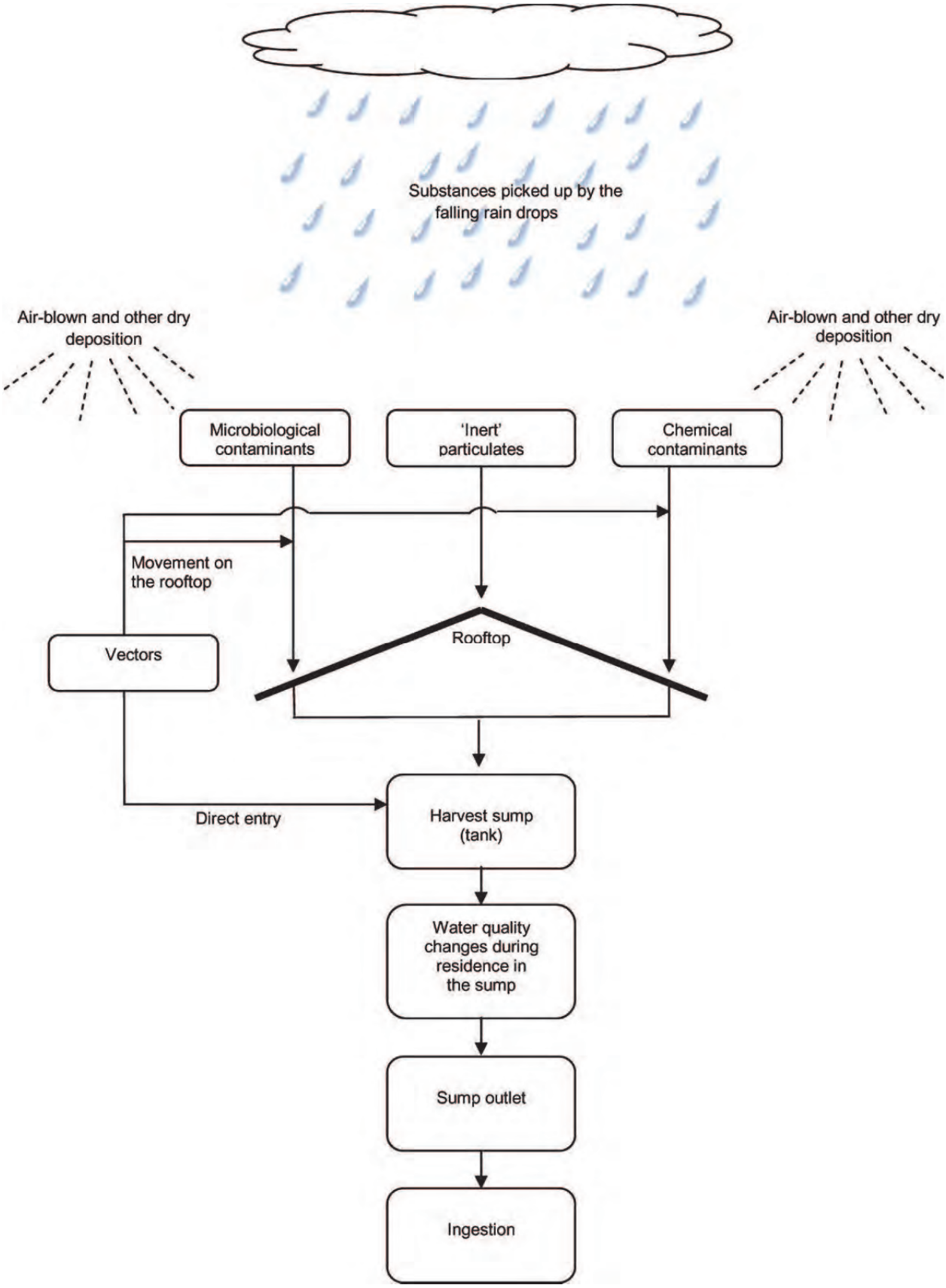

Figure 1.2: The different pathways through which chemical and microbial contaminants can enter a DRWH system (Abbasi and Abbasi, 2011)

Table 1.2: A summary of the reports on the chemical quality of water collected from DRWH tanks.

Location/ Country	No. of samples	Average pH	Average concentrations of cations	Average concentrations of anions	Reference
Korea	5	7.5	Fe: 0.033 mg/L; Cu: 0.054 mg/L; Zn: 0.15 mg/L	PO_4: 0.2 mg/L; N_2: 1 mg/L	Kim et al. (2005)
Mexico	9	6.6	Ca: 7.58 mg/L; K: 1.52 mg/L; Mg: 0.36 mg/L; Na: 3.23 mg/L;	F: 0.23 mg/L; Cl: 1.48 mg/L; NO_3: 3.84 mg/L; SO_4: 4.51 mg/L	Adler et al. (2011)
Hebron, Palestine	100	8.2	Ca: 94.6 mg/L; Mg: 64.6 mg/L; NH_4: 1.4 mg/L;	NO_3: 4.2 mg/L; Cl: 42.3 mg/L;	Al-Salaymeh et al. (2011)
Northern Jordan	90		Pb: 0.01 mg/L, Fe: 0.01 mg/L, Cr: 0.012 mg/L; NH_4: 0.06 mg/L	NO_3: 1.56 mg/L; PO_4: 1.27 mg/L	Radaideh et al. (2009)
Bermuda	112	7.81	Ca: 13 mg/L; K: 0.61 mg/L; Mg: 1 mg/L; Na: 7.7 mg/L; Fe: 7.8 µg/L; Al: 110 µg/L; Ba: 4.2 µg/L; Mo: 0.13 µg/L; Pb: 0.15 µg/L; Sb: 0.82 µg/L; Sr: 110 µg/L; V: 2 µg/L; Zn: 9.2 µg/L	Cl: 15 mg/L; NO_3: 1.6 mg/L; SO_4: 6.4 mg/L;	Peters et al. (2008)
Kefalonia Island, Greece	156	8.31	Ca: 15.2 mg/L; Mg: 0.6 mg/L; Na: 6 mg/L: K: 2.4 mg/L; Fe: 11 µg/L; Mn: 1 µg/L; Cd: 0.05 µg/L; Zn 10 µg/L	NO_3: 7.04 mg/L; NO_2: 0.013 mg/L; PO_4: 0.09 mg/L; SO_4: 8 mg/L; Cl: 7 mg/L;	Sazakli et al. (2007)
Northern China	76	7.39	Na: 3.02-11.2 mg/L; K: 3.36-8.658 mg/L; Ca: 11.2-31.15 mg/L; Mg: 0.930-1.143 mg/L; B: 0.011-0.056 mg/L; Fe: 0.01-0.083 mg/L; Mn: 0.048-0.112 mg/L; Cu: 0.0011-0.016 mg/L; Al: 0.093-0.336 mg/L; Pb: 0.003-0.041 mg/L; NH_4: 0.01 mg/L	Cl: 6.13-79.20 mg/L; SO_4: 2.4-15.62 mg/L; F: 0.071-0.163 mg/L; P: 0.247 mg/L; N: 1.188 mg/L	Zhu et al. (2004)

Table 1.3 (continued): A summary of the reports on the chemical quality of water collected from DRWH tanks.

Location/ Country	No. of samples	Average pH	Average concentrations of cations	Average concentrations of anions	Reference
Batoka, Zambia	2	7.15	Zn: 0.645 mg/L	SO_4: 1.675 mg/L; Cl: 10.5 mg/L; NO_3: 4.63 mg/L	Handia et al. (2003)
Ayudhaya, Thailand	10	6.4	Ca: 10.30 mg/L; Cu: 0.03 mg/L; Fe: 0.54 mg/L; Mn: 0.001 mg/L; Pd: 0.017 mg/L; Zn: 0.15 mg/L	NO_3: 14.1 mg/L; Cl: 1.45 mg/L; SO_4: 3.24 mg/L; PO_4: 0.86 mg/L	Areeracha-kul et al. (2009)
Dertig Village, South Africa	13	6.85	Fe: 0.013 mg/L; Al: 0.014 mg/L;	NO_3: 2.40 mg/L; NO_2 0.006 mg/L; F: 0.270 mg/L	Nevondo and Cloete (1999)
Gangneung, Korea	90	5.3	NH_4: 0.02 mg/L; Ca: 1.6 mg/L; Na: 1.1 mg/L; K: 2.1 mg/L; Mn: 40 µg/L; Pb: 20 µg/L; Cu: 35 µg/L; Cr: 1 µg/L; Zn: 60 µg/L; Al: 100 µg/L	NO_3: 2.2. mg/L; Cl: 3 mg/L;	Lee et al. (2010c)
Auckland, New Zealand	125	7.3	Pd: < 0.01 mg/L; Cu: 0.06 mg/L; Zn: 0.4 mg/L	-	Simmons et al. (2001)
National Survey, Australia	70	6.7	Ca: 3.7 mg/L; Mg: 0.6 mg/L; K: 0.6 mg/L; Na: 3.5 mg/L; NH_4: 0.0074 mg/L; Al 41.6 µg/L; Ar: 1 µg/L; Ba: 6.4 µg/L; Cd: 0.9 µg/L; Cr: 9.8 µg/L; Co: 0.7 µg/L; Cu: 18.4 µg/L; Fe: 44.8 µg/L; Pb: 3.8 µg/L; Li: 3.5 µg/L; Mn: 10.2 µg/L; Zn 1790 µg/L	SO_4: 3.2 mg/L; NO_2: 0.006 mg/L; NO_3: 1.2 mg/L;	Chapman et al. (2008)
Brisbane, Australia	165-354	6.10	Ca: 2400 µg/L; Mg: 500 µg/L; K: 900 µg/L; Na: 2800 µg/L; Li: 0.55 µg/L; Al: 60 µg/L; V: 0.32 µg/L; Cr: 0.53 µg/L; Mn: 8.70 µg/L; Fe: 68 µg/L; Co: 0.17 µg/L; Ni: 1.3 µg/L; Cu: 21 µg/L; Zn: 770 µg/L; As: 0.25 µg/L; Sr: 30 µg/L; Sb: 0.15 µg/L; Ba: 12 µg/L; Pb: 5.4 µg/L; Sn: 0.51 µg/L	Cl: 3900 µg/L; NO_2: 100 µg/L; NO_3: 1600 µg/L; PO_4: 100 µg/L; SO_4: 1600 µg/L	Huston et al. (2012)

As mentioned, rainwater that traverses through air in areas with high industrial or agricultural activities can result in the collected water being contaminated with chemical concentrations exceeding the respective country's drinking water standards. A study in Brisbane, Australia, concluded that atmospheric deposition was responsible for 21% of the incidences where lead levels in rainwater tank samples exceeded the Australian Drinking Water Guidelines (ADWG). Sources for atmospheric deposition, include; traffic (exhaust fumes and discharges), industrial and secondary aerosols (Huston et al., 2012). The town Port Pirie in Australia is also affected by lead smelter emissions. Research has shown that the contamination of collected rainwater as a result of the lead accumulated in RWH tanks contributed to the elevated lead levels in the blood of children (Body, 1986). Consequently the use of rainwater tanks in the community has since been strongly discouraged through community education campaigns (Maynard et al., 2003).

Air pollution that can have an impact on rainwater quality is not however, restricted to local pollution sources, thus complicating the monitoring thereof. High levels of aluminium, possibly originating from anthropogenic sources, were observed in rainwater collected in Istanbul, Turkey. The observed aluminium levels of 7.66 mg/L (Uyger et al., 2010) were above drinking water standards as stipulated by the South African Water Quality Guidelines for Domestic Use (between 0 and– 0.15 mg/L) (DWAF, 1996) and the ADWG (0.2 mg/L) (NHMRC and NRMMC, 2011). Rainwater samples were also moderately to extremely laden with the trace elements Cr, Co, Ni, V and Pb. It was concluded that the heavy metal pollution observed was mainly influenced by the transport of the pollutants from Western Europe and Russia (Uyger et al., 2010).

Although there are concerns about the presence of organic compounds associated with herbicides and pesticides, applied in agricultural farming, filtering into the DRWH tanks, in the studies that analysed rainwater for these compounds, the observed concentrations were well below the local water drinking guidelines (Sazakli et al., 2007; Chapman et al., 2008; Huston et al., 2009). The only exception was observed during a national survey performed in Australia. The herbicide CPA (4-chlorophenoxy acetic acid) was detected (366 µg/L) in water sampled from one tank in Brisbane (Chapman et al., 2008). Another study, also performed in Brisbane, Australia, detected low levels of the herbicides, atrazine, simazine and diuron in the bulk deposition and water sampled from RWH tanks. However, the levels detected were well below the ADWG guidelines (Huston et al., 2009).

Catchment areas including rooftop and drainage pipes are the second major sources of contamination. The materials used to construct the roof, materials deposited onto the roof and roof maintenance, influence the quality of the roof runoff. Lead-based and acrylic based paints should be avoided since this could lead to roof runoff with high lead levels and dissolved chemicals such as detergents, respectively (Abbasi and Abbasi, 2011). In a study conducted in Lusaka, Zambia, higher zinc concentrations were observed in harvested rainwater collected from roofs constructed from galvanised iron sheets than those constructed from asbestos cement roofs (Handia et al., 2003). The corrosion of galvanised iron sheets has also been proposed to contribute to lead contamination in harvested rainwater (Simmons et al., 2001). Contradicting results were obtained from a study in South Korea where the suitability of four types of roofing materials (wooden shingles, concrete tiles, clay tiles and galvanised steel) that were widely used in the area were investigated. Galvanised steel was found to be the most suitable roofing material for use in the harvesting of rainwater for domestic use as the physical, chemical and microbiological parameters evaluated met the Korean and WHO guidelines for drinking water (Lee et al., 2012).

Harvested domestic rainwater is stored in water tanks, which can be built above or underground (Kahinda et al., 2007). Depending on the requirements, storage tanks differ in size and shape. For storage of smaller quantities of water, tanks are typically made of bricks, stabilised soil, rammed earth, plastic sheets and mortar jars. In order to store larger quantities of water, pottery, ferrocement, or polyethylene are materials used to construct the tanks. The material of storage tanks can however, influence the quality of harvested rainwater. Higher pH levels have been observed in rainwater stored in concrete tanks when compared to non-concrete tanks (plastic, wood, fibreglass or galvanised iron) (Simmons et al., 2001; Despins et al.,

2009). The increase in pH could be attributed to the leaching of calcium carbonate from the concrete walls of the cistern (Zhu et al., 2004).

The location of DRWH tanks can also influence the chemical quality of the rainwater. A correlation between the magnitude of rainfall in a region and the quality of harvested rainwater has been observed. In Jordan, higher concentrations of heavy metals were detected in areas with lower rainfall levels (Radaideh et al., 2009). The number of days between rainfall events also has an impact on the quality of harvested rainwater. More contaminants are deposited onto the catchment areas during longer dry periods. Therefore higher levels of contaminants will be present in the harvested rainwater after a dry spell than during periods of frequent rainfall events (Yaziz et al., 1989).

1.1.1.2 *Microbial Quality of Water Collected By Domestic Rainwater Harvesting*

Animals including, squirrels, birds, possums and rats, for example, may deposit faecal matter on the roof surface, which implies that undesired bacteria, viruses and protozoan pathogens can filter into the rainwater tank. Pathogens that occur in the faeces of birds, insects, mammals and reptiles can contaminate the water from rooftops. The rain then allows pathogens associated with animal droppings and other organic debris, to be flushed into the tanks via the gutters and inlet tank system. A study performed in Southeast Queensland, Australia, demonstrated that identical biochemical phenotype profiles of *E. coli* strains were isolated from RWH tanks and from bird and possum faeces collected from the roof surface. Their results thus suggested that the faeces could have been the source of *E. coli* contamination in the RWH tanks (Ahmed et al., 2012b).

Rainwater is susceptible to various sources of pollution, however, no guidelines for routine rainwater analysis and monitoring currently exists internationally as well as in South Africa. It is thus common practice, in assessing the quality of the water, to use guidelines of drinking water to monitor the rainwater quality. The Department of Water Affairs and Forestry (DWAF) uses the South African Water Quality Guidelines to monitor the quality of water use for various purposes (DWAF, 1996). These general purposes can be sub-divided into four categories which include water for industrial, agriculture, recreational and domestic purposes. The water quality requirements for each of these four categories have been developed to ensure that the water has no negative health impact on humans, no aesthetic impacts on the water and no economic impact, which may include the increased cost of water treatment (DWAF, 1996). Table 1.3 summarises the water standards for domestic use as established by the DWAF (1996).

Table 1.4: Domestic water quality standards according to DWAF (1996).

Indicator Organism	**Target range for water Quality**
Heterotrophic plate count	<100 CFU/mL
Total coliforms	≤5 CFU/100 mL
Faecal coliforms	0 CFU/100 mL
Escherichia coli	0 CFU/100 mL
Enterococci	0 CFU/100 mL

As indicated by the Department of Water Affairs and Forestry (DWAF) standards for drinking water, it would be technically and economically impractical to assess the safety of water by testing for all of the currently known pathogens that may be present in the water. For this reason, indicator organisms are used for monitoring the presence of pathogens in harvested rainwater (HRW) and most studies use the presence or absence of indicator organisms to assess the quality of the water (Ahmed et al., 2011a).

The testing for indicator organisms has its disadvantages. The duration of incubation for analyses is long, there can be antagonistic organisms that interfere with their growth and it can be difficult to detect stressed coliforms (Rompré et al., 2002). A study by Ahmed et al. (2011a) also showed that there can be a poor

correlation between faecal indicators and pathogenic bacteria. To allow for a reliable indication of the potential risks of infection, a combination of indicators, such as heterotrophic plate counts and total coliforms, are thus tested for.

Heterotrophic plate counts (HPC) determine the number of culturable bacterial organisms in a particular water source (Lye et al., 2002). This then allows for the monitoring of the disinfection efficiency in a system, which implies that if the plate counts are above a particular standard, measures can be implemented to lower the level of microbial contamination.

Total coliforms (TC) serve as indicators of the general hygienic quality of the water. Coliforms are bacteria belonging to the family, *Enterobacteriaceae* and the genera associated with this bacterial family include, *Escherichia*, *Citrobacter*, *Klebsiella*, *Enterobacter*, *Serratia* and *Rahnella* (Ahmed et al., 2011a). Coliforms are defined based on the biochemical characteristics that they exhibit and total coliforms are classified as rod-shaped, non-spore-forming, Gram-negative, oxidase-negative, aerobic or facultative anaerobic bacteria, that are able to grow in the presence of bile and that ferment lactose with gas and acid production within 48 h at 37^0C (Rompré et al., 2002). Total coliforms that are found in water are microorganisms that not only originate from faecal matter, but can originate from other sources such as soil and vegetation. Therefore their presence in rainwater tanks does not necessarily indicate faecal pollution.

The enumeration of the Faecal coliforms (FC) serves as an indicator of the level of faecal pollution in the water source. Faecal coliforms are microorganisms that are only from a faecal origin and can grow at higher temperatures than total coliforms. They are defined as thermo-tolerant coliforms that have the same fermentation properties as total coliforms and can grow at a temperature of 44^0C (Rompré et al., 2002).

Escherichia coli (E. coli) is included as a specific indicator organism of faecal pollution from warm-blooded animals (Rompré et al., 2002). This Gram-negative organism is a thermo-tolerant bacterium that produces indole from tryptophan at 44^0C, yields a positive methyl red test, does not use citrate as its sole carbon source and cannot produce acetyl-methyl carbinol. *Escherichia coli* are also part of the normal bacteria found in the lower intestine of humans and while most *E. coli* strains are harmless, certain serotypes are associated with serious gastroenteritis and urinary tract infections. The five major pathogenic strains are then classified according to the virulent factor they express and include Enteroaggregative *E. coli* (EAEC), Enterohaermorrhagic *E. coli* (EHEC), Enteropathogenic *E. coli* (EPEC), Enterotoxigenic *E. coli* (ETEC) and Enteroinvasive *E. coli* (EIEC) (Todar, 2008).

Enteroaggregative *E. coli (*EAEC) adhere in an aggregative manner to the intestinal mucosa by producing aggregative adherence fimbriae (AAF) (Tobias & Vutukuru, 2012). They cause watery, mucoid, bloody or non-bloody diarrhoea with low fever and little or no vomiting in children and adults (Tobias & Vutukuru, 2012) without invading or causing inflammation (Todar, 2008). This suggests that the EAEC strain must produce an enterotoxin of some kind. An Entero-aggregative ST (EAST) heat-labile toxin, which is plasmid encoded, has been isolated from this strain, but the role of the toxin has not yet been proven (Todar, 2008).

The enteropathogenic *E. coli* (EPEC) causes watery diarrhoea and is associated with vomiting and low fever (Todar, 2008). It has the biggest impact on infants and young children in developing countries (Tobias & Vutukuru, 2012). Outbreaks of this disease have been linked to the consumption of contaminated water and meat products (Todar, 2008). Enteropathogenic *E. coli* also has a plasmid-encoded protein called the EPEC adherence factor (EAF), which enables them to adhere to the intestinal cells. Intimin is an outer membrane protein that they encode for that assists in the final stages of the adherence process. The adherence process is however, complicated and includes the rearrangement of the actin in the intestinal cells at the site of adherence. This strain of *E. coli* is said to be moderately invasive since they are not as invasive as *Shigella*, but they do cause an inflammatory response (Todar, 2008).

The enterotoxigenic *E. coli* (ETEC) strain causes watery diarrhoea without blood, and in a few cases it can also cause vomiting and fever. Infants and travellers in underdeveloped countries are the most susceptible to this disease (Tobias & Vutukuru, 2012). The symptoms of the disease can vary from slight discomfort to harsh cholera-like symptoms. An estimated number of 10^3 cells are sufficient to cause illness. The bacteria will colonise the intestinal tract by a fimbrial adhesion mode of action and are non-invasive. The disease requires the production of a plasmid encoded heat-labile enterotoxin (LT) and the heat stable toxin (ST) (Todar, 2008). The LT enterotoxin has a function and enzymatic activity similar to that of the cholera toxin and also binds to the ganglioside receptors that the cholera toxin binds to. The ST has a molecular weight of 4 000 Daltons which could explain their resistance to heat. Heat stable enterotoxins are also not inactivated when they are subjected to boiling for 30 min. The ST1b and STh are the predominant variants of the ST toxin that is found in humans. The ST functions by causing an increase in the cyclic guanosine monophosphate, which has the same effects as an increase in cyclic adenosine monophosphate (Todar, 2008).

Enteroinvasive *E. coli* (EIEC) has a pathogenic mode of action similar to that of *Shigella*. It causes dysentery-like diarrhoea in humans along with a high fever. Studies have shown that 10^6 organisms are necessary to cause illness in healthy adults. The EIEC strain will penetrate and multiply within the epithelial cells of the colon and are therefore invasive. This will lead to the destruction of the epithelial cells (Todar, 2008). The infection is mediated by a plasmid encoded invasion associated loci (Parsot, 2005)

Enterohaermorrhagic *E. coli* (EHEC) can cause watery diarrhoea followed by bloody diarrhoea, with little or no fever (Tobias & Vutukuru, 2012). This strain can also cause haemorrhagic colitis that may progress to haemolytic-uraemic syndrome (HUS); affecting the kidney and liver if the disease is left untreated (Todar, 2008). The pathogenicity of EHEC is due to the production of Shiga-like toxins (SLTs) (Cebula et al., 1995). The SLT1 and SLT2 are variants most frequently encountered in humans (Todar, 2008). The presence of a 60 MDa plasmid is also characteristic of the EHEC strains (Cebula et al., 1995). There are more than a hundred *E. coli* strains that produces the SLTs, but the predominant serotype is 0157:H7 (Cebula et al., 1995). An estimated number of 10-100 cells are necessary to infect an individual (Todar, 2008). In 1993 there was a large outbreak and it was discovered that hamburgers that were undercooked were contaminated with 0157:H7. More than 700 people throughout four states in America were infected. Fifty one cases resulted in haemolytic – uraemic syndrome (HUS) and four of the cases were fatal.

Enterococci or faecal streptococci survive longer in water compared to coliforms and may be specific indicators of faecal pollution. Species include *Enterococcus faecalis*, *E. faecium, E. durans*, *E. hirae*, *E. cecorum*, *E. columbae, E. avium* and *E. gallinarum* together with *Streptococcus bovis* and *S. equinus* (ISO, 1998; Ashbolt et al., 2001; WHO, 2003).

In many incidences HRW is not suitable for drinking purposes without prior treatment. For instance, as indicated by Sazakli et al. (2007), the presence of coliforms, *Escherichia coli* and enterococci in rainwater were found to be 80.3%, 40.9% and 28.8%, respectively. Table 1.4, adapted from Ahmed et al. (2011a), summarises the studies undertaken to indicate the general quality of rainwater in various regions around the world by monitoring the presence of indicator organisms.

The presence of indicator organisms and pathogens vary amongst reports with some studies indicating levels of up to thousands of CFU/100 mL. Certain studies have thus indicated that rainwater is not suitable for drinking (Yaziz et al,. 1989; Zhu et al,. 2004; Sazakli et al,. 2007). However, a study conducted by Dillaha and Zolan (1985), found that rainwater was generally acceptable for drinking and household purposes.

A study conducted in Micronesia also showed low numbers of faecal indicators. Thirty nine percent of the samples had TC numbers <10 CFU/100 mL, which did not exceed the World Health Organisation (WHO, 2004) guidelines. The authors suggested that the water was fit for drinking, even though 61% of the samples did not comply with the guidelines. In contrast, a study conducted in Victoria, Australia, showed elevated

numbers of faecal indicators present in the water source. Forty nine rainwater tanks were tested and the results showed that 33% of the samples tested positive for *E. coli* and 73% tested positive for enterococci (Ahmed et al., 2011a). A study performed in South Queensland, Australia, by the same research group, detected *E. coli* strains in 63% of the collected rainwater samples and enterococci in 92% of the rainwater samples (Ahmed et al., 2012b). The presence of virulence genes in 200 *E. coli* strains isolated from rainwater samples have also been investigated to determine the pathogenicity of these strains. Forty percent of the *E. coli* strains were carrying one virulence gene, 37.5% were carrying two virulence genes, 18% were carrying three virulence genes and 3% were positive for the presence of four or more virulence genes. The virulence genes detected in these strains belonged to enteropathogenic *E. coli*, Shiga-toxigenic *E. coli*, enterotoxigenic *E. coli* and extraintestinal pathogenic *E. coli*. This study raises concerns about the presence of potentially clinically significant *E. coli* strains in RWH tanks (Ahmed et al., 2011b).

It has been documented that New Zealand relies predominantly on roof-collected rainwater as a potable domestic water supply, especially in rural households where this collected rainwater can be seen as the only available domestic water source. A study completed by Simmons et al. (2001) however, showed that the rainwater is not always safe and cannot always be considered potable. Simmons et al. (2001) found a positive correlation between *Aeromonas* spp. and bacterial indicator organisms. For this study, HPC, TC, FC and enterococci (ENT), representing indicator organisms, were enumerated and 56% of the water supplies exceeded the criteria as stipulated in the New Zealand Drinking Water Standards (NZDWS) of <1 FC/100 mL.

In South Africa studies focus predominantly on the optimisation of the tank system. Factors such as water availability and requirements, and technical constraints such as the limitation of space and the socio-economic pressure, need to be considered (Nevondo and Cloete, 1999). Nevondo and Cloete (1999) however, conducted a study on the quality of rainwater in Hammanskraal, which is situated 55 km north of Pretoria. Heterotrophic plate counts were recorded at between 1.0×10^1 and 1.63×10^4 CFU/mL, with an average count of 3.27×10^3 CFU/mL. The enumeration of TC ranged from 4.7×10^2 to 1.0×10^3 CFU/mL, with the FC count ranging between 9.0×10^1 and 2.6×10^2 CFU/mL. As a result of the HPC, TC and FC counts the general quality of the rainwater source, was deemed unacceptable. The quality of the rainwater however, depends on several factors such as the weather conditions, proximity of the pollution source, the maintenance of the water tanks, the type of catchment area and the topography of the area where the RWH tanks are located (Mwenge Kahinda et al., 2007).

Pseudomonas aeruginosa is a Gram-negative, flagellated rod, belonging to the family *Pseudomonadaceae*. Although it is commonly found in faeces, soil, some foods and water, it cannot be used as a faecal indicator as it is not universally present in faeces and sewage. In addition, suitable surfaces of organic material that come into contact with water and result in the enrichment of water, will allow *P. aeruginosa* to multiply. In Australia, the presence of *P. aeruginosa* indicates the general cleanliness of water (NHMRC and NRMMC, 2011). *Pseudomonas aeruginosa* colonises damaged systems of its host, for instance burn wounds, making it a classical opportunistic pathogen. Colonisation of *P. aeruginosa* in wounds, could then lead to the creation of critical lesions or septicaemia (NHMRC and NRMMC, 2011).

Klebsiella spp. are Gram–negative rods, do not produce spores and are oxidase-negative. They are environmental organisms and are able to multiply in certain water sources. They have also been found in the faeces of humans and have been associated with the roots of plants and leaves of vegetables. These organisms are as sensitive to disinfection as *E. coli* and several other bacterial enteric pathogens. They therefore serve as indicators of the competence of a drinking water disinfection process. The two opportunistic pathogenic strains *K. pneumoniae* and *K. oxytoca*, have been associated with pneumonia in healthy patients and infections in compromised patients, in for example the elderly or infants (NHMRC and NRMMC, 2011).

Clostridium spp. are classified as anaerobic, sulphite-reducing bacteria. These bacilli produce spores and in particular *C. perfringens* is rarely able to multiply in water environments. *Clostridium perfringens,* uniquely found in faeces, is relatively common in dogs and forms part of the naturally occurring intestinal flora of 13 – 35% of humans (Leeming et al., 1998). They are predominantly resistant to disinfection such as chlorination, and changes in pH and temperature extremes. For this reason *C. perfringens* spores have been suggested as potential indicators of enteric viruses and protozoa in drinking water (Payment and Franco, 1993), but their concentrations are lower than *E. coli* in faeces and sewage and the survival of *C. perfringens* is much longer than that of viruses and protozoa. For this reason, drinking water needs to be treated with vigilance, as the spores could potentially be present long after faecal pollution and death of other enteric pathogens (WHO, 2004).

Legionella forms part of the single genus belonging to the family *Legionellaceae*. *Legionella* are found in natural freshwater sources and soils, and in man-made water systems such as hot water and cooling systems. *Legionella pneumophila* serogroup 1 is one of 26 species that is repeatedly and predominantly associated with human disease. *Legionella* spp. are thought to infect humans by inhalation, rendering their presence in drinking water irrelevant. But their growth is amplified under certain conditions, usually by thermal enhancement, for example hot water systems can form aerosols in the nozzle heads of showers. There are two types of disease associated with *Legionella* infections, legionellosis, also known as Legionnaires' disease and Pontiac fever (NHMRC and NRMMC, 2011).

Shigella is a Gram–negative, non-motile, rod-shaped bacterium, that does not produce spores and is closely related to *E. coli*. *Shigella* spp. are highly pathogenic towards humans and cause bacillary dysentery, which is an infectious disease of the intestinal tract. *Shigella* infection is not usually water-borne, but major outbreaks have been reported as a result of water-borne transmission. The isolation of *Shigella* from water is an indication of recent human faecal contamination. There is no enrichment or selective media for these bacteria and this could be the reason why they are very rarely detected, even though they could be present in various water bodies (NHMRC and NRMMC, 2011).

Aeromonas spp. are also Gram-negative, rod–shaped bacteria that do not produce spores. They are found in freshwater, soil and food sources such as meat, fish and milk. They are classified in the family *Vibrionaceae*. The genus is subdivided into two groups, the psychrophilic, nonmotile group consisting of *A. salmonicida* (a fish pathogen) and a mesophilic, motile group consisting of *A. hydrophila, A. sobria* and *A. caviae*. The mesophilic group also causes infections in cold-blooded animals, and has been implicated in infections in immune-compromised patients. They are able to cause septicaemia and have been linked with gastroenteritis in children (Gracey et al., 1982).

Campylobacter spp. are Gram-negative, spiral shaped, microaerophilic bacteria. Thermophilic (growing at 42°C) *Campylobacter* spp. can cause gastrointestinal illness. Many strains are pathogenic including *C. jejuni, C. coli* and *C. fetus* while non-pathogenic strains include *C. sputorum* and *C. concisus* (Penner, 1988). *Escherichia coli* can be used to indicate the presence of *Camplylobacter* in water and their presence should not be detected in water sources according to the Australian Drinking Water Guidelines (NHMRC and NRMMC, 2011). Wild birds and poultry are the major carriers of *Campylobacter* spp. However, other domestic animals, including pigs, cattle, dogs and cats, can also harbour the thermophilic *Campylobacter* bacteria. Depending on rainfall, temperature, and the presence of birds, *Campylobacter* spp. can be found in surface waters as they survive well at low temperatures just like many other bacterial pathogens. A number of outbreaks of campylobacteriosis have been reported in the last decade due to the presence of *Campylobacter* spp., but only two of them were as a result of a contaminated un-chlorinated water source (NHMRC and NRMMC, 2011).

Table 1.5: Percentage of harvested rainwater samples that tested positive (>1 CFU/100 mL) for total bacteria and faecal indicators (number of samples selected) (adapted from Ahmed et al., 2011a).

Country	Total Bacteria	Total coliforms	Faecal coliforms	*E. coli*	Enterococci	Reference
Australia	NR	52 (100)	38 (100)	NR	NR	Verrinder and Keleher (2001)
Australia	NR	90 (49)	NR	33 (49)	73 (49)	Spinks et al. (2006b)
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	63 (15)	92 (22)	Ahmed et al. (2012a)
Australia	100 (67)	91 (46)	78 (41)	57 (67)	82 (67)	CRC for Water Quality and Treatment (2006)
Australia	NR	NR	83 (6)	NR	NR	Thomas and Green (1993)
Australia	100 (77)	63 (81)	63 (81)	NR	NR	Evans et al. (2006)
Canada	NR	31 (360)	14 (360)	NR	NR	Despins et al. (2009)
Greece	NR	80 (156)	NR	41 (156)	29 (156)	Sazakli et al. (2007)
Denmark	100 (14)	NR	NR	79 (14)	NR	Albrechtsen (2002)
Micronesia	NR	70 (176)	43 (155)	NR	NR	Dillaha and Zolan (1985)
New Zealand	NR	NR	56 (125)	NR	NR	Simmons et al. (2001)
Nigeria	100 (6)	100 (6)	ND	NR	ND	Uba and Aghogho (2000)
South Korea	NR	92 (90)	NR	72 (90)	NR	Lee et al. (2010c)
Thailand	NR	NR	NR	40 (86)	NR	Pinfold et al. (1993)
USA	100 (30)	93 (30)	NR	3 (30)	NR	Lye (1987)
U.S. Virgin Islands	86 (45)	57 (45)	36 (45)	NR	NR	Crabtree et al. (1996)
U.S. Virgin Islands	NR	NR	59 (17)	NR	NR	Rushkin and Krishna (1990)
Bermuda	NR	90 (102)	NR	66 (102)	NR	Lévesque et al. (2008)
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)
Hawaii- USA	NR	NR	89 (9)	NR	NR	Fujioka et al. (1991)
Zambia	NR	100 (5)	100 (5)	NR	NR	Handia (2005)

*NR = Not Reported

*ND = Not Determined

Salmonella are predominately motile, Gram-negative, rod-shaped bacteria that do not produce spores. The presence of *Salmonella* in water bodies is as a result of faecal contamination from animals and inadequately treated waste discharges. *Salmonella* are broadly dispersed in the environment and gain access to water systems in this way. These are also the main causes of salmonellosis outbreaks. *Salmonella* enterica serovar Typhi is a human pathogen, whereas other strains use animals as reservoirs in aiding them to affect humans (Lloyd, 1983).

Cryptosporidium is an obligate parasite that is capable of reproducing sexually and asexually. The presence of thick-walled oocysts in faeces is accountable for this protozoan species transmission. Oocysts can survive for weeks in fresh water under cool conditions (King and Monis, 2007). *Cryptosporidium* is now regarded as one of the most significant water-borne human pathogens in developed countries. North America and Britain have reported 30 outbreaks, and these have all been associated with drinking water. According to Mackenzie et al. (1994) the largest number of people that were affected was estimated to be around 403 000 people. The methods for detecting for the presence of the human pathogen remain demanding and relatively expensive, but have become increasingly reliable. *Cryptosporidium hominis* and *C. parvum* have been acknowledged as the major causes of disease, known as cryptosporidiosis, in humans. Although, *C. hominis* affects humans, the *C. parvum* strains that infect humans can also occur in cattle and sheep.

Giardia has been considered a serious water-borne, human pathogen since the 1960s. However, the tests for identifying the presence of human infectious species in water are limited. *Giardias*' lifecycle consists of two phases. In the intestine, they are able to multiply and contain flagella. Whereas in faeces they are seen in elevated numbers and appear as thick-walled cysts that shed sporadically. Wallis et al. (1996) reported 240 cysts per litre in surface water, and in Australia, sewage typically presented much larger prominent numbers of *Giardia* than *Cryptosporidium*. Just like *Cryptosporidium*, this protozoan has robust cysts that are able to survive in water for weeks. Outbreaks of human infections (giardiasis) occur when untreated water is ingested. *Giardia* species are found in a wide range of hosts from birds (*G. psittaci)*, other mammals (*G. muris)*, to amphibians (*G. agilis),* with *Giardia lamblia* (syn. *Giardia intestinalis, Giardia duodenalis*) considered the primary pathogen of humans and other mammals.

The detection of certain bacterial pathogens has been determined in most studies assessing the quality of water as indicated in Table 1.5, adopted from Ahmed et al. (2011a). Simmons et al. (2001) investigated for the presence of *Salmonella* spp., *Legionella* spp., *Campylobacter* spp., *Aeromonas* spp. and the protozoan species, *Cryptosporidium* and *Giardia*. *Salmonella* spp. and *Aeromonas* spp. were detected in 0.9% and 20% of the rainwater samples, respectively. *Legionella* spp. and *Campylobacter* spp. were not detected. The presence of protozoan species were only determined in samples that contained elevated levels of FC or ENT, for this 50 samples were selected. *Cryptosporidium* oocysts were detected in 4% of these 50 samples and *Giardia* was not detected. Ahmed et al. (2010) detected *Aeromonas* spp., *Legionella* spp., *Campylobacter* spp., *Salmonella* spp. and *Giardia* spp. in rainwater samples in Australia. In South Africa, Nevondo and Cloete (1999) found *Aeromonas hydrophila, Aeromonas caviae*, *Bordetella species, Alcaligenes* spp. and the possibility of *Vibrio fluvialis* present in rainwater samples.

Table 1.6: Other bacterial pathogens associated with harvested rainwater from DRWH tanks (adapted from Ahmed et al., 2011a).

Country	Positive percentage of samples	Pathogenic Bacteria Detected	Reference
New Zealand	20 0.9 4	*Aeromonas* spp. *Salmonella* spp. *Cryptosporidium* spp.	Simmons et al. (2001)
Nigeria	83 67 67 67	*Pseudomonas* spp. *Salmonella* spp. *Shigella* spp. *Vibrio* spp.	Uba and Aghogho (2000)
U.S Virgin Islands	45 23	*Cryptosporidium* spp. *Giardia* spp.	Crabtree et al. (1996)
U.S Virgin Islands	80	*Legionella* spp.	Broadhead et al. (1988)
Australia	15 26 45 11 19	*Aeromonas* spp. *Legionella* spp. *Campylobacter* spp. *Salmonella* spp. *Giardia* spp.	Ahmed et al. (2008)
Australia	7 8 20 17 15	*Aeromonas* spp. *Legionella* spp. *Campylobacter* spp. *Salmonella* spp. *Giardia* spp.	Ahmed et al. (2010)
Australia	21 4 13	*Campylobacter* spp. *Salmonella* spp. *Giardia lamblia*	Ahmed et al. (2012a)
Australia	32 15 1.5 3	*Aeromonas* spp. *Legionella* spp. *Campylobacter* spp. *Salmonella* spp.	CRC for Water Quality and Treatment (2006)
Denmark	14 7 71 12 7 35	*Aeromonas* spp. *Pseudomonas* spp. *Legionella* spp. *Campylobacter* spp. *Mycobacterium* spp. *Cryptosporidium* spp.	Albrechtsen (2002)
New Zealand	37	*Campylobacter* spp.	Savill et al. (2001)
Palestine	2	*Cryptosporidium parvum*	Abo-Shehada et al. (2004)

Adopted from Ahmed et al. (2011a), Table 1.6 shows the various pathogenic bacteria that have been shown to cause diseases in many individuals. Gastroenteritis associated with the consumption of HRW has been recorded in many cases in literature, with several authors demonstrating the elevated health threat correlated to harvested rainwater (Koplan et al., 1978; Murrell and Stewart, 1983; Schlech et al., 1985; Brodribb et al., 1995; Simmons and Smith, 1997; Merritt et al., 1999; Simmons et al., 2001; Ahmed et al., 2008; Simmons et al., 2008; Franklin et al., 2009;).

Birds nesting in the catchment areas of rainwater collection systems in New Zealand were hypothesised to be causing campylobacteriosis in certain cases where the water was consumed (Eberhart-Phillips et al., 1997). Microbiological studies were undertaken after a group of tourists were reported to have contracted Legionnaire's disease whilst visiting the U.S. Virgin Islands. The exact mode of transmission was not established, but the hotel acquired its potable water from a DRWH system, which seemed to be the most likely cause as an identical serogroup of *Legionella pneumophila* was isolated from the patients, the stored harvested rainwater and the hot and cold water outlets. The hotel then resorted to chlorinating the water and no further outbreaks were reported. *Salmonella mississippi* was implicated in the cause of infections in Tasmania, Australia, after individuals had consumed water from contaminated harvested rainwater tanks (Ashbolt and Kirk, 2006). The salmonellosis was as a result of contaminated harvested rainwater, as it was confirmed that the native animals were not the cause, as was initially assumed.

Table 1.7: Reported cases of disease associated with the consumption of HRW (adapted from Ahmed et al., 2011a).

Country	Pathogenic microorganism	Contracted disease	Individuals affected	Reference
Australia	*C. botulinum*	Not specified	3	Murrell and Stewart (1983)
Australia	*Campylobacter fetus*	Diarrhoea, vomiting	1	Brodribb et al. (1995)
Australia	*Campylobacter* spp.	Diarrhoea, abdominal pain	23	Merritt et al. (1999)
Australia	*S. typhimurium* phage 1	Diarrhoea, abdominal pain, nausea	27	Franklin et al. (2009)
New Zealand	*S. typhimurium* phage 1	Diarrhoea	2	Simmons and Smith (1997)
New Zealand	*L. pneumophila*	Legionnaires' disease	1	Simmons et al. (2008)
U.S. Virgin Islands	*L. pneumophila* sero group 1	Legionnaires' disease	27	Schlech et al. (1985)
West Indies	*S. arechevalata*	Diarrhoea, headache, fever, vomiting	48	Koplan et al. (1978)

Ahmed et al. (2010) suggested that it may be likely that the incidences of gastrointestinal outbreaks associated with RWH tanks are not always reported as not every individual seeks medical advice or attention. In addition most faecal specimens that are collected are not always extensively analysed in hospitals. They also suggested that most communities considered HRW quality equal to potable water and would rather blame other sources for infection before implicating DHRW as the potential source of a disease. It was also reported that only between 8 and 11% of *Campylobacter*- and *Salmonella*-linked food-borne gastroenteritis cases are reported in Australia and only 10 to 33% of gastroenteritis cases associated with water are reported in America. Few cases of disease, which could be associated with RWH tanks, are reported as many communities consider the water to be of a good quality. This implies that detailed monitoring studies need to be undertaken to accurately determine the microbial quality of the rainwater. Where applicable, measures such as the implementation of filtering systems and solar panels can then be used to improve the quality of the water to within drinking water standards.

1.1.2 Methods Used For Disinfecting Water Collected By Domestic Rainwater Harvesting

The WHO strongly discourages the direct consumption of untreated rainwater (WHO, 1997) due to evidence of microbial and chemical contamination (section 1.1.2). Despite the development of natural resistance to certain pathogens in healthy individuals after long-term exposure, water-borne disease outbreaks have been linked to the consumption of contaminated rainwater (Lye, 2002). The treatment of rainwater therefore is of paramount importance and two approaches for treating harvested rainwater will be discussed in this section. In the first approach water is treated directly in the DRWH tank. In the second approach harvested rainwater is removed from the tanks and then treated separately. The treatment of rainwater in rural communities, especially in the developing world, should be inexpensive, simple and easy to use. Boiling, chlorine, slow sand filtration and pasteurization by solar technology have been proposed for treating harvested rainwater. In each case the rainwater is removed from the tank and treated separately (Meera and Ahammed, 2008; Helmreich and Horn, 2009).

1.1.2.1 *Treatment Systems Connected to Rainwater Harvesting Tanks*

Screens and filters are generally employed as a first step to improve the quality of harvested rainwater. Debris that collects on catchment areas not only serves as a source of chemical contamination but also as a nutrient source for bacterial survival and growth. A course leaf screen or fine filter can then be effectively employed, anywhere between the rooftop and the inlet to the rainwater storage tank, to collect the debris and in so doing prohibit the pollutants from entering a DRWH tank. It is however, imperative that the screen or filter can withstand high intensity rainfall, while optimally collecting the rooftop particles or debris. In addition, it is recommended that the filter or screen be durable, easy to clean and cost-effective (Abbasi and Abbasi, 2011). The first flush of harvested rain has a higher concentration of contaminants due to the washing off of particles that were deposited onto the collection surface (Yaziz et al., 1989). By eliminating the first flush of the rainfall event the quality of harvested rain can be improved. First flush diverters operate automatically and are easy to install. Another benefit is that first flush diverters reduce tank maintenance (Helmrich and Horn, 2009). A study in Australia observed that the diversion of the first 1 to 2 mm of rainfall through first flush diverters, harvests water compliant with most of the water quality parameters of the ADWG. Lead and turbidity were the only two parameters that did not comply, but the problem was overcome by diverting approximately the first 5 mm of rainfall water. It was also shown that the concentration of organic matter decreased in the water with increasing volumes of roof runoff (Kus et al., 2010). First flush systems can also be fitted with fibre filters to remove particles and nutrients such as phosphate and nitrogen (Kim et al., 2007).

1.1.2.2 *Application of Settling Tanks, Disinfection and Membrane Filtration for the Treatment of Rainwater Harvesting*

Disinfection systems for treating water directly from a DRWH tank have been investigated. The use of antimicrobial silver ions in combination with a settling tank and conventional filtration has been evaluated by Alder et al. (2011). The system is illustrated in Figure 1.3 and consists of a 450 L settling tank (1), a tank or cistern (2), a stainless steel filter (3), a silver ionizing unit (4) and a refillable filter that contains a mixture of granular activated carbon and Kinetic Degradation Fluxion (KDF) filtration media (5). Nine such systems were installed and evaluated in a rural setting in Mexico. The treatment systems were able to reduce the total coliforms by between 62.5 and 99.9%. The settling tank that serves as a first flush system reduced the chemical oxygen demand (COD) by 77%. The filtering treatment system reduced the COD by a further 41%, although additional settling in the tank could also have played a role. The researchers recommended that the routine cleaning of the treatment system was vital for the effective treatment of rainwater. Currently the role of the antimicrobial silver ions in the disinfection system is being evaluated in a laboratory-scale model alongside further developments for improved treatment efficiencies (Adler et al., 2011).

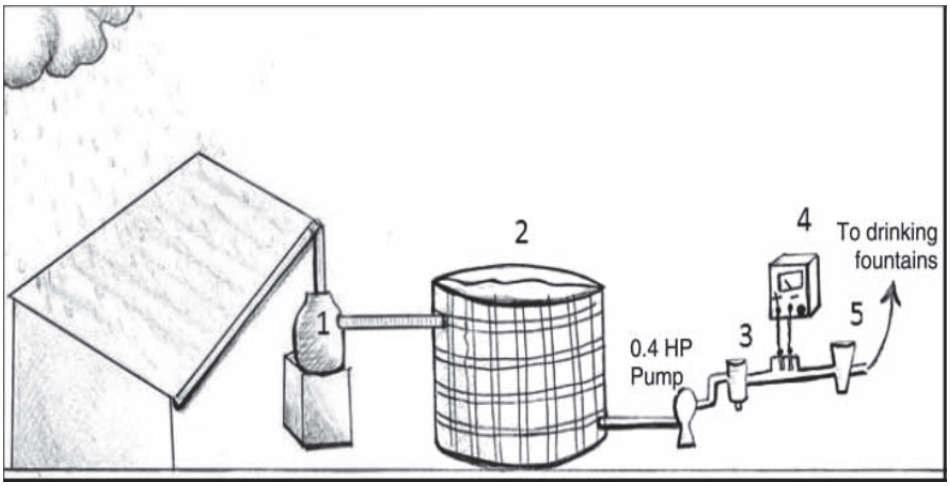

Figure 1.3: A schematic diagram of a rainwater disinfection system that consisted of a stainless steel filter (3), silver ionizing unit (4) and a refillable filter (5) (Adler et al., 2011)

Kim et al. (2005) designed a DRWH disinfection system with a metal membrane submerged into a tank (Figure 1.4). Permeate that flows into the metal membrane was drawn with a peristaltic pump. An ozone generator was installed in the feed side for chemical disinfection of water and to reduce membrane fouling. The ozone treats the organic compounds present in the rainwater through a chemical oxidation process. It was concluded that the system was effective in reducing microbial and particulate pollutants in rainwater. However, membrane fouling due to pore blockage still remained a hurdle during continuous usage (Kim et al., 2003; 2005).

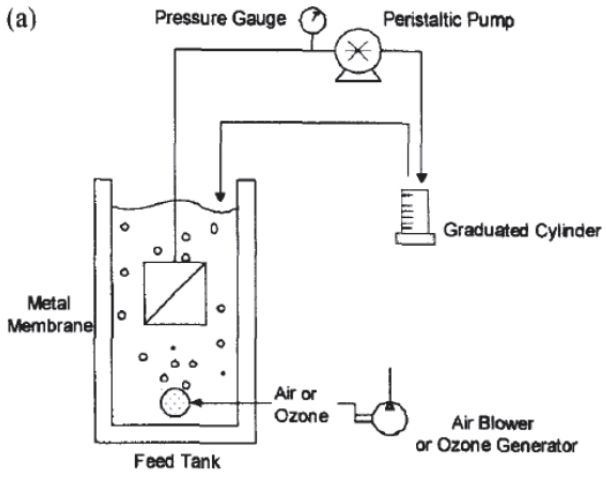

Figure 1.4: A schematic diagram of a submerged metal membrane system combined with ozonation (Kim et al., 2005)

Filtration in combination with UV-disinfection has also been utilised in a DRWH treatment system for privately owned cisterns in the USA. Three filters, namely a 20 µm spun polypropylene progressive density cartridge filter, 5 µm spun polypropylene progressive-density cartridge filter and an activated carbon filter were used. The high capacity ultraviolet steriliser was equipped with a 22 W UV lamp (Figure 1.5). The system was effective in reducing total coliforms, *E. coli* and enterococci numbers but had a marginal impact in reducing total heterotrophic plate counts (Jordan et al., 2008).

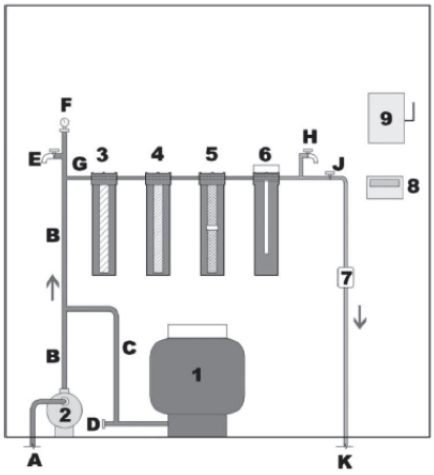

Figure 1.5: A schematic diagram of a point of use rainwater disinfection system that consists of the following: pressure tank (1), pump (2), 20 µm filter (3), 5 µm filter (4), charcoal filter (5), ultraviolet filter (6), backflow preventer (7), controller (8), electrical disconnect (9), 1" pipe to cistern w/foot valve (A), 1" pipe (B), 1" pipe to pressure tank (C), system drain (D), sample tap (E), pressure relief valve (F), ¾" pipe to filters (G), filtered water sample tap (H), cutoff valve (J) and ¾" pipe to house (K) (Jordan et al., 2008)

Granular activated carbon may also be employed in the treatment of rainwater. The activated carbon has a large surface area, which allows for the removal of microbial and chemical pollutants. A laboratory-scale activated carbon treatment system, also based on membrane filtration, was developed by Areerachakul et al. (2009). The pre-filter consisted of granular activated carbon and was used to remove the dissolved organic solids (DOCs) (Figure 1.6A). After the initial formation of a biofilm layer on the activated carbon, the removal efficiency of DOCs was 40, 35 and 15% for bed filter depths of 15, 10 and 5 cm, respectively. The effluent was treated with a hollow fibre membrane microfiltration with a pore size of 0.1 µm (Figure 1.6B). Microfiltration alone reduced DOC by only 10% compared to the 45-50% when used in combination with a biofilter pre-treatment. Microfiltration however, removed all heterotrophic bacteria present in the rainwater. The biofilter was also shown to decrease biofouling of the microfilter system (Areerachakul et al., 2009).

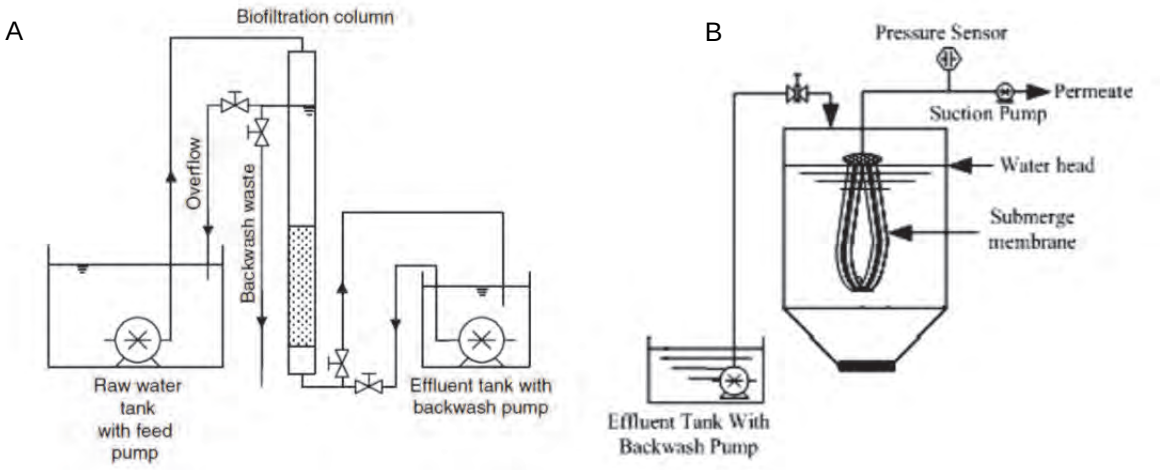

Figure 1.6: Schematic diagrams of the biofilter experimental set-up (A) and of the submerged membrane experimental set-up (B) (Areerachakul et al., 2009).

Treatment of rainwater in hot storage tanks also enables the inactivation of microorganisms (Spinks et al., 2003; 2006a; Despins et al., 2009). Spinks et al. (2006a) suggested that the temperature range of 55 to 65ºC is critical for thermal inactivation of bacterial species due to the presence of heat resistant bacteria. An optimal temperature of 60ºC for hot water systems was thus proposed for the effective elimination of bacteria (Spinks et al., 2006a). Chlorination of water is a common, inexpensive and easily applied method for disinfecting water. It is recommended to chlorinate water after it has been removed from the tank since chlorine may react with organic material present in the sludge that has settled at the bottom of the storage tank and form hazardous by-products (Gordon et al., 1995). The recommended dosage for chlorination of water is 0.4-0.5 mg/L free chlorine for at least 15 minutes. Researchers in Greece successfully treated rainwater in tanker trucks that were used to distribute water to consumers. However, care must be taken with the storage of treated water to prevent re-contamination (Sazakli et al., 2007).

Slow sand filtration is another method used to treat water, especially in the developing world, since it is a simple and inexpensive method. Slow sand filters, also referred to as biofilters, consist of layers of graded sand with the coarsest fraction on the top and the finest at the bottom. A thin biofilm layer on the filter surface is responsible for the filtration efficiency of the filter and therefore it functions as a biological treatment rather than a physical filtration process. A constant flow of water through the filter is essential for a slow sand filter to function effectively (Fewster et al., 2004). The sand can also be coated to further functionalise the filter for enhanced bacteria and heavy metal removal. A dual filter consisting of manganese oxide- and iron hydroxide-coated sand, for the treatment of rainwater, has been investigated (Ahammed and Meera, 2010). Manganese oxide-coated sand has been shown to remove heavy metals from water (Liu et al., 2005) and iron hydroxide-coated sand is effective against microorganisms and turbidity (Ahammed and Chaudhuri, 1996; Chen et al., 1998; Lukasik et al., 1999; Ahammed and Meera, 2006). The dual filter removed 99% of bacteria and 96% of zinc from roof-harvested rainwater. No leaching of iron or manganese was observed during the filtration period. The efficiency of the dual filter was also higher when compared to an iron hydroxide-coated sand filter and uncoated sand filter (Ahammed and Meera, 2010).

The treatment of water with the use of solar irradiation, commonly referred to as solar disinfection (SODIS), is not a new phenomenon. The effectiveness of SODIS in treating microbial contaminated water has been demonstrated in numerous studies (Sommer et al., 1997; Lonnen et al., 2005; Martin-Dominiguez et al., 2005). The simplicity of the technique and the fact that it is inexpensive makes SODIS an ideal method for treating harvested rainwater in rural communities. In Seoul, South Korea, PET (polyethylene terephthalate) bottles were filled with harvested rainwater from underground tanks and exposed to the sun to investigate the efficiency of SODIS. Total and faecal coliforms, *E. coli* and heterotrophic plate counts were used as indicators for water quality. Even under strong weather condition SODIS was ineffective in reducing any of the microbial indicators to below drinking water standards (Amin and Han, 2009a). Strong and weak weather conditions were defined as weather conditions with irradiance ranges of between 650 and 1000 W/m^2 and between 100 and 400 W/m^2, respectively. The same research group repeated the study but placed the PET bottles filled with rainwater samples in a solar collector. The solar collector disinfection (SOCO-DIS) system had a rectangular base and reflective open wings. Disinfection with SOCO-DIS was 20-30% more effective when compared with SODIS even under moderate weather conditions. This was due to the combined effects of sunlight radiation, thermal properties and optical inactivation. SOCO-DIS completely disinfected rainwater with low turbidity under strong weather conditions. The method was more effective if the pH of the rainwater was lowered to 5 with HCl. Re-growth of microorganisms was also lowered during SOCO-DIS (Amin and Han, 2009b).

Individuals at a household level do not have access to HCl and therefore the effect of lowering the pH of harvested rainwater with inexpensive and easy available food products/preservatives was investigated. Vinegar and lemon were used as catalysts during SODIS and SOCO-DIS experiments. Results obtained showed that the addition of either vinegar or lemon increased the efficiency of SODIS by 40%. The addition of vinegar and lemon resulted in the complete inactivation of harvested rainwater during SOCO-DIS, even under weak weather conditions. Vinegar was a more effective catalyst since heterotrophic bacteria were still

observed during SOCO-DIS treatment with lemon (Amin and Han, 2011). A SODIS study in India observed that exposure of roof-harvested rainwater for 6 hours (solar intensity of more than 500 W/m^2) resulted in effectively inactivating all the coliforms present, however heterotrophic bacteria were still present in the rainwater (Meera and Ahammed, 2008).

1.1.2.3 *Natural Treatment Processes within a Rainwater Harvesting Tank*

Biofilms have been proposed as a natural disinfection system in DRWH tanks. Microbial cells, embedded within an extracellular polymeric matrix, can aggregate on a biotic or abiotic surface, and effectively remove metals and organics from the water thereby decreasing the survival rate of planktonic cells (Spinks et al., 2003; 2005). A study by Evans et al. (2009) isolated and identified *Pseudomonas, Shingomonas, Bacillus, Arthrobacter* and *Rhodococcus* species in harvested rainwater. These bacterial communities, through nutrient cycling and other metabolic activities, have been shown to degrade or facilitate the removal of halogenated, aromatic and heavy metal contaminants from different water sources (Aislabie and Lloyd-Jones, 1995; Remoudaki et al., 2003; Jackson et al., 2009).

Although roof runoff can have very high turbidities (Yaziz et al., 1989), levels can be reduced during storage as particulates settle out in a tank within 24 hours. The sludge that forms during this process has been linked to the aforementioned biofilm assisted cleaning process (Spinks et al., 2005). Another natural cleaning mechanism is the precipitation of metal ions out of the rainwater. Rainwater stored in metal tanks may leach calcium from the wall of the tanks which lowers the pH of the water. This in turns leads to dissolved metals precipitating out of the water and settling on the bottom of the tank. Studies have shown that the concentration of metals is higher in the bulk deposition than the water samples collected from RWH tanks (Spinks et al., 2005; Peters et al., 2008; Huston et al., 2012).

1.1.3 Summary

The wide-spread implementation and use of DRWH in South Africa has its limitations. The challenges that the South African Government is faced with include socio-economic pressure, the lack of clear rainwater usage legislations and the need for a national management body that co-ordinates the expansion of roof rainwater harvesting (Mwenge Kahinda and Taigbenu, 2011). In addition, 67% of all rural households are below the poverty line and cannot afford the installation of a rainwater tank (Mwenge Kahinda et al., 2007). Guidelines should be developed for the proper use and maintenance of DRWH, as there is a significant lack of information on the potential public health risks associated with untreated rainwater due to chemical and microbiological contamination (Ahmed et al., 2011a).

In order for DRWH to be sustainable, there needs to be co-operation between the government, private sector and the rural households. The sustainability of the rainwater system can also only be achieved when all the physical attributes (location, rainfall) and the socio-economic attributes are taken into account during the designing of such a system (Mwenge Kahinda et al., 2007). In addition, detailed information on the microbial and chemical quality of rainwater in South Africa must be obtained before the tank systems can be implemented on the national level as a sustainable water source for domestic and irrigation purposes. If the quality of the water however, does not meet potable water standards, various cost-effective treatment systems can be implemented to improve the rainwater quality.

CHAPTER 2: A SURVEY OF THE QUALITY OF WATER COLLECTED IN DRWH IN KLEINMOND HOUSING SCHEME

2.1 INTRODUCTION

DRWH is practised worldwide in many countries such as Australia (Heyworth et al., 1998), Jordan (Rabi and Abo-Shehada, 1995), Bermuda Islands (Lévesque et al., 2008) and Greece (Sazakli et al., 2007) and is often used as an alternative drinking water source. Harvested rainwater does not always meet the standards of the local water authorities' drinking water guidelines and researchers have isolated a range of microbial contaminants from rainwater including *Escherichia coli*, *Aeromonas* spp., *Campylobacter* spp., *Giardia* spp., *Cryptosporidium* spp., *Enterococcus* spp., *Salmonella* spp. and *Legionella* spp., amongst many others (Simmons et al., 2001; Albrechtsen, 2002; Ahmed et al., 2012a, b). Chemical contaminants observed in harvested rainwater, where concentrations exceeded drinking water guidelines, included lead, copper and zinc (Simmons et al., 2001; Lee et al., 2010c; Huston et al., 2012). This raises concern, as often harvested rainwater is utilised for drinking and certain domestic purposes without prior treatment. In 2010 it was reported that rural communities in South Africa predominantly used DRWH for potable purposes (Statistics South Africa, 2010). There is, however, limited information available on the microbial and chemical quality of harvested rainwater in South Africa (Nevondo and Cloete, 1999), and before DRWH can be widely implemented more information on the possible contaminants associated with this water source is required. The aim of this study was to survey the quality of water collected by DRWH. The aim of this project was to assess the microbial and chemical quality of harvested rainwater. The enumeration of total coliforms, *Escherichia coli*, enterococci and *Pseudomonas aeruginosa*, was used to assess the microbial quality of the water. Additionally, microbial indicators, such as total heterotrophic bacteria, total Gram-negative enteric bacteria and *Legionella* spp., were also enumerated. Chemical parameters investigated, included the concentration of metal ions, anions and cations present in the harvested rainwater. The chemical oxygen demand (COD) of the harvested rainwater samples was also determined. The physicochemical parameters investigated included temperature and pH of the rainwater samples.

2.2 MATERIALS AND METHODS

2.2.1 Study site

The Kleinmond Housing Scheme Project an initiative of the Council for Scientific and Industrial Research (CSIR), together with the Department for Science and Technology (DST) was used as a study site for the collection and monitoring of harvested rainwater. The Kleinmond Housing Scheme Project is situated in an urban coastal area in the Western Cape, South Africa (Figure 2.1). The Kleinmond study site consists of 40 m^2 houses, which are part of the Government's initiative to provide low cost sustainable development houses in South Africa and are fitted with alternative technologies such as solar panels and rainwater harvesting tanks (CSIR, 2011; De Villiers, 2011) (Figure 2.2). From a cluster of 411 houses, 29 houses were selected for sampling and the selection process was conducted by consulting with an official from the Kleinmond Municipality. The study site enabled the monitoring of a cluster of 29 DRWH tanks in one primary location.

Figure 2.1: The Kleinmond housing scheme was established in a coastal town, Kleinmond, Western Cape, South Africa.

Figure 2.2: Houses fitted with alternative technologies in the Kleinmond Housing Scheme.

2.2.2 Sample Collection

For the microbial and chemical analysis, water samples were collected in 2 L sterile polypropylene bottles that had been sterilised with 70% ethanol, rinsed with tap water and stored on ice to maintain a low temperature. The temperature and pH of the rainwater at the sampling locations were measured using a hand-held mercury thermometer and colour-fixed indicator sticks with a pH range of 0 - 14 (ALBET®, Barcelona, Spain). Rainfall patterns were obtained from the South African Weather Services (SAWS, 2012). In total eight sampling sessions were conducted for the duration of the study with a total rainfall recorded for each month. Control water samples (i.e. treated municipal water) were collected from the Kleinmond Water Treatment Plant for the first, seventh and eighth sampling sessions. A map of the cluster of houses used in this study is depicted in Figure 2.3.

Figure 2.3: A map of the Kleinmond Housing Scheme (Western Cape, South Africa). GPS coordinates: 34˚20'11.81" S 19˚00'59.74" E. The houses selected for sampling throughout the study are indicated by black circles. Green circles indicate the houses that were replaced with alternative, new houses (red circles) that were sampled from the third and sixth sampling sessions.

The DRWH tanks connected to these houses became unavailable for sampling during the study period due to unforeseen circumstances. As can be seen from the map, there are no obstacles obstructing the roofs, i.e. trees or electrical power lines. While the risk of contamination may still occur, the lack of apparent areas (no trees) for birds and other animals to nest in, may be an added advantage in lowering the risk for the contamination of the rainwater (Ahmed et al., 2011b; 2012a, b). The vertical, polyethylene rainwater tanks, have a capacity of 2000 litres and were installed at the housing sites at the end of 2011 (Figure 2.3) and the tanks were therefore less than a year old at the time of sampling. The catchment area consisted of concrete roof tiles, namely double roman standard plus. No first flush diverters were installed to eliminate the first flush of debris from the roof surface into the tanks. During the low rainfall period sampling was conducted every three weeks (March to May 2012) and thereafter one to four days after a rain event [high rainfall period (June to September 2012)]. Table 2.1 indicates the dates on which samples were collected from the rainwater tanks at the Kleinmond Housing Scheme.

Table 2.1: Sampling dates for the study period March to August 2012.

Sampling Session	Date
1	5 March 2012
2	28 March 2012
3	19 April 2012
4	22 May 2012
5	5 June 2012
6	19 June 2012
7	7 August 2012
8	21 August 2012

The house and sampling numbers were recorded as indicated in Table 2.2. It should be noted that, as indicated in Table 2.2, for sample numbers 8 and 28, the sampling of the rainwater tank at house 8390 was replaced with house 8395 (19 April – third sampling session) and house 8352 was replaced with house 8351 (19 June – sixth sampling session) as indicated by the red circles in Figure 2.3, respectively, due to unforeseen circumstances.

Table 2.2: Sample numbers correlating to the house numbers at the Kleinmond pilot plant used in this study.

Sample Number	House number	Sample Number	House number
1	8220	16	8399
2	8217	17	8402
3	8216	18	8473
4	8212	19	8404
5	8208	20	8466
6	8506	21	8408
7	8387	22	8344
8	8390 replaced with 8395	23	8339
9	8392	24	8345
10	8498	25	8337
11	8497	26	8347
12	8394	27	8335
13	8494	28	8352 replaced with 8351
14	8477	29	8332
15	8401	30	Control

2.2.3 Microbiological Analysis

The microbial quality of the water was assessed in a longitudinal study conducted over a six month period, from March to August 2012, to cover the autumn and winter rainfall period in the region. The enumeration of total coliforms, *Escherichia coli*, enterococci and *Pseudomonas aeruginosa*, was used to assess the microbial quality of the water. Additional microbial indicators added to the study included the enumeration of total heterotrophic bacteria, total Gram-negative enteric bacteria and *Legionella* spp. The isolates collected from the above mentioned culture media were identified using molecular techniques (16S rRNA sequencing). The total number of viable and non-viable cells present in the rainwater samples was also determined by flow cytometry analysis. In addition, the isolation of virulent *E. coli* strains from the harvested rainwater samples, including enteropathogenic *E. coli*, Shiga-toxigenic *E. coli*, enterotoxigenic *E. coli* and extraintestinal *E. coli* (Ahmed et al., 2011) was incorporated into the study. Isolates of these strains were identified using 16S rRNA and DNA sequencing.

2.2.3.1 *Enumeration of Total Heterotrophic Bacteria and Faecal Indicators*

Various conditions and media were used to enumerate *Escherichia coli,* total coliforms, faecal coliforms, enterococci and total heterotrophic bacteria (Table 2.3). Each medium was prepared according to the manufacturer's instructions. Samples were processed within 4 hours of sampling in order to obtain accurate total coliform and *E. coli* counts. For each of the 29 tanks and control samples, an undiluted and diluted (10^{-1}) rainwater sample was spread plated onto various media as indicated in Table 2.3.

Table 2.3: Media and conditions of incubation for the identification of indicator organisms.

Organism/s	Medium	Temperature	Duration of Cultivation (hours)
Heterotrophic Plate Count	Nutrient Agar (Merck)	35 ± 2°C	18 – 24
Total Coliforms	m-Endo Agar (Merck) ChromoCult® Coliform Agar (CCA) (Merck)	35 ± 2°C	18 - 24
Faecal Coliforms	m- FC Agar (Merck)	44.5 ± 0.5°C	22–24
Enterococci	Slanetz and Bartley Agar (Oxoid, Hampshire, England)	36 ± 22°C	44-48

Nine millilitres of 0.9% NaCl was used for the serial dilution with an inoculum of 1 mL. Subsequently 100 µL of the dilution series samples were spread plated onto ChromoCult® Coliform Agar (CCA) (Merck, Biolab, Wadeville, Gauteng) to obtain total coliform and *E. coli* numbers after the plates were incubated at 35 ± 2°C for 18 - 24 hours. For the enumeration of faecal coliforms and enterococci the samples were plated onto the various media within 36 hours of sampling. For faecal coliforms a series dilution was prepared as mentioned above with 100 µL spread plated onto m-FC Agar (Merck) and plates incubated at 44.5 ± 0.5°C for 22–24 hours. From the second sampling session onwards enterococci were enumerated in the same manner as the faecal coliforms; however 100 µL of each dilution of each sample was additionally spread plated onto Slanetz and Bartley Agar (Oxoid, Hampshire, England) and incubated at 36 ± 22°C for 44-48 hours.
In order to analyse the general microbial quality of the rainwater, total heterotrophic bacteria were enumerated through the pour plate method. A serial dilution of each sample was made (as indicated previously) and 1 mL of each dilution, for each sample, was added to Nutrient Agar (NA) (Merck) plates which were then incubated at 37°C for 18 - 24 hours.

2.2.3.2 *Total coliform counts*

Membrane filtration was used to obtain total coliform counts and the procedure was performed in duplicate within 4 hours of sampling. For sampling sessions one and two, undiluted samples were filtered, but no single colonies were visible as the sample plates were overgrown with bacteria, thus a Too Numerous to Count (TNTC) value was obtained. From sampling session three a 1:4 dilution was made of each sample in duplicate. The method consisted of filtering 100 mL (25 mL rainwater sample plus 75 mL sterile distilled water) of each sample 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. The filtration flow rate was approximately ≥ 65 mL/min/cm^2 at 0.7 bar (10 kPa, 10 psi). The filters were then incubated on m-Endo Agar (Merck) at 35 ± 2°C for 18 - 24 hours (Table 2.3) (U.S. Environmental Protection Agency, 2009).

2.2.3.3 *Enumeration of Bacterial Pathogens*

For each rainwater sample an undiluted and diluted (10^{-1}) sample was processed within 36 h, with the collected water samples stored at 4ºC. Table 2.4 shows the conditions under which the various organisms were incubated. For example an undiluted and diluted (10^{-1}) sample was spread plated onto Cetrimide Agar (Merck), and incubated at 35 ± 2°C for 18–24 h, in order to isolate *Pseudomonas aeruginosa*. The dilutions were the same for isolating Gram-negative enteric bacteria on *Salmonella Shigella* Agar (Merck), *Campylobacter* spp. and *Legionellaceae.* Media were prepared according to the manufacturer's instructions.

Table 2.4: Media utilised for the cultivation of various pathogenic bacteria.

Organism	Medium	Temperature	Duration of Cultivation (h)
Pseudomonas aeruginosa	Cetrimide Agar (Merck)	35 ± 2°C	18–24
Gram-negative enteric bacteria	*Salmonella Shigella* Agar (Merck)	35 ± 2°C	18 – 24
Campylobacter spp.	*Campylobacter* Blood-free Selective Medium (Oxoid)	37°C	48
Legionellaceae	*Legionella* CYE Agar base (GVPC) (Oxoid)	35°C	≥ 10 d

<u>*Legionella* spp.</u>

Legionella species are unable to grow on a range of commonly used standard laboratory media. This genus requires special additives such as the amino acid L-cysteine and iron salts. For this reason, glycine, vancomycin, polymyxin B and cycloheximide (GVPC) agar are used which is the *Legionella* CYE Agar base supplemented with two vials. One vial consists of glycine (3 g/l), vancomycin hydrochloride (1 mg/l), polymyxin B sulphate (80000 IU) and cycloheximide (80 mg/l), which was added according to the manufacturer's instructions (Oxoid, SR0152), and the second vial consists of buffer/potassium hydroxide (10 g/l), ferric pyrophosphate (0.250 g/l), L-cysteine HCl (0.4 g/l) and α -ketoglutarate (1 g/l) which was also added according to the manufacturer's instructions (Oxoid, SR0110). The selective detection was then increased by pre-incubating the agar plates at 50°C for 30 min before cultivation (Feeley et al., 1979).

<u>*Campylobacter* spp.</u>

Campylobacter Blood-free Selective Medium (Oxoid) is based on the formulation described by Bolton et al. (1984). Charcoal, ferrous sulphate and sodium pyruvate act as a replacement for blood. Selective supplement SR0155 (Oxoid), which consists of cefoperazone (16 mg/l) and amphotericin B (20 mg/l), was added according to the manufacturer's instructions. Plates were incubated under micro-aerophilic conditions with the use of an Anaeropack® Anaero (Davies Diagnostics, Randburg, South Africa) in a sealed container. This container was incubated at 35°C for approximately 10 d.

2.2.3.4 *Isolation and confirmatory tests of E. coli Isolates*

ChromoCult® Coliform Agar (CC agar) was developed for the simultaneous detection of total coliforms and *E. coli*. Chromogenic substrates that are used to distinguish between *E. coli* strains and total coliforms include P-galactosidase (LAC) and P-glucoronidase (GUS). Total coliforms cleave the Salmon-GAL (6-Chloro-3-indolyl-β-D-galactopyranoside) substrate and this reaction causes a salmon to red colour. *Escherichia coli* can cleave both Salmon-GAL and X-glucuronide and the positive colonies appear as a dark blue to violet colour (Appendix A, Table 1). Tergitol 7 inhibits accompanying flora, but does not affect the growth of coliforms (Manafi, 2000). Rainwater samples (100 µL each of undiluted and 10^{-1} dilution) were spread plated onto the Chromocult® Coliform Agar (Merck). The plates were incubated at 37^0C for approximately 18 - 24 h. *Escherichia coli* colonies were selected and re-streaked onto CC agar for further selection.

2.2.3.5 *Genomic DNA Extractions from Plate Isolates*

Selected isolates, which were collected from the 29 tanks during the sampling period (March to August 2012) utilising the culture media were identified using molecular techniques (16S rRNA sequencing). In addition, the isolation of virulent *E. coli* strains from the harvested rainwater samples (10 tanks within the 29 tank cluster), including enteropathogenic *E. coli*, Shiga-toxigenic *E. coli*, enterotoxigenic *E. coli* and extraintestinal *E. coli* was incorporated into the study. Isolates of these strains were identified using 16S rRNA and DNA sequencing. Once all the desired organisms were isolated based on colour reactions and morphological characteristics, various techniques were used to isolate genomic DNA. The isolates were re-streaked onto Nutrient Agar at least three times before glycerol stocks were made of each isolate. For this, colonies were selected and inoculated in 5 mL of Nutrient Broth (Merck) and 750 µL of the culture was added to 750 µL of 80% glycerol (Saarchem). To extract total genomic DNA from the isolates, a modified version of the boiling method proposed by Watterworth et al. (2005) was used.

A single colony was inoculated into Luria Bertani (LB) broth (Merck) and was grown at 37^0C for 12 h. Cells were harvested from 1 mL of the cell suspension by centrifuging at 14 000 rpm for 10 min. The supernatant was discarded and the pellet was re-suspended in 100 µL sterile MilliQ water. The re-suspended cells were then boiled at 95^0C for 15 min, followed by cooling on ice for 10 min. The sample was subjected to centrifuging at 14 000 rpm for 5 min and the supernatant was transferred to a sterile eppendorf tube. The genomic DNA was then visualised on a 0.8% agarose gel stained with 0.5 µg/mL ethidium bromide. Electrophoresis was conducted at 80 volts for approximately an hour with the use of Tris/Borate/EDTA (TBE) buffer (Sambrook et al., 1989). Extraction of DNA was also performed using the ZR™ Soil microbe DNA Miniprep Kit (Zymo Research). Genomic DNA was isolated according to the manufacturer's instructions.

2.2.3.6 *Molecular identification of isolates*

Once genomic DNA had been extracted from the various isolates, polymerase chain reactions (PCR) were used to amplify the 16S rRNA conserved sequence (Table 2.5). The PCR mixture consisted of a final volume of 50 µL and contained 10 µL of 5X Green GoTaq® Flexi Buffer (Promega) (1X), 4 µL $MgCl_2$ (2.0 mM), 0.5 µL of each dNTP (0.1 mM) (Thermo Scientific, Lithuania), 2.5 µL of each PCR primer (0.5 µM) (Table 2.5), and 0.3 µL (1.5 U) of GoTaq® Flexi DNA Polymerase (Promega, Madison, USA). A DNA Thermal Cycler (Bio-Rad, USA) was used. Amplification was performed using an initial template denaturation step at 94°C for 3 min and then 30 cycles of denaturation at 94°C for 30 s, annealing at 53°C for 30 s and extension at 72°C for 1.5 min, extension was conducted for 5 min at 72°C (Rawlings, 1995). The annealing temperature of the *E. coli* isolates was changed to 59°C to decrease nonspecific binding.

Table 2.5: Sequences of universal primer sets utilised (Rawlings, 1995).

Organism	Primer name	Primer sequence (5'-3')	Gene (Size)
Universal	fDD2 rPP2	CCGGATCCGTCGACAGAGTTTGATCITGGCTCAG CCAAGCTTCTAGACGGITACCTTGTTACGACTT	16S rRNA (1.6 Kb)

PCR products were analysed by gel electrophoresis in 2% agarose (Bio- Rad) containing 0.5 µg/mL ethidium bromide in TBE buffer. DNA bands were confirmed by UV illumination and photographed using the Gel Doc 1000 documentation system (Bio-Rad). Once the size and the concentration of the PCR products had been confirmed, the products were cleaned and concentrated using the DNA Clean & Concentrator™-5 Kit (Zymo Research) as per manufacturer's instructions. The cleaned products were then sent to the Central Analytical Facility at Stellenbosch University for sequencing. Sequences were then aligned and analysed using DNAman TM version 4.1.2.1 software. Sequence analysis (16S rRNA) was completed using the National Center for Biotechnology Information (NCBI) and The Basic Local Alignment Search Tool (BLAST) (http://blast.ncbi.nlm.nih.gov/Blast.cgi) to find the closest match of local similarity between isolates and the international database in GenBank, EMBL, DDBJ, and PDB sequence data.

2.2.3.7 *Phylogenetic analysis*

Phylogenetic trees of the results obtained for the 16S rRNA sequences were analyzed as outlined in Jackson et al. (2009). The sequences of representative isolates, that showed > 97% similarity (<3% diversity) to organisms recorded on the international databases, such as Genbank, were used in the construction of the phylogenetic trees. The 16S rRNA sequences were aligned using the default settings and BLOSUM matrix (for the correction of multiple base changes) of Clustal X (1.81) (Higgins and Sharpe, 1988). To calculate the distances of relatedness between each sequence, unrooted trees were assembled using the neighbor-joining method and Maximum Composite Likelihood function (Saitou and Nei, 1987). Phylogenetic analysis according to Tamura et al. (2004) was done using the program Molecular Evolutionary Genetics Analysis Version 3.1 (MEGA version 3.1) where bootstrap values were set at 1000. Positions that contained missing data were eliminated from the dataset using the complete deletion option.

2.2.4 Chemical Analysis

Metal and anion concentrations were determined for the first sampling session. For the determination of the metal concentrations, Falcon™ 50 mL high-clarity polypropylene tubes containing polyethylene caps were pre-treated with 1% nitric acid before sampling. The concentrations of metals such as aluminium (Al), vanadium (V), chromium (Cr), manganese (Mn), iron (Fe), copper (Cu) and zinc (Zn), amongst others, were determined. Metal concentrations were determined using inductively coupled plasma atomic emission spectrometry (ICP-AES) according to Saleh et al. (2000) and nitric acid digestion. All chemical analyses were performed at the Central Analytical Facility (CAF), Stellenbosch University. Anions detected included chloride (Cl), nitrate (NO_3) and sulphate (SO_4). High-Performance Ion Chromatography (HPIC) was used to determine the concentration of anions. The concentration of organic compounds in the water samples was also determined for the last sampling session. Water samples were sent to the CAF, in order for the Chemical Oxygen Demand (COD) to be determined for each rainwater sample.

2.2.5 Statistical Analysis

The data obtained from the microbial and chemical analysis of the collected rainwater samples was assessed using the statistical software package Statistica™ Ver. 11.0 (Stat Soft Inc, Tulsa, USA). In each

data set, analysis of the residuals revealed that the data was not normally distributed, which pointed to the requirement for the Spearman Rank Order Correlation as non-parametric correlation technique to test the significance of the data set. In this test, a Restricted Maximum Likelihood solution (REML) with type Ш decomposition was performed on all data recorded to establish whether or not there was variation between sampling sessions. Once it was established that variation was indeed present, Variance Estimation, Precision and Comparison (VEPACK) analysis was performed, however the data for pH, temperature and average rainfall were set as fixed variables and time and sample were set as grouping variables. Data pairs that showed significant differences were subsequently further analysed using the Least Squares Difference (LSD) test and probabilities for Post-hoc pair-wise comparisons. Data that did not present variation was not analysed using this method. For example, data obtained for faecal coliforms was analysed by applying the Repeated measures ANOVA, using Holm's Sequential Bonferroni method. In all hypothesis tests, a significant level of 5% was used as standards (Dunn and Clark, 1974). In all tests a P-value smaller than 0.05 was considered as statistically significant.

2.3 RESULTS AND DISCUSSION

2.3.1 Rainfall

The overall rainfall patterns recorded for Kleinmond during the sampling period were obtained from the South African Weather Services (2012). Initially sampling sessions were conducted every three weeks, and once the rainfall events had started to increase (during the rainy season); sampling sessions were performed three to four days after a rain event. Table 2.6 indicates the total rainfall for each month (March to August) during the sampling period.

Table 2.6: The total rainfall for each month (March to August) during the sampling period.

Month	Total rainfall (mm per month) during the Sampling Period
March 2012	16.8
April 2012	56.5
May 2012	30.6
June 2012	74.7
July 2012	90.7
August 2012	198.1

The total monthly rainfall (mm) pattern observed for sampling periods 1 to 4 (March 2012 to May 2012) was lower than sampling periods 5 to 8 (June 2012 to August 2012). An increase of water and debris flowing into the tanks before the fifth to eight sampling sessions would thus have been expected. It is also expected that the contaminants, such as debris, bird droppings etc., that would have collected over the summer and autumn months would be washed into the tanks during the rainy season as no first flush diverters were installed. A study in Australia observed that diverting the first 2 - 5 mm of rain with the use of flush diverters improved the quality of the harvested rainwater by lowering the concentration of lead and organic matter (Kus et al., 2010). As the rain continued to fall during the rainy season, the bacterial numbers could have either decreased due to the inflow of rain diluting the water in the tanks and so diluting the amount of bacteria, or increased due to added debris, collected during the winter months, being flushed into the tanks.

2.3.2 Microbiological quality of harvested rainwater

Where applicable, the counts obtained in this study for each indicator group were compared to the drinking water standards stipulated by the SANS 241 (SABS, 2005), the World Health Organisation (WHO, 2011), the South African Water Quality Guidelines for Domestic Water Use of the Department of Water Affairs and Forestry (DWAF, 1996) and the Australian Drinking Water Guidelines (ADWG) (NHMRC and NRMMC, 2011). The percentages of the rainwater samples collected throughout the study that exceeded the DWAF (1996) guideline are indicated in Table 2.7.

Table 2.7: Indicator bacteria used to determine the quality of harvested rainwater as indicated by drinking water standards.

Indicator	DWAF standard (CFU/100 mL)	Samples exceeding standards (%)
Total Coliforms (*SP)	≤5	90
Total Coliforms (*MF)	≤5	97
E. coli (*SP)	0	38
E. coli (*MF)	0	60
Faecal Coliforms	0	38
Enterococci	0	8
HPC	10000	96

*MF: Membrane Filtration technique
*SP: Spread Plate method

2.3.2.1 *Total Coliforms*

Total coliform counts, utilising the spread plate technique, for the first to fourth sampling period (low average rainfall recorded) are represented in Figure 2.4. On average the total coliform counts recorded during this period ranged from 5.96×10^4 CFU/100 mL (sampling one) to 1.03×10^5 CFU/100 mL (sampling four), while for sampling five to eight (results not shown), where the rainfall events started to increase, on average the spread plate counts ranged from 1.75×10^4 CFU/100 mL in the fifth sampling period to 5.56×10^4 CFU/100 mL in the eighth sampling period. Overall the total coliform results obtained, by spread plating onto ChromoCult® Coliform Agar, for sampling periods one to four were higher than the average total coliform counts obtained in sampling periods five to eight, with no total coliform counts (0 CFU/mL) also recorded sporadically throughout the last four sampling sessions for numerous tanks. For all the rainwater samples collected from the DRWH tanks (1 to 29) for sampling one to eight, 90% of the total coliform counts then exceeded the recommended values as stipulated by the DWAF (1996) drinking water guidelines (Table 2.7).

Total coliform counts were also enumerated utilising m-Endo Agar (Merck, Biolab Diagnostics) and membrane filtration (Table 2.8). For the first two sampling sessions, undiluted samples of 100 mL were filtered. The number of total coliforms could however, not be distinguished as the filters were over grown with bacteria and the values were recorded as > 250 CFU/100 mL (results not presented). From the third to the eighth sampling period a 1:4 dilution was performed and coliform numbers were recorded for most samples by membrane filtration, however the filters for a few rainwater tank samples were consistently overgrown with bacteria and results were recorded as > 250 CFU/100 mL.

Figure 2.4: Enumeration of total coliform counts (CFU/mL) in the rainwater samples (Kleinmond) obtained for sampling one to four (March to May 2012) utilising the spread plating technique (ChromoCult® Coliform Agar).

Table 2.8: Total coliform counts obtained through membrane filtration for the DRWH tanks sampled in the Kleinmond Housing Scheme.

Total Coliforms (CFU/100 mL)						
Sample	**3rd Session**	**4th Session**	**5th Session**	**6th Session**	**7th Session**	**8th Session**
1	368	8	1000	1152	1000	560
2	1000	148	2304	1000	1344	0
3	1080	0	1888	624	960	288
4	416	24	216	1000	1280	124
5	288	0	2176	704	1120	656
6	300	576	2624	1000	964	3456
7	176	20	1024	1056	512	392
8	0	8	1408	672	1000	1024
9	1000	12	1216	800	176	1024
10	1000	768	1504	768	192	24
11	1000	124	56	960	120	20
12	536	408	1280	1216	928	192
13	1000	0	1984	1152	1000	240
14	1000	1056	2944	352	928	416
15	1000	0	208	832	1184	2656
16	1000	112	1760	1024	1024	48
17	24	52	2112	1280	768	1984
18	76	0	2048	1600	240	976
19	656	0	1000	1000	432	624
20	928	724	3264	1056	1440	808
21	976	624	1952	1312	800	144
22	1000	1000	1000	1248	1000	992
23	268	160	1312	480	800	432
24	120	496	496	1024	1000	2304
25	1000	52	52	960	1152	1184
26	1000	1000	1000	960	672	32
27	160	16	16	320	848	432
28	268	24	24	608	120	2080
29	176	1000	1000	1120	576	24

For samplings one, two, five, six and seven, all the rainwater samples collected from tanks 1 to 29 yielded total coliform numbers above the standards recommended by DWAF (1996) and the ADWG (NHMRC and NRMMC, 2011). During sampling three and eight, total coliforms present in 97% of the rainwater samples were above the guidelines, while 79% of the counts were higher than the guidelines during sampling four. Throughout the whole sampling period (one to eight) 97% of the rainwater samples exceeded the DWAF (1996) recommended guideline for total coliforms (Table 2.7). With the exception of a few samples, overall the results recorded for total coliforms utilising the spread plate technique (ChromoCult® Coliform Agar) and membrane filtration (m-Endo Agar) significantly exceeded ($p < 0.05$) the stipulated guidelines (DWAF, 1996). High total coliforms counts indicate that the general sanitary quality of the water is compromised and should not be used for potable purposes (DWAF, 1996). These results correlate to previous studies, conducted on the quality of rainwater, where high total coliform counts were recorded in harvested rainwater samples (Spinks et al., 2006; Lévesque et al., 2008; Al-

Salaymeh et al., 2011). Sazakli et al. (2007), found that coliforms were present in 80.3% of all of their rainwater samples analysed, with the authors indicating that microbial and chemical parameters exhibited seasonal fluctuations. From a pilot study, Spinks et al. (2006) found that 90% of their 49 samples analysed were contaminated with total coliforms. They also hypothesised that there was no significant relationship between the levels of microbial indicator organisms and the use of first flush diverters, cleaning the gutters or cleaning the holding tank.

2.3.2.2 *Escherichia coli*

The total *E. coli* counts obtained in CFU per 100 mL for sampling one to four (the graph for samplings five to eight is not presented) utilising the spread plate technique with ChromoCult® Coliform Agar are presented in Figure 2.5. For the first to fourth sampling period, on average the spread plate *E. coli* counts ranged from 2.5×10^3 CFU/100 mL to 2.0×10^3 CFU/100 mL, while during the fifth to eighth sampling periods lower *E. coli* counts were obtained overall with averages ranging from 1.0×10^2 CFU/100 mL (sampling five) to zero *E. coli* detected during sampling eight. Significantly high ($p < 0.05$) *E. coli* counts were also recorded during sampling sessions one and four, with the highest count of 1×10^4 CFU/100 mL recorded for numerous tanks (9, 11, 12 and 29) during sampling four (Figure 2.5). According to the DWAF (1996) ADWG (NHMRC and NRMMC, 2011) and WHO (2011), *E. coli* should not be present in water sources utilised for drinking purposes, however utilising the spread plate technique and Chromocult® Coliform agar, 38% of all the samples exceeded the recommended drinking water guidelines of 0 CFU/100 mL, as stipulated by DWAF (2006), the ADWG (NHMRC and NRMMC, 2011) and the WHO (2011) (Table 2.7). Results for the DRWH tanks also varied with fluctuating *E. coli* counts obtained during the respective sampling periods and between sampling occasions.

The membrane filtration (MF) technique (utilising m-Endo agar) was also utilised to enumerate the *E. coli* counts. The MF technique is a routine monitoring technique applied by local municipalities and water treatment facilities for the monitoring of the microbial quality of drinking water. Compared to the multiple tube fermentation technique, it is more accurate, time- and cost-effective. The inability of the MF to recover coliforms that have been injured or stressed can however be a disadvantage. Exposure to chemical treatment such as chloride can also cause sub-lethal damage to the cells, preventing the cell forming a colony on the selective media (Rompré et al., 2002).

While low *E. coli* counts were detected in sampling two using ChromoCult® Coliform Agar, no *E. coli* was detected during this sampling period using membrane filtration and m-Endo agar. Throughout sampling one to four numerous tanks also had no *E. coli* present with 23% of the DRWH tanks sampled exceeding the drinking water guidelines. During sampling five to eight, *E. coli* counts ranged from 6.0×10^1 CFU/100 mL to 7.0×10^1 CFU/100 mL, respectively (Figure 2.6). The lowest *E. coli* counts of 0 CFU/100 mL (utilising m-Endo and the membrane filtration technique) were recorded in sampling periods five (tank 7 and 28), six (tank 2) and seven (tank 1 and 8), while the highest count of 2.2×10^2 CFU/100 mL was recorded in sampling five (tank 21). However overall, for sampling five to eight, the highest *E. coli* counts utilising the membrane filtration technique were obtained for sampling eight, while the lowest counts were obtained for sampling seven. In addition, 96% of the tanks sampled exceeded the recommended *E. coli* count of 0 CFU/mL during this sampling period (five to eight), with 60% of the DRWH tanks sampled overall (sampling one to eight) exceeding the stipulated guideline using the membrane filtration technique (Table 2.7). Generally the *E. coli* results obtained, by spread plating onto ChromoCult® Coliform Agar, for sampling periods one to four, were higher than the average *E. coli* counts obtained during the same sampling period using the membrane filtration technique.

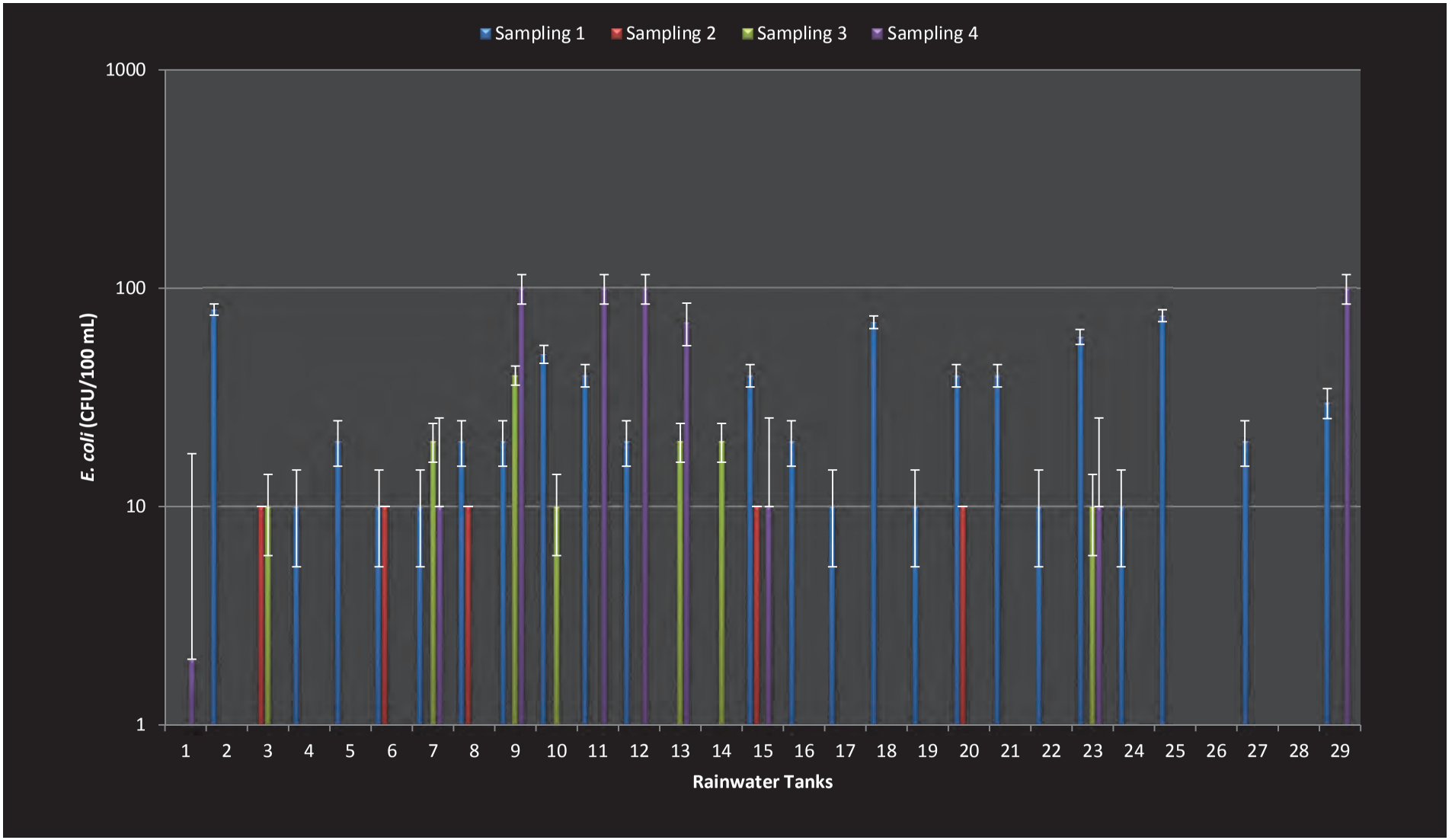

Figure 2.5: Enumeration of *E. coli* numbers (CFU/100 mL) in the rainwater samples (Kleinmond) obtained for sampling one to four utilising the spread plating technique (ChromoCult® Coliform Agar).

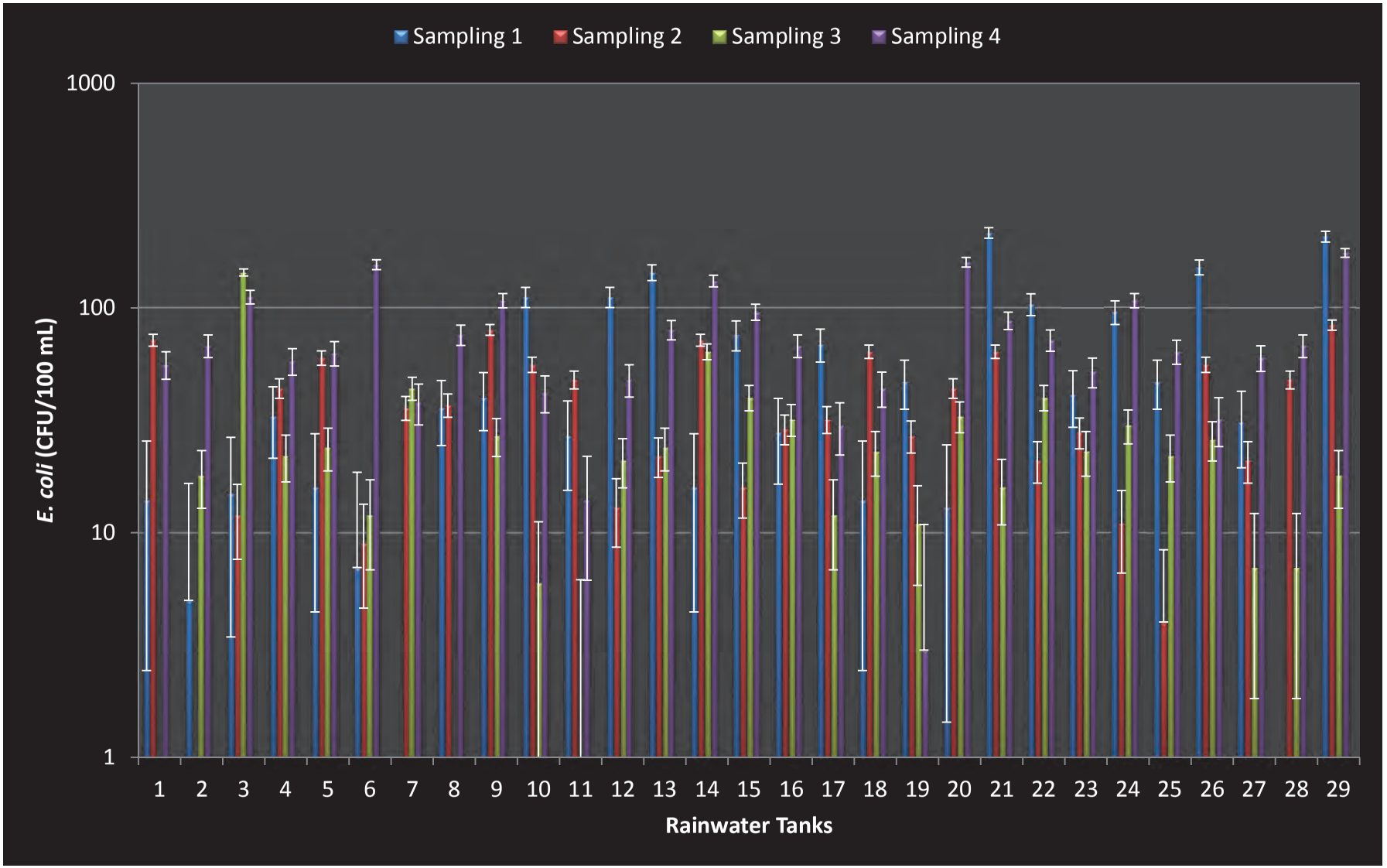

Figure 2.6: Enumeration of *E. coli* numbers (CFU/100 mL) in the rainwater samples (Kleinmond) obtained for sampling five to eight utilising membrane filtration (mEndo agar).

The multivariate tests of significance and one way ANOVA revealed significant variation ($p < 0.05$) between *E. coli* numbers utilising the two techniques (membrane filtration and the spread plate technique). For the entire sampling period (March to August 2012) the lowest average *E. coli* count recorded, using the spread plating technique was obtained in sampling eight (0 CFU/100 mL), while the highest average count was obtained in sampling one (2.5×10^3 CFU/100 mL). While results fluctuated between the sampling occasions, high *E. coli* counts were also recorded for tanks 9 and 29 (sampling one to eight). In contrast, for the membrane filtration technique, the lowest average *E. coli* count was obtained in sampling two (0 CFU/100 mL) with the highest average count obtained in sampling eight (7.0×10^1 CFU/100 mL). While the *E. coli* results also fluctuated between sampling occasions, the highest count was similarly recorded for tank 29 (sampling one to eight).

Escherichia coli is included as a specific indicator organism of faecal pollution from warm-blooded animals (Pinfold et al., 1993; Rompré et al., 2002; Sazakli et al., 2007). This study therefore suggests that the water should not be used for drinking purposes as faecal contamination from warm-blooded animals may be present in the harvested rainwater tanks (DWAF, 1996). The enumeration of *E. coli* in numerous studies also varied, with Spinks et al. (2006) indicating that *E. coli* was present in 33% of the samples analysed, Ahmed et al. (2012a) indicating that *E. coli* was present in 63% of the collected rainwater samples and Albrechtsen (2002) finding *E. coli* in 79% of the samples tested. Ahmed et al. (2012a) also found that wild animals, such as possums and birds could be the main contributors to faecal contamination in DRWH tanks.

2.3.2.3 *Faecal Coliforms*

As total coliforms do not necessarily represent the contamination of faecal origin, the presence of faecal coliforms (FC) (also referred to as thermotolerant coliforms) was also monitored, with the results obtained for the rainwater samples collected from tanks one to twenty-nine, for sampling two to eight represented in Figure 2.7. For the second to eighth sampling period, on average the spread plate counts for FC ranged from 6.9×10^1 CFU/100 mL to 1.2×10^3 CFU/100 mL, respectively. For numerous tanks throughout the entire sampling period, no faecal coliforms were recorded, while the highest count of 2.8×10^4 CFU/100 mL was recorded in sampling seven (tank 21).

The drinking water standards, according to DWAF (1996), the SANS 241 for drinking water (SABS, 2005), WHO (2011) and ADWG (NHMRC and NRMMC, 2011) stipulate that there should be no faecal coliforms present if the water is to be used for potable purposes. While during the second sampling, the majority of the tanks had no faecal coliforms present, 6.9% of the rainwater samples did not conform to the standards stipulated by DWAF (1996), SANS 241 (SABS, 2005), WHO (2011) and the ADWG (NHMRC and NRMMC, 2011) with FC numbers exceeding acceptable levels. Of the rainwater samples analysed in sampling three and four, 34.5% and 41.4% had FC numbers that did not comply with the respective drinking water guidelines, while during the fifth, sixth, seventh and eighth sampling periods 34.5%, 55.2%, 51% and 41.4%, respectively, of the samples collected from the rainwater tanks had faecal contamination above the stipulated standard.

A total of 37.9% of the rainwater tanks thus contained elevated faecal coliform numbers (numbers above the standards as mentioned previously), which implies that these tanks may possibly be contaminated with faecal pollution and are therefore not suitable for potable purposes (DWAF, 1996). A study conducted by Despins et al. (2009) in Canada however, found that only 14% of 360 samples analysed had FC contamination and observed that cold weather significantly improved the microbial quality of harvested rainwater. Similarly, Handia et al. (2003) also found that in their study conducted in Zambia, 14% of their samples showed elevated levels of FC from rainwater stored in ferrocement tanks.

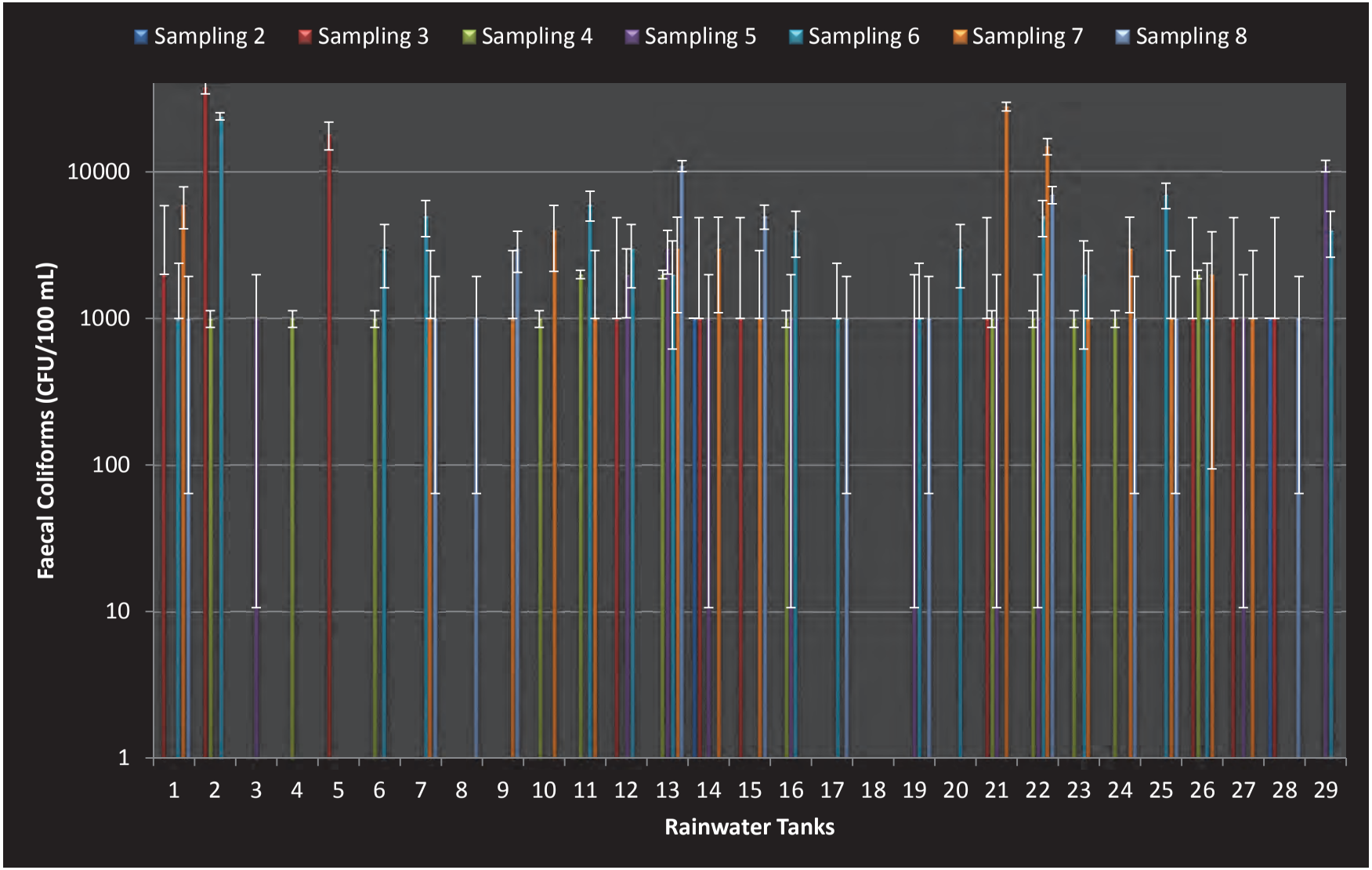

Figure 2.7: Enumeration of faecal coliform numbers (CFU/mL) in the rainwater samples (Kleinmond) obtained for sampling two to eight (March to August 2012) utilising the spread plating technique (m-FC Agar).

2.3.2.4 *Enterococci*

Enterococci results were obtained by spread plating and culturing the rainwater samples on Slanetz and Bartley Agar. No enteroccoci counts were recorded for samplings two, five and six and very few tanks contained significant enterococci counts in sampling three, seven and eight, with the highest average count of 8.9×10^2 CFU/100 mL recorded in sampling four. Enterococci should not be present in water samples according to guidelines stipulated by DWAF (1996) and the ADWG (NHMRC and NRMMC, 2011). During samplings three and four, 3.4% and 34.5%, of the rainwater samples, respectively, exceeded this specified guideline value, while in sampling seven and eight, 6.9% and 10.3% of the samples, respectively, exceeded the enterococci standards. Enterococci, including predominantly faecal streptococci, originate from human or animal faeces. Therefore enterococci serve as an indicator of faecal pollution but are present in lower numbers than total and faecal coliforms. Based on the results obtained the faecal streptococci contamination in a majority of the DRWH tanks sampled was thus below the stipulated guidelines (DWAF, 1996; NHMRC and NRMMC, 2011) as on average only 7.9% of the rainwater tanks sampled during March to August 2012 had elevated numbers of enterococci present. These numbers were however; lower than the enterococci counts recorded in harvested rainwater samples in many other studies (Spinks et al., 2006; Ahmed et al., 2008; Ahmed et al., 2012c). For example Ahmed et al. (2012c), found that 83% of 100 DRWH tanks sampled did not conform to enterococci standards set for drinking water purposes as the rainwater samples were contaminated with various *Enterococcus* species including *E. faecalis, E. mundtii, E. casseliflavus, E. faecium, E. hirae, E. avium,* and *E. durans,* all of which contained virulence genes.

2.3.2.5 *Heterotrophic Plate Count*

HPC results obtained are represented in Figure 2.8. Repeated measures ANOVA was then used to analyse and compare results obtained for HPC. For the first to fourth sampling period, on average the pour plate counts ranged from 6.8×10^4 CFU/100 mL in the first sampling period to 4.6×10^5 CFU/100 mL in the fourth sampling period. Overall, for sampling one to four, the highest average HPC counts utilising the pour plate technique were obtained for samplings four while the lowest counts were obtained for sampling one. For sampling period five to eight, on average the counts ranged from 6×10^4 CFU/100 mL in the fifth sampling period to 4.3×10^5 CFU/100 mL in the eighth sampling period. Overall, for sampling five to eight, the highest average HPC counts were obtained for sampling eight while the lowest counts were obtained for sampling five. The DWAF (1996) and ADWG (NHMRC and NRMMC, 2011) guidelines stipulate that the heterotrophic bacteria should not exceed 100 CFU/mL.

During the first sampling, 100% of the rainwater samples exceeded these standards. The percentage of rainwater samples where the HPC count exceeded the acceptable levels, as stipulated by the respective guidelines, were 100, 93.1, 100, 79.3, 100, 100, and 96.6% for the second, third, fourth, fifth, sixth, seventh and eight sampling, respectively. During this study 96.12% of the rainwater tanks sampled thus exceeded the drinking water standards (Table 2.7) due to elevated heterotrophic bacterial numbers recorded. Even though the percentage of HPC numbers that exceeded the guidelines was lower during sampling five in comparison to the other seven sampling sessions, the differences between HPC values recorded for all sampling sessions was not significant ($p = 0.52$). With the exception of sampling five, on average the heterotrophic numbers in the present study were comparable to a number of previous studies conducted. Evans et al. (2006) found that all of the 67 rainwater samples collected had an elevated HPC with the same results observed by Albrechtsen (2002), Uba and Aghogho (2000) and Lye (1987) where again, all of their samples contained elevated numbers of heterotrophic bacteria.

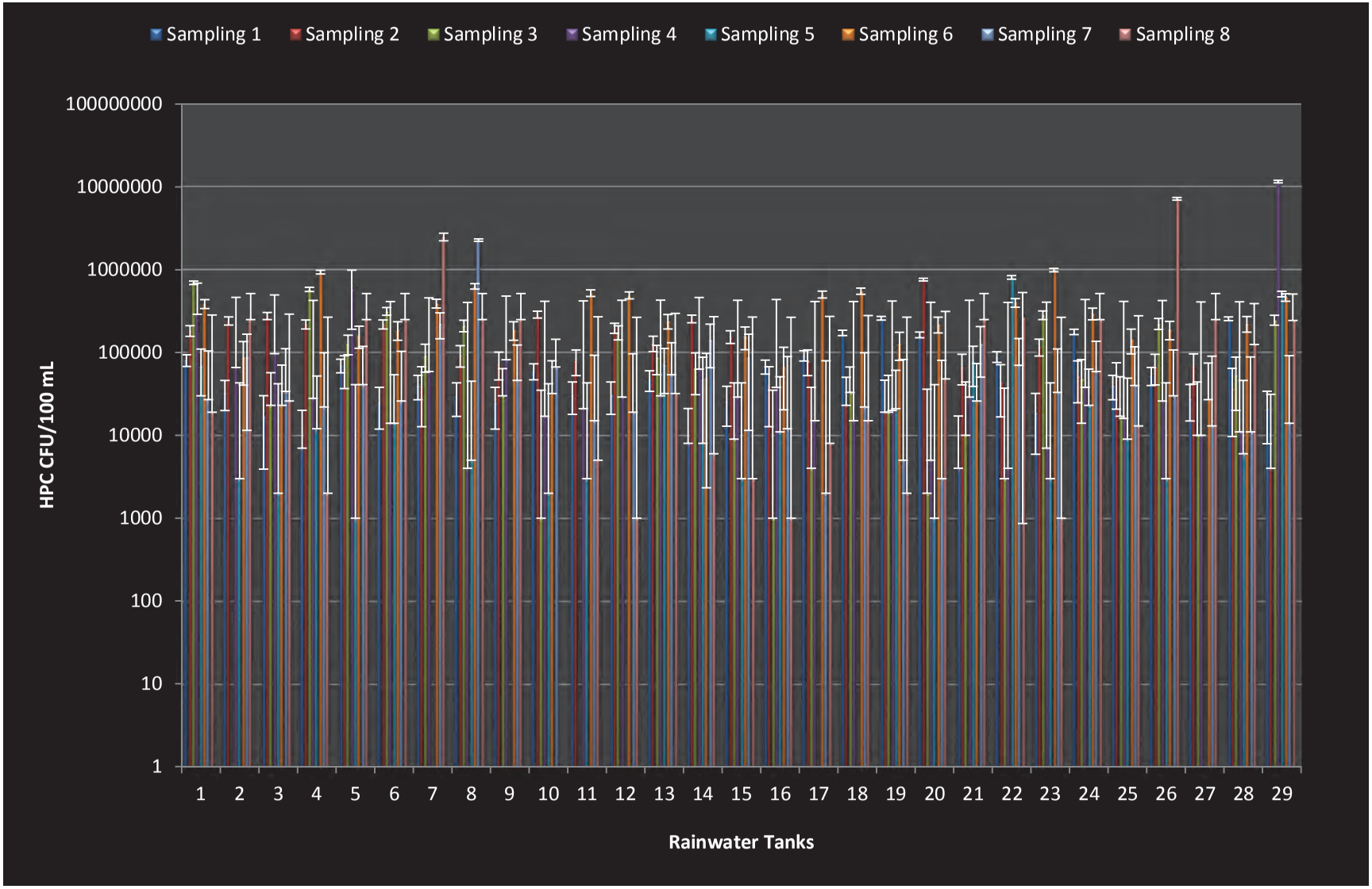

Figure 2.8: Enumeration of heterotrophic bacteria (CFU/100 mL) in the rainwater samples (Kleinmond) obtained for sampling one to eight using the pour plate technique (Nutrient Agar).

2.3.2.6 *Enumeration of Bacterial Pathogens Present in the Rainwater Samples*

Pseudomonas spp.

No bacterial colonies were observed on the Cetrimide Agar plates during the course of this study. A study in Kefalonia Island, Greece, was also unable to detect *Pseudomonas* spp. in the 156 harvested rainwater samples with the use of culture methods (Sazakli et al., 2007).

Gram negative enteric bacterial pathogens

Results obtained for Gram Negative Bacteria (GNB) by spread plating and culturing the rainwater samples on *Salmonella Shigella* (SS) Agar are represented in Figure 2.9 (sampling one to four). For the first to fourth sampling period, on average the spread plate counts ranged from 3.2×10^4 CFU/100 mL in the first sampling period, to 1.2×10^5 CFU/100 mL in the fourth sampling period. The lowest average GNB count recorded in the first four sampling periods was obtained in sampling two with an average of 1.5×10^3 CFU/100 mL, while the highest average count of 1.2×10^5 CFU/100 mL was recorded in sampling four. A significantly high ($p < 0.05$) GNB count was also obtained in sampling four for tank 29 (3×10^6 CFU/100 mL).

For sampling period five to eight, on average the counts ranged from 5.5×10^3 CFU/100 mL in the fifth sampling period to 5.4×10^4 CFU/100 mL in the eighth sampling period. Low GNB counts of 0 CFU/100 mL was recorded throughout sampling periods five to eight, for numerous tanks while the highest count of 2.5×10^5 CFU/100 mL was recorded in sampling five for tank 5, 6, 9, 21, 24 and 27. However overall, for sampling five to eight, the highest average GNB counts were obtained for sampling eight while the lowest counts were obtained for sampling six.

Overall the GNB results obtained for sampling periods one to four were higher than the average GNB counts obtained in sampling five to eight. For the entire sampling period (March to August 2012) the lowest average GNB count recorded was obtained in sampling six, and the highest average count was obtained in sampling four. The highest GNB count was also recorded in tank 29 (sampling one to eight). *Salmonella Shigella* Agar has been used by other researchers to enumerate Gram negative bacteria present in environmental water samples including river water (Shittu et al., 2008) and rainwater samples (Akharaiyi et al., 2007). Shittu et al. (2008) made use of, amongst others, *Salmonella Shigella* Agar to analyse river water samples with different proximities to a refuge dump site in Southwest Nigeria. With the use of Gram staining and conventional biochemical tests they were able to confirm presumptive colonies. Another research group, Akharaiyi et al. (2007) in the Ondo State of Nigeria made use of, amongst others, *Salmonella Shigella* agar to test the quality of rainwater samples and isolated *Shigella dysenteriae* from the rainwater samples utilising this media.

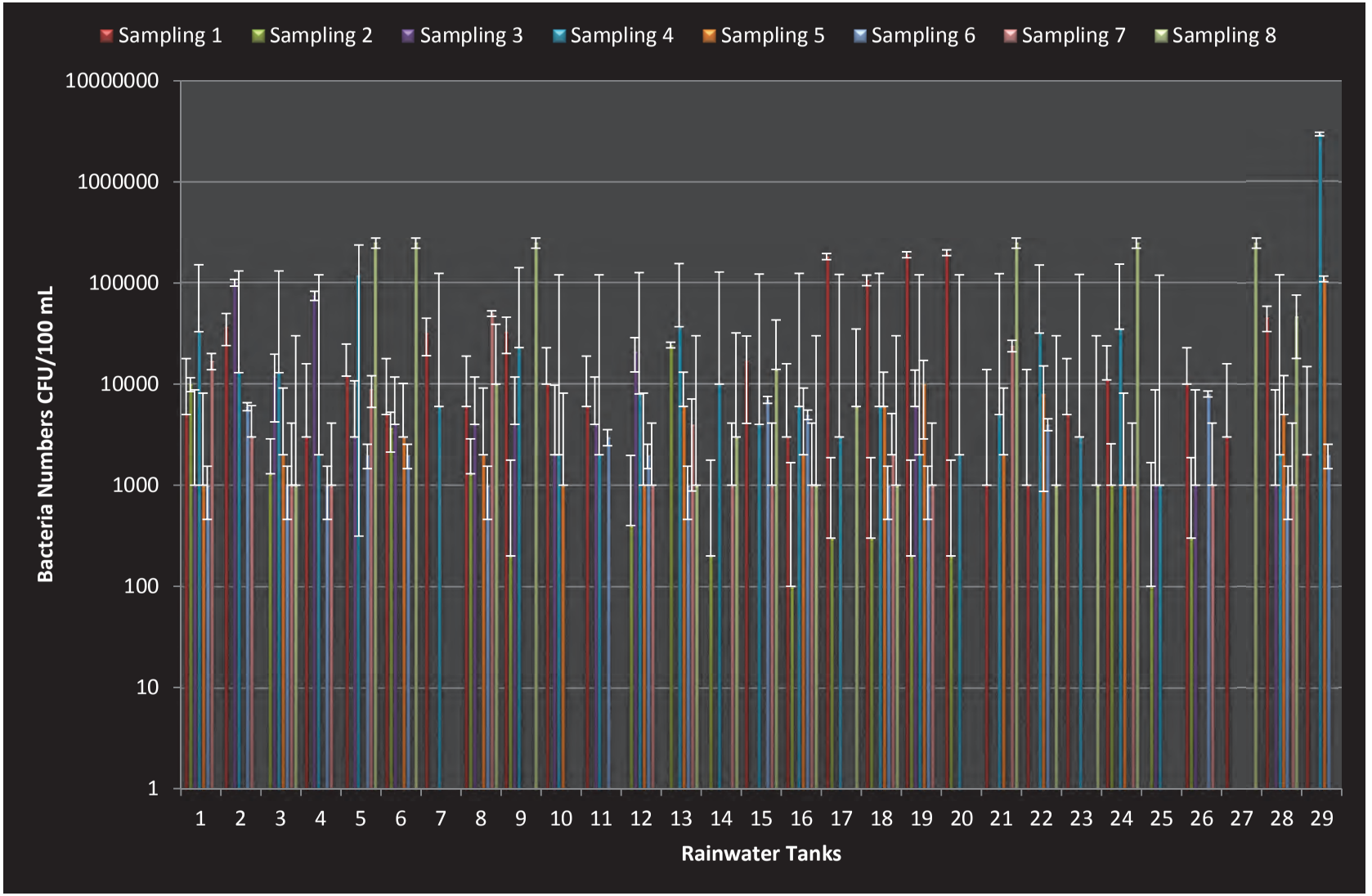

Figure 2.9: Enumeration of Gram negative bacteria numbers (CFU/ 100 mL) in the rainwater samples (Kleinmond) obtained for sampling one to eight using the spread plate technique (*Salmonella Shigella* Agar)

Legionella spp.

During this study, no *Legionella* species were detected through culturing methods. Each of the colonies that grew on *Legionella* CYE Agar base (GVPC) (Oxoid) were re-streaked onto Nutrient Agar (NA) and incubated at 37^0C for 24 h. *Legionella* spp. require cysteine, present in the *Legionella* CYE Agar base, for growth and therefore the re-growth on NA was used as a preliminary identification test. Isolates from sampling three and six that grew on *Legionella* CYE Agar base and on Nutrient Agar (Merck) were then categorised based on morphological similarities and representatives from each category were identified with the use of molecular techniques. Harvested rainwater samples, in two separate studies in Canada and New Zealand, were also screened for the presence of *Legionella* spp. In both studies no *Legionella* spp. were observed above the detection limit (Simmons et al., 2001; Despins et al., 2009). The use of quantitative PCR has also enabled researchers in other studies to observe and quantify the estimated numbers of *Legionella pneumophila* in harvested rainwater samples (Ahmed et al., 2008, 2010).

Campylobacter spp.

No *Campylobacter* species were isolated during the course of this study with the use of culturing methods. Isolates that represented a group with morphological similarities were chosen and re-streaked onto Nutrient Agar and incubated at 37^0C aerobically. *Campylobacter* spp. require micro-aerobic conditions for growth and therefore the re-growth in aerobic conditions was used as a preliminary identification test. All isolates, which were selected from *Campylobacter* Blood-free Selective Medium (Oxoid), were culturable in aerobic conditions (see Materials and Methods). From the fourth to the sixth sampling isolates were identified with the use of molecular techniques to identify the microorganisms that were able to grow on *Campylobacter* media under micro-aerobic conditions. Similar results were obtained by researchers in New Zealand and Canada that were unable to detect *Campylobacter* spp. in harvested rainwater samples using culture methods (Simmons et al., 2001; Despins et al., 2009). *Campylobacter* spp. have however been detected in harvested rainwater by using molecular techniques, such as species specific PCR on whole DNA isolated from the water samples (Ahmed et al., 2008, 2012).

2.3.3 Molecular Identification of Isolates

The identification of microorganisms isolated from *Salmonella Shigella* Agar during the sampling period [5 March to 7 August 2012 (sampling one to seven)] was determined by amplifying the 16S rRNA gene through PCR and subsequent sequencing. In addition to the *Salmonella Shigella* Agar, during the third and sixth sampling periods microorganisms were also isolated from *Legionella* CYE Agar, while during the fourth, fifth and sixth sampling sessions microorganisms were isolated from *Campylobacter* Blood-free Selective Medium. These microorganisms were also identified with 16S rRNA PCR and subsequent sequencing. All the isolates were identified based on the highest value of identity and query coverage to organisms on the NCBI database. The BLAST result for each isolate is indicated in Appendix A (Tables 2.7 to 18), including the accession number, percentage identity and the query coverage. Table 2.9 summarises the major genera and species isolated and identified during the seven sampling sessions from the rainwater at the Kleinmond Housing scheme utilising the three selective media. It should be noted that while numerous species of various genera were isolated (Table 2.9), the main genera only are indicated. The identification of the microorganisms isolated from *Salmonella Shigella* Agar is discussed for sampling one through to sampling seven. After sampling one, organisms of the genera *Acidovorax* spp., *Aeromonas* spp., *Enterobacter* spp., *Lutiella* spp., *Pseudomonas* spp., *Salmonella* spp., *Serratia* spp., *Shigella* spp. and *Yersinia* spp. were isolated from this selective agar (Table 2.9 and Appendix A).

Sampling two yielded the lowest diversity of organisms cultured, with only two organisms of the genera *Aeromonas* spp. and *Klebsiella* spp. (Table 2.9 and Appendix A) identified. *Klebsiella* spp. were also isolated for the first time in this study during sampling two.

Table 2.9: Summary of major organisms isolated from the various selective media for sampling periods one to seven from the rainwater at the Kleinmond Housing Scheme.

Isolate	Sampling Session						
	One	Two	Three	Four	Five	Six	Seven
Acidovorax spp.	Yes	-	Yes	-	-	-	-
Achromobacter piechaudii	-	-	Yes	-	-	-	-
Acinetobacter spp.	-	-	-	-	Yes	Yes	-
Aeromonas caviae	Yes	Yes	Yes	Yes	Yes	-	Yes
Aeromonas hydrophila	-	-	-	-	-	Yes	-
Aeromonas salmonicida	-	-	-	Yes	Yes	-	-
Brucella abortus	-	-	-	-	Yes	Yes	-
Comamonas terrigena	-	-	-	-	Yes	-	-
Chryseobacterium gleum	-	-	-	-	Yes	-	-
Citrobacter freundii	-	-	-	-	Yes	Yes	-
Comamonas testosterone	-	-	Yes	-	-	-	-
Enterobacter cloacae	Yes	-	Yes	Yes	Yes	-	Yes
Enterococcus faecalis	-	-	-	-	Yes	-	-
Enterobacter hormaechei	-	-	-	-	Yes	-	-
Enterobacter mori	-	-	-	-	Yes	-	-
Escherichia coli DEC11D	-	-	Yes	-	Yes	-	-
Escherichia coli EPEC C342-62	-	-	-	-	-	Yes	-
Escherichia coli O104:H4	-	-	-	-	-	Yes	-
Klebsiella oxytoca	-	Yes	Yes	Yes	Yes	Yes	Yes
Klebsiella pneumonia	-	-	-	-	Yes	-	-
Lutiella nitroferrum	Yes	-	-	-	-	-	-
Morganella morganii	-	-	-	-	Yes	-	-
Orchrobactrum intermedium	-	-	-	Yes	Yes	-	-
Paenibacillus polymyxa	-	-	-	-	Yes	-	-
Proteus mirabilis	-	-	-	-	Yes	-	-
Proteus penneri	-	-	Yes	-	Yes	-	-
Providencia alcalifaciens	-	-	-	-	Yes	Yes	-
Providencia rettgeri	-	-	Yes	-	-	-	-
Providencia stuartii	-	-	-	Yes	Yes	-	-
Pseudomonas aeruginosa	Yes	-	-	-	-	-	-
Pseudomonas extremaustralis	-	-	-	-	Yes	Yes	-
Pseudomonas fluorescens	-	-	-	-	Yes	Yes	-
Pseudomonas pseudoalcaligenes	Yes	-	-	-	-	-	-
Pseudomonas psychrophila	-	-	-	-	-	-	Yes
Pseudomonas putida	Yes	-	-	-	-	Yes	-
Pseudomonas synxantha	-	-	-	-	-	Yes	-
Pseudomonas syringae	-	-	-	Yes	-	-	Yes
Salmonella enterica	Yes	-	Yes	Yes	Yes	-	-
Serratia fonticola	-	-	-	-	-	Yes	-
Serratia odorifera	Yes	-	-	-	-	-	-
Serratia marcescens	-	-	Yes	Yes	-	-	-

Shigella flexneri	Yes	-	-	-	-	-	-
Isolate	**Sampling Session**						
	One	**Two**	**Three**	**Four**	**Five**	**Six**	**Seven**
Yersinia enterocolitica	Yes	-	Yes	-	-	-	-
Yersinia massiliensis	-	-	-	-	-	Yes	-
Yersinia pestis biovar	-	-	-	-	Yes	-	-
Yersinia pestis CA88-4125	-	-	-	-	-	Yes	-
Yersinia rohdei	Yes	-	Yes	-	-	Yes	-

* "-" indicates organism not detected

White: indicates isolation of organism from SS Agar

Blue: indicates isolation of organism from *Legionella* medium

Yellow: indicates isolation of organism from *Campylobacter* medium

Green: indicates the isolation of organisms from SS Agar and *Legionella* medium

Orange: indicates the isolation of organisms from SS Agar and *Campylobacter* medium

Organisms of the genera *Achromobacter* spp., *Acidovorax* spp., *Aeromonas* spp., *Comamonas* spp., *Enterobacter* spp., *Escherichia* spp., *Klebsiella* spp., *Proteus* spp. and *Yersinia* spp. were then isolated and identified during sampling three (Table 2.9 and Appendix A). The fourth sampling again yielded organisms of the genera *Aeromonas* spp., *Enterobacter* spp., *Klebsiella* spp., *Pseudomonas* spp., *Salmonella* spp. and *Serratia* spp., with the genus *Providencia* spp., isolated for the first time in the study (Table 2.9 and Appendix A). Sampling five yielded the highest genus diversity amongst the sampling sessions for SS agar with species belonging to the genera *Aeromonas* spp., *Chryseobacterium* spp., *Citrobacter* spp., *Enterobacter* spp., *Escherichia* spp., *Klebsiella* spp., *Morganella* spp., *Proteus* spp., *Providencia* spp., *Salmonella* spp. and *Yersinia* spp. isolated and identified. *Citrobacter* spp., *Klebsiella* spp., *Providencia* spp., *Pseudomonas* spp., *Serratia* spp. and *Yersinia* spp. were then isolated and identified after sampling six had been completed (Table 2.9 and Appendix A). The presence of four organisms was also confirmed after the seventh sampling and included organisms of the genera *Aeromonas* spp., *Enterobacter* spp., *Klebsiella* spp. and *Pseudomonas* spp. (Table 2.9 and Appendix A).

Organisms from the genera *Klebsiella* spp., *Aeromonas* spp., *Providencia* spp., *Serratia* spp. and *Salmonella* spp. were isolated from harvested rainwater that had been spread plated onto *Legionella* CYE Agar base during the third sampling (Table 2.9 and Appendix A). During sampling six, presumptive *Aeromonas hydrophila, Pseudomonas* spp. and *Yersinia* spp. were isolated and identified from this selective medium (Table 2.9 and Appendix A).

During the fourth, fifth and six sampling isolates were identified from *Campylobacter* Blood-free Selective Medium with the use of molecular typing techniques. Presumptive *Aeromonas caviae* and *Ochrobactrum intermedium* were isolated and identified after sampling four from this selective medium (Table 2.9 and Appendix A). As with the isolation and identification of organisms from the SS Agar, during sampling five a more diverse array of organisms was isolated from the *Campylobacter* Blood-free Selective Medium (Table 2.9 and Appendix A) than during sampling four. The major genera identified included, *Pseudomonas* spp., *Enterococcus* spp., *Acinetobacter* spp., *Brucella* spp., *Paenibacillus* spp. and *Comamonas* spp., with *Orchrobactrum intermedium* isolated and identified during both sampling four and five. During sampling session six two isolates of *Escherichia coli* were isolated and identified. *Acinetobacter* spp. and presumptive *Brucella abortus* were also isolated and identified during sampling six from *Campylobacter* Blood-free Selective Medium.

Many bacteria that occur naturally in water environments may be considered as opportunistic pathogens for humans. People with impaired health or with compromised immune systems are most vulnerable to such pathogens. In addition, people such as the elderly and the very young, people with burns or who have, in the recent past, undergone surgery or who have endured a serious injury, are more susceptible to opportunistic pathogens. Examples of opportunistic pathogens include *Aeromonas* spp. (isolated throughout the study, except in sampling six), *Klebsiella* spp. (isolated from sampling two through to sampling six), presumptive *Pseudomonas aeruginosa* (isolated during sampling one) (Table 2.9 and Appendix A), and certain slow- growing mycobacteria (NHMRC and NRMMC, 2011).

The guidelines (DWAF (DWAF, 1996) and the Drinking Water Specification 241 of the South African National Standards (SANS) (SABS, 2005)), do not stipulate guidelines for the general pathogenic bacterial groups. The guidelines stipulated by the ADWG (NHMRC and NRMMC, 2011) will thus be noted accordingly. While various *Aeromonas* spp. were identified throughout the sampling period (utilising the various selective media), presumptive *Aeromonas caviae* was one of the predominant species isolated throughout the current study and was detected in every sampling session, except for sampling six (Table 2.9 and Appendix A). The ADWG have previously indicated that certain difficulties have arisen in determining the degree of pathogenicity amongst *Aeromonas* isolates, and recommended that further studies be performed to clarify the pathogenicity associated with this organism. This has hindered the establishment of a specific guideline value being assigned to *Aeromonas* by the Australian Government. It is stipulated however, that if the presence of *Aeromonas* is suspected then water must be tested directly for *Aeromonas* spp. (Gracey et al., 1982; Jana and Duffy, 1988; Cunliffe and Adcock, 1989; NHMRC and NRMMC, 2011).

The *Aeromonas* genus is highly diverse with 16 DNA hybridization groups currently being recognised (Figueras et al., 2000). In our study, *A. hydrophila* and *A. caviae* were of clinical significance (Janda, 1991; Janda and Abbott, 1998). *Aeromonas* species cause a wide range of diseases, including gastrointestinal infections as well as extra-intestinal infections, such as cellulitis, wound infections, septicaemia, urinary tract infections, hepato-biliary and ear infections and diarrhoea (Janda et al., 1995). The *Aeromonas* spp. most commonly associated with gastrointestinal infections is *A. hydrophila* (Kühn et al., 1997), which was isolated from harvested rainwater samples during the sixth sampling. *Aeromonas* spp. have also been identified in previous studies that monitored the microbial quality of harvested rainwater (Simmons et al., 2001; Albrechtsen, 2002; CRC for Water Quality and Treatment 2006; Ahmed et al., 2010). The values varied amongst the studies, for example Simmons et al. (2001) found that 20% of their samples contained *Aeromonas* spp. and Ahmed et al. (2010) found that only 7% of their samples were positive for *Aeromonas hydrophila.* Along with *Aeromonas*, *Klebsiella* spp. were also consistently isolated and identified in the current study. Presumptive *Klebsiella oxytoca* was identified in every sampling session, except for the first session (Table 2.9 and Appendix A). *Klebsiella* spp. usually originates from the environment and are often associated with the gastrointestinal system, while they are not necessarily of faecal origin. Organisms such as *Pseudomonas, Klebsiella* and *Legionella* spp. are classified as opportunistic pathogens. This implies that they can cause disease when certain conditions are favourable (NHMRC and NRMMC, 2011).

Amongst the opportunistic pathogens isolated in hospital environments, *K. pneumoniae* (identified during sampling five) and *K. oxytoca* are prevalent in Australian drinking water sources (Table 2.9 and Appendix A). People that are at added risk of acquiring *Klebsiella* associated diseases are, amongst others, those with compromised defence mechanisms, people with serious wounds, or those with Acquired Immune Deficiency Syndrome (AIDS). In some instances *Klebsiella* spp. (*K. pneumoniae* and *K. oxytoca*) have been identified as the causative agents in infections, where research showed that this organism was able to colonise the desired area and cause, for example, destructive pneumonia in healthy individuals (Grimont et al., 1991; NHMRC and NRMMC, 2011).

Based on information obtained from the ADWG, no guideline value has been assigned to the limit of *Klebsiella* spp. allowed in drinking water. They do however mention that relevant historical data and characteristics of the system should be noted and taken into consideration when determining whether the water body is potable or not. However, during standard testing of water for indicator organisms, *Klebsiella* spp. form a large part of the coliform microbial community and the ADWG do stipulate guidelines for coliforms (NHMRC and NRMMC, 2011). No previous reports have identified specific *Klebsiella* contamination in rainwater collected from domestic rainwater harvesting tanks. Kaushik et al. (2012), however, recently showed that of the 50 rainwater samples tested in Singapore, 12% were positive for *Klebsiella* spp.

Various *Yersinia* spp. were isolated during the study period, including presumptive *Yersinia enterocolitica* strains (isolated during sampling one and three), a presumptive *Yersinia massiliensis* strain (isolated during sampling six) and presumptive *Yersinia pestis* strains (isolated during sampling five and six) (Table 2.9 and Appendix A). This is of particular concern as the three pathogenic species belonging to the genus *Yersinia*, include *Y. pestis*, *Yersinia pseudotuberculosis* and *Y. enterocolitica*. The genus *Yersinia* falls under the family *Enterobacteriaceae*. *Yersinia enterocolitica* is able to grow at low temperatures, even as low as 4°C and when these organisms are ingested they can cause gastrointestinal disease. They have shown to endure long-term survival in water bodies and this renders the establishment of the origin of contamination difficult. *Yersinia enterocolitica* have also been predominantly isolated from many domestic and wild animals, which are considered possible reservoirs. Transmission of this bacterium is generally through meat products (Lloyd 1983), however *Y. enterocolitica* has been isolated from a number of environmental samples, particularly from water, but the serotypes are different to those that cause disease in humans. Strains of *Y. enterocolitica* found in the environment are yet to be defined based on epidemiological importance, and for this reason it is recommended that *Y. enterocolitica* strains isolated from the environment should be recorded as *Y. enterocolitica*- like organisms. Based on biochemical characteristics they can be divided into *Y. intermedia, Y. frederekseni, Y. kristensenii* and *Y. aldovae* and are not pathogenic towards humans. As it is not yet possible to determine the infectious dose of *Yersinia* the ADWG suggests that *E. coli* or thermotolerant coliforms should be used to indicate the presence of *Yersinia*, but if *Yersinia* is explicitly sought, pathogenic *Yersinia* spp. should not be detected (NHMRC and NRMMC, 2011).

Yersinia pestis is generally associated with disease in rodents and wild animals and is transmitted by fleas. This species is subdivided into three biovars, namely Antiqua, Medievalis and Orientalis. *Yersinia pestis* was identified as the causative agent of the plague that occurred during 541 - 767 AD, with the biovar Antiqua implicated (hypothesised to be a descendent of the bacterium that caused the Justinians plague) (Brossolet et al., 1994). Medievalis is descended from the bacterium that caused the second pandemic, known as the Black Death, which occurred from 1346 into the 19th century. The third pandemic started in the mid- 19th century and has been linked to Orientalis. It was only after the last pandemic that *Y. pestis* was identified in 1984 (Yersin, 1894; Perry and Fetherston 1997; Achtman et al., 1999). *Acinetobacter* spp. were isolated during sampling five and six from rainwater samples that were spread plated onto *Campylobacter* Blood-free Selective Medium (Table 2.9 and Appendix A). *Acinetobacter* are Gram negative, aerobic coccobacilli and according the Bergery's Manual of Systematic Bacteriology the genus *Acinetobacter* belongs to the family *Neisseriaceae* (Juni, 1984). Many studies have shown that *Acinetobacter baumannii* is the main species associated with nosocomial outbreaks of infection (Bouvet and Grimont, 1987; Seifert et al., 1993a) and *Acinetobacter johnsonii* has been associated with catheter-related bacteraemia (Seifert et al., 1993b). *Acinetobacter lwoffii* and *A. johnsonii* have also been identified as natural inhabitants of human skin (Bergogne-Bérézin and Towner, 1996). *Acinetobacter* spp. have also previously been identified in harvested rainwater and a study in the West Bank, Palestinian Authority, identified *Acitenobacter* spp. in 78% of the harvested rainwater samples collected from storage tanks (Daoud et al., 2011).

Salmonella enterica strains were isolated during sampling one, three, four and five (Table 2.9 and Appendix A). Salmonellosis waterborne outbreaks are usually as a result of the consumption of water that has been contaminated by the faeces of livestock and native animals or by wastewater discharges that have not been adequately treated. However, most diseases usually associated with *Salmonalla* spp. originate from contaminated foodstuffs and to a lesser extent, water. According to the ADWG, *Salmonella* spp. should not be detected in a drinking water source, "if explicitly sought for" (NHMRC and NRMMC, 2011). If not, *Escherichia coli* or thermotolerant coliforms should be used to indicate the possible presence of *Salmonella* spp. (NHMRC and NRMMC, 2011).

Enterobacter cloacae was isolated during sampling one, three, four, five and seven (Table 2.9 and Appendix A). The genus *Enterobacter* belongs to the family *Enterobacteriaceae*, and there are 14 species belonging to this genus according to the Manual of Clinical Microbiology (Farmer, 1995). *Enterobacter aerogenes* and *Enterobacter cloacae* are the most commonly encountered human pathogens. However *Enterobacter agglomerans* and *Enterobacter sakazakii* have also been implicated as causes of diseases in humans (John et al., 1982; Burchard et al., 1986; Gaston, 1988; Gallagher, 1990; Chow et al., 1991; Haddy et al., 1991; Hawkins et al., 1991; Stenhouse 1992; Rubinstien et al., 1993; Andresen et al., 1994; Schonheyder et al., 1994; Farmer, 1995; Farmer et al., 1985). Studies have shown that *Enterobacter* spp. are important nosocomial pathogens, although it is less commonly encountered than *Escherichia coli* and *Klebsiella* spp. (McGowan, 1985; Jarvis et al., 1992).

Organisms that are considered non-pathogenic were also identified in this study, for example *Acidovorax* spp. were isolated in the first and third samplings (Table 2.9 and Appendix A). *Acidovorax* spp. are members of the family *Comamonadaceae*, in the class Proteobacteria. Until recently members of the genus *Acidovorax* were assigned to *Pseudomonas*, but a number of studies have indicated that certain species are distinct from *Pseudomonas* sensus stricto (Palleroni, 1984; Kersters et al., 1996). *Acidovorax* spp. are plant pathogens that cause leaf blight and leaf spots. The occurrence of these organisms should be taken into consideration if the rainwater from the rainwater harvesting tanks is to be used for growing vegetables or fruit. As described previously, DRWH can provide uplifting activities for communities such as vegetable gardening, which could potentially alleviate food scarcity.

2.3.3.1 *Phylogenetic analysis of Escherichia coli strains*

Various methods were employed to select for *E. coli* isolates from 10 different domestic rainwater tanks (within the cluster of 29 tanks routinely monitored) at the Kleinmond Housing Scheme during the sampling period of 5 March to 7 August 2012 (sampling sessions one to seven). The rainwater tanks selected for investigating the presence of pathogenic *E. coli* strains were connected to houses 8220, 8216, 8208, 8387, 8392, 8497, 8494, 8401, 8402 and 8404, which corresponded to sampling numbers 1, 3, 5, 7, 9, 11, 13, 15, 17 and 19, respectively (Table 2.2). In order to distinguish between *E. coli* and total coliforms, serial dilutions of the collected rainwater samples were spread plated onto ChromoCult® Coliform Agar, m-Endo agar and MLGA agar, which specifically selects for the growth of *E. coli* and suppresses the growth of other enteric species. Presumptive positive *E. coli* colonies were selected from these plates according to their specified criteria and re-streaked onto nutrient agar until pure colonies were obtained. However, colonies selected from m-Endo agar and MLGA agar were first grown in 5 mL Luria Bertani broth and spread plated onto ChromoCult® Coliform Agar before being re-streaked onto nutrient agar.

A total of 168 isolates were obtained using these three selective media. These isolates were then subjected to the IMViC test for the further selection of *E. coli* strains (Harley and Prescott, 1993). Of these 168 isolates, 65% (109 isolates) tested positive for *E. coli*. The identity of these *E. coli* strains were then confirmed through universal 16S rRNA PCR with subsequent sequencing, which confirmed the presence

of 98 *E. coli* isolates in the rainwater sampled in Kleinmond (Appendix A, Table 19 and 20). This result indicated that the IMViC test was only 93% effective in identifying *E. coli* as other enteric species, from the genera *Enterobacter, Serratia, Shigella,* and *Proteus,* were also positive for the IMViC test. *Shigella* was predominantly identified from sampling 5 to sampling 7 (high total rainfall recorded). This high frequency of *Shigella* isolates identified can be explained by the fact that the genus is closely related to *E. coli* and that certain *Shigella* species share the same IMViC profile as *E. coli* (Cowan and Steel, 1961). According to Powers and Latt (1977), *Proteus* species also share an identical IMViC profile with *E. coli,* which could have resulted in the false positive IMViC result. The phylogeny of the representative organisms isolated in GenBank for samplings one, three and six (majority of the *E. coli* strains were isolated during these sampling times) were analysed using the Neighbour-joining algorithm in ClustalX (Figures 2.10, 2.11 and 2.12).

Appendix A, Tables 19 and 20, represent the organism names, isolated during sampling one to seven, as well as the accession numbers. Among the 98 *E. coli* isolates that were identified using GenBank, 4% were positively identified as the enterotoxigenic *E. coli* (ETEC), which contains the heat stable toxin (ST1). These presumptive positive ETEC isolates were identified predominantly in sampling one and three (Figures 2.11 and 2.12) and were isolated from tanks 3, 6, 7 and 9. The ETEC strain causes watery diarrhoea without blood and in a few cases it can also cause vomiting and fever. Infants and travellers in underdeveloped countries are the most susceptible to this *E. coli* strain (Tobias and Vutukuru, 2012). In addition 6% of the total *E. coli* isolates were identified as *E. coli* 0157:H7 (sampling one, two and three) and were consistently isolated from tanks 3, 4 and 7. *Escherichia coli* 0157:H7 is the predominant serotype of enterohaemorrhagic *E. coli* (EHEC) that produces the Shiga-like toxin that is responsible for causing watery and subsequent bloody diarrhoea in humans. A study conducted by Ahmed et al. (2012), showed that 4% of the *E. coli* strains isolated from 22 rainwater tanks contained the ST1 genes, associated with ETEC strains, and none of the isolates contained any of the virulent genes that EHEC produces. As indicated above, the majority of the *E. coli* strains were isolated and identified during sampling one, three and six (Figures 2.10, 2.11 and 2.12). When more than one specific strain was identified during a sampling, it was allocated a specific number corresponding to the number of times it was identified for that particular sampling time (indicated in brackets following the organism's name on Figures 2.10, 2.11 and 2.12).

From Figure 2.10 it is clear that the *E. coli* strain W (categorised as W complete genome) was the predominant strain isolated from the 10 different domestic rainwater harvesting tanks during sampling one. Three ETEC strains were identified during sampling one, with two of the strains clustering together with a 99% homology. In this phylogenetic tree the pathogenic *E. coli* strains did cluster together with the non-pathogenic strains and isolates identified as the same strain often did not group together.

The *E. coli* strains most frequently isolated and identified during sampling two (results not shown) and three (Figure 2.11) were the *E. coli* 0157:H7 strains WAB1892 and TW14359, respectively and *E. coli* 0111:H- strain 11128 (sampling three). The lowest diversity of *Escherichia coli* strains was isolated during sampling two. This correlates with the results obtained during the isolation and identification of the general bacterial species present in the harvested rainwater samples, with the lowest species diversity of bacterial isolates also observed during sampling two. From Figure 2.11 it is clear to see that these two strains, namely *E. coli* 0157:H7 strain TW14359 and *E. coli* 0111:H- strain 11128 cluster together. *Escherichia coli* 0111:H- and 0157:H7 are both main serotypes that produce Shiga toxins which could explain their similar homology (Mainil, 1999).

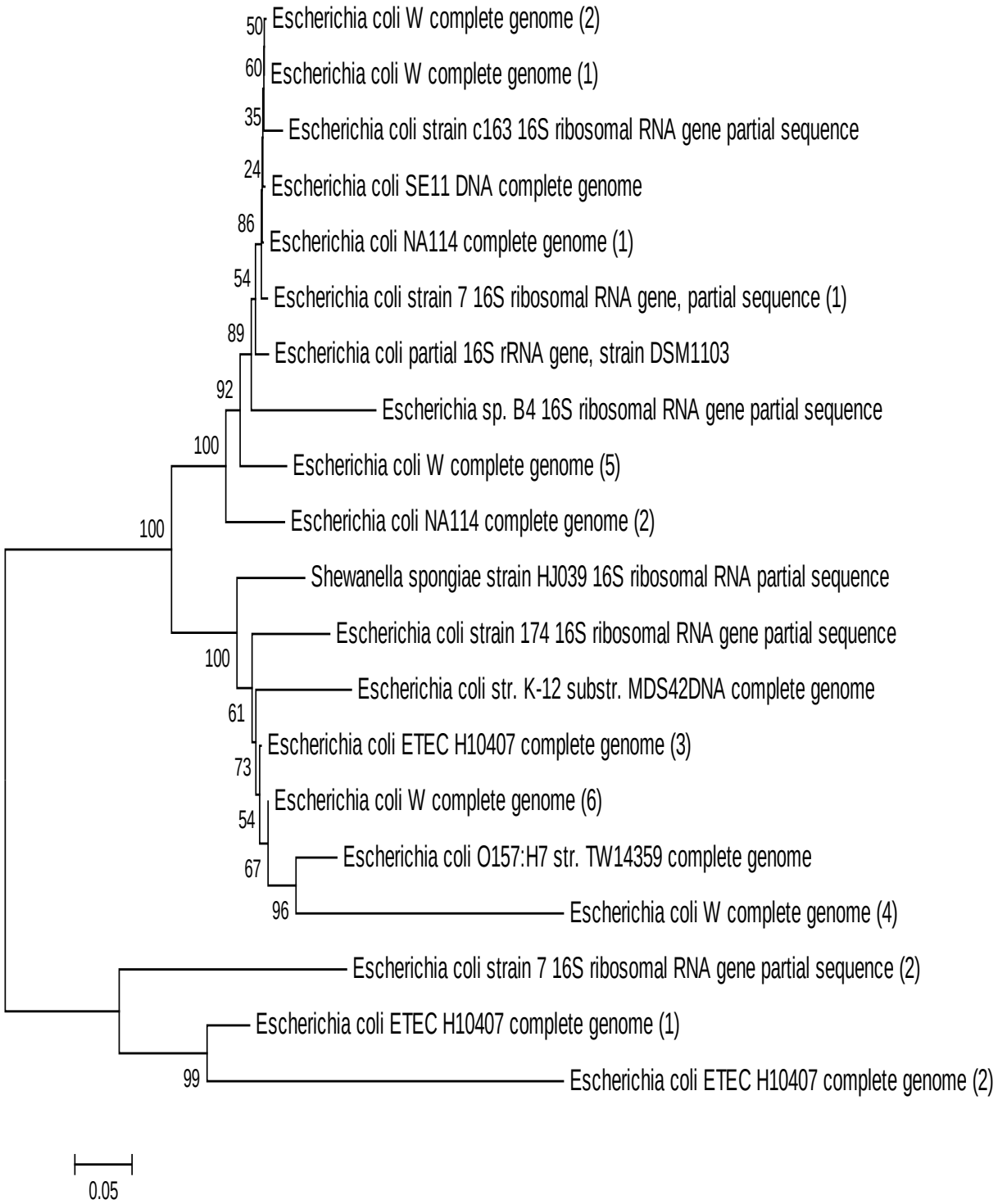

Figure 2.10: An unrooted phylogenetic tree of organisms isolated during sampling one. The tree of isolates was constructed using the Neighbour-joining algorithm of ClustalX. Bootstrap values are shown at the nodes.

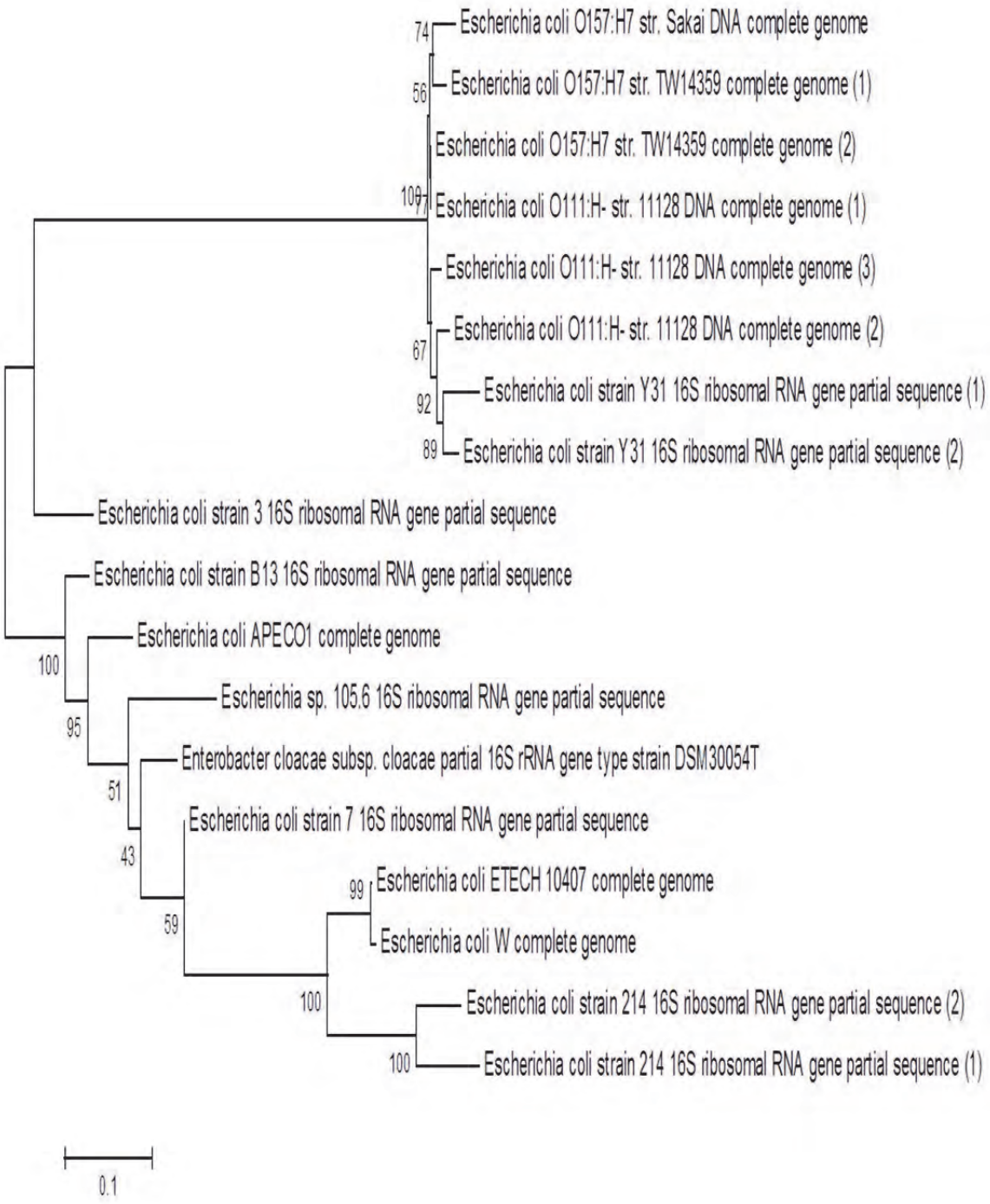

Figure 2.11: An unrooted phylogenetic tree of organisms isolated during sampling three. The tree of isolates was constructed using the Neighbour-joining algorithm of ClustalX. Bootstrap values are shown at the nodes.

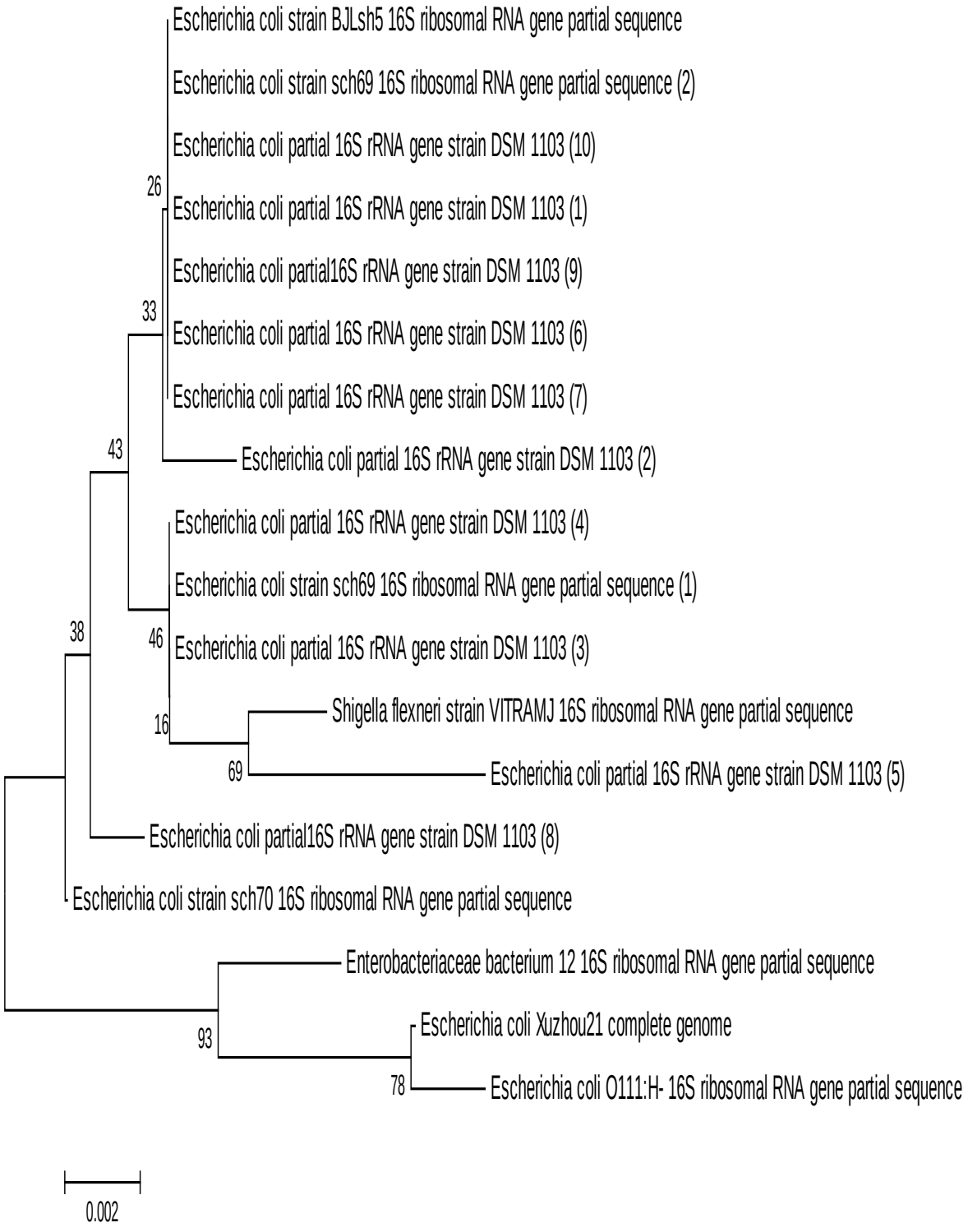

Figure 2.12: An unrooted phylogenetic tree of organisms isolated during sampling six. The tree of isolates was constructed using the Neighbour-joining algorithm of ClustalX. Bootstrap values are shown at the nodes.

An avian isolate *E. coli* APECO1 was also identified during the third sampling period. This strain most likely originated from bird faeces and may contain many virulent genes belonging to the extra-intestinal pathogenic *E. coli* (ExPEC). These strains are associated with human disease and primarily cause infections such as diarrhoea, soft tissue infections, bacteraemia and urinary tract infection (Ahmed et al., 2011). In a study conducted by Ahmed et al. (2011) ExPEC was also detected in 15 (68%) of the 22 rainwater tanks sampled. The dominant strain identified in sampling six was *E. coli* DSM 1103 (Figure 2.12). Figure 2.12 also clearly indicates that the two genera*, Shigella* and *Escherichia* grouped together and this could be attributed to the fact that they are closely genetically related (Lukjancenko et al., 2010). No pathogenic *E. coli* were isolated during sampling six as can be seen from the phylogenetic tree (Figure 2.12). This was also the case for samplings four, five and seven. Thus, pathogenic strains of *E. coli* were only isolated during sampling one, two and three, during the warmer temperatures where the total rainfall was low.

From the information presented in Figures 2.10, 2.11 and 2.12 and Appendix A (Tables 19 and 20) it is clear that a wide variety of *E. coli* strains were isolated from the 10 different rainwater tanks in the Kleinmond Housing Scheme during the sampling period. While only a small percentage of these strains were identified as possible human pathogens (isolated predominantly during sampling one to three) it is crucial that the harvested rainwater should not be utilised without prior treatment as the consumption of this water source could be associated with public health risks and human disease.

The faeces of warm-blooded animals could serve as a possible source of *E. coli* contamination in the rainwater tanks, as warm blooded animals have been shown to carry a high number of pathogenic *E. coli* strains in their intestines (Ishii et al., 2007). Ahmed et al. (2012b) conducted a study in Southeast Queensland, Australia, and successfully isolated *E. coli* species with identical biochemical phenotype profiles from rainwater tanks as well as from bird and possum faeces found on the roof surfaces where the tanks were installed. A recent study conducted in Singapore confirmed the presence of pathogenic microorganisms, including *E. coli*, in airborne particulate matter which can serve as another source of contamination (Kaushik and Balasubramanian, 2012). The presence of *E. coli* strains also implies that these tanks do not comply with the guidelines stipulated by the WHO (2011), DWAF (1996) and the ADWG (NHMRC and NRMMC, 2011), which indicate that *E. coli* should be absent from any water source utilised for drinking purposes. *Escherichia coli* is included as a specific indicator organism of faecal pollution from warm-blooded animals (Rompré et al., 2002). In the current study however, 67% of the *E. coli* counts exceeded the recommended values as stipulated by the respective drinking water guidelines. In addition, *Escherichia coli* was also isolated and identified by molecular analysis throughout the study period from the tanks sampled. These results, thus clearly indicate that the water harvested in the rainwater tanks at the Kleinmond Housing Scheme is not suitable for drinking purposes.

2.3.4 Chemical Analysis

Harvested Rainwater samples collected on the 5th of March 2012 (sampling one) were chemically analysed to determine the concentrations of cations, anions and metal ions present. The drinking water guidelines as stipulated by the Drinking Water Specification 241 of the South African National Standards (SANS) (SABS, 2005), the South African Water Quality Guidelines for Domestic Water Use of the Department of Water Affairs and Forestry (DWAF, 1996), the Guidelines for Drinking-water Quality the World Health Organisation (WHO, 2011) and the Australian Drinking Water Guidelines (NHMRC and NRMMC, 2011), were used to evaluate the quality of the harvested rainwater for potable purposes, as shown in Table 2.10. Appendix A (Table 2.6) shows the raw data for the chemical analysis on the rainwater samples.

Table 2.10: Metal ion and cation concentrations (mean and range) obtained from the rainwater samples vs recommended concentrations as stipulated by the respective water guidelines (n = 29). The p value was calculated for the statistical difference between the samples.

Cation/ Metal ion	Mean	Range	Control	P value	SANS 241	DWAF	ADWG	WHO
Al (µg/L)	78.27±34.07	37.87 – 180.20	1220.26	0.80	300	150	200	-
V (µg/L)	0.91±0.19	0.58 – 1.54	0.59	0.98	200	1000	-	70
Cr (µg/L)	0.80±0.24	0.47 – 1.70	0.38	0.40	100	50	50	50
Mn (µg/L)	0.38±0.40	0.11 – 1.82	13.61	0.53	100	50	500	-
Fe (µg/L)	19.29±11.31	7.36 – 61.34	232.22	0.90	200	100	300	-
Co (µg/L)	0.02±0.01	0.01 – 0.06	0.28	1	500	-	-	
Ni (µg/L)	0.14±0.06	0.07 – 0.32	1.96	1	150	-	20	70
Cu (µg/L)	1.90±0.83	0.75 – 3.73	0.82	0.86	1000	1000	2000	2000
Zn (µg/L)	3.86±3.41	0.57 – 15.77	1.20	0.65	5000	3000	3000	-
As (µg/L)	0.48±0.11	0.32 – 0.80	0.12	0.96	10	10	10	-
Se (µg/L)	0.41±0.16	0.11 – 0.79	0.22	0.89	20	20	10	40
Mo (µg/L)	0.08±0.02	0.06 – 0.14	0.02	1	-	-	50	-
Cd (µg/L)	0.15±0.11	0.06 – 0.58	0.01	1	5	5	2	3
Ba (µg/L)	3.67±1.43	1.95 – 7.35	3.71	0.96	-	-	2000	700
Hg (µg/L)	0.08±0.05	0.03 - 0.19	0.07	1	-	10	1	6
Pb (µg/L)	0.18±0.08	0.08 – 0.39	0.05	1	20	10	10	10
Li (µg/L)	Nd	-	7.31	-	-	-	-	
Be (µg/L)	Nd	-	Nd	-	-	-	60	-
Sn(µg/L)	Nd	-	Nd	-	-	-	-	
B (mg/L)	0.04±0.03	0.02 – 0.18	0.01	1	-	-	4	2.4
Ca (mg/L)	11.58±0.83	10.52 – 14.19	12.14	1	150	32	-	
K (mg/L)	2.68±0.91	1.75 – 6.31	0.69	0.97	50	50	-	-
Mg (mg/L)	1.60±0.24	1.30 – 2.15	2.27	1	70	30	-	
Na (mg/L)	15.37±2.76	11.81 – 22.27	22.01	1	200	100	180	-
P (mg/L)	0.02±0.01	0.01 – 0.03	0.01	1	-	-	-	-
Si (mg/L)	3.42±0.50	2.79 – 4.98	1.52	1	-	-	-	
Sr (mg/L)	0.14±0.02	0.10 – 0.18	0.05	1	-	-	-	

Nd – not detected

The average concentrations of the metal ions and cations, vanadium (V), chromium (Cr), manganese (Mn), iron (Fe), cobalt (Co), nickel (Ni), arsenic (As), selenium (Se), molybdenum (Mo), cadmium (Cd), barium (Ba), mercury (Hg), lead (Pb), copper (Cu), zinc (Zn), boron (B), calcium (Ca), potassium (K), magnesium (Mg), sodium (Na), phosphorus (P), silicon (Si) and strontium (Sr) in the rainwater samples were below the recommended guidelines as stipulated by SABS, DWAF and ADWG. Beryllium (Be), tin (Sn) and lithium (Li) were not detected in any of the rainwater samples (Table 2.10). No significant difference ($p > 0.05$) in the concentrations of the respective metals ions and cations for the 29 tanks sampled were also recorded. The concentration of Al (1220.26 µg/L) and Fe (232.22 µg/L) in the control sample, collected from the Kleinmond Municipality Water Treatment Plant, was however higher than the recommended concentrations stipulated by SANS, DWAF and ADWG. The Kleinmond Water Treatment Plant chemically doses the water with alum during the coagulation treatment process (Overstrand Municipality, 2011), which could have contributed to the high Al concentrations detected.

The total hardness of the water is defined as the total concentration of Ca and Mg present in the water and can be calculated using Equation 1 (DWAF, 1996). The harvested rainwater samples had a total hardness of 35.50 and according to the classification by Kunin (1972) the harvested rainwater can be described as soft. This value was calculated by using the average concentration of Ca (11.58 mg/L) and Mg (1.60 mg/L) for the 29 collected rainwater samples in Equation 1. Sample 7 had the lowest total hardness with a concentration of 31.83 mg/L $CaCO_3$/L, and sample 27 had the highest total hardness concentration of 44.29 $CaCO_3$/L. Soft water can be corrosive when in contact with metal surfaces such as plumbing, tanks and geysers (DWAF, 1996). The RWH tanks and fittings in the Kleinmond Housing Scheme, however, are constructed from polyethylene and are therefore better suited for the storage of the harvested rainwater than metal or concrete materials.

Equation 1: Total hardness (average) (mg $CaCO_3$/L) = 2.497 × (mg Ca/L) + 4.118 × (mg Mg/L)
(2.497 × 11.58) + (4.118 × 1.60) = 35.50

Nitrate (NO_3), phosphate (PO_4) and fluoride (F) were not detected in any of the rainwater samples (Table 2.11). Fluoride is an important element in drinking water since it reduces dental decay (Satur et al., 2010). However, a study in India indicated that drinking water containing fluoride concentrations exceeding the guideline stipulated by the World Health Organisation resulted in dental and skeletal fluorosis (Hussain et al., 2012). Potable water is provided to the residents in the Kleinmond Housing Scheme by the Kleinmond Municipality. The rainwater is thus not utilised as the primary drinking water source. Fluoride supplementation is therefore not required, which is the case when harvested rainwater is used as the primary drinking water source (Sazakli et al., 2007). The concentrations of chloride [(range of 16.70 (tank 7) to 29.90 mg/L (tank 14)], nitrate [(range of 1 (tank 5) to 2.30 mg/L (tank 24)] and sulphate [(range of 3.70 (tank 7) to 19.50 mg/L (tank 14)] detected, were well below the respective drinking water guidelines (Table 2.11). The only anions detected in the control sample were SO_4 (31.4 mg/L) and Cl (24.7 mg/L). The concentration of SO_4 in the control sample, although higher than the average SO_4 concentration detected in the rainwater samples, was still lower than the recommended values stipulated by the respective drinking water guidelines. The concentration of chlorine in the control sample was also lower than the values stipulated by the recommended drinking water guidelines.

Table 2.11: Anion concentrations (mean and range) obtained from the rainwater samples compared to the recommended concentrations as stipulated by the respective drinking water guidelines (n = 29). The p value was calculated for the statistical difference between the samples.

Anion	Mean	Range	Control	P value	SANS 241	DWAF	ADWG	WHO
Cl (mg/L)	21.20±3.50	16.70 – 29.90	24.7	1	200	100	250	5
NO_3 (mg/L)	1.52±0.31	1 – 2.30	Nd	0.99	10	6	50	50
NO_2 (mg/L)	Nd	-	Nd	-	10	6	3	30
SO_4 (mg/L)	7.95±4.36	3.70 – 19.50	31.4	0.90	400	200	500	-
PO_4 (mg/L)	Nd	-	Nd	-	-	-	-	-
F (mg/L)	Nd	-	Nd	-	1	1	1.5	1.5

Nd – not detected

The chemical oxygen demand (COD) of the harvested rainwater samples collected on the 21st of August 2012 (Sampling 8) was also determined. The kit used to determine the COD had a detection range of 4 to 40 mg/L. The COD values of some of the rainwater samples were however lower than 4 mg/L and therefore the range of COD values described were between 4 and 9.5 mg/L. No significant difference between the COD of the rainwater samples ($p > 0.05$) was also recorded. A low COD implicates that the

concentration of organic matter in the harvested rainwater samples is low as COD refers to the amount of oxygen required to oxidise all the organic matter present (SABS, 2005).

The pH of the rainwater samples was within the SANS 241 (pH 5 – 9.5) guidelines for drinking water (Table 2.12). However, the pH levels recorded from sampling three to eight were lower than the recommended guidelines stipulated by DWAF (6 - 9) and ADWG (6.5 – 8.5). There is insufficient data to stipulate a health guideline for pH levels, although drinking water with a pH less than 4 and higher than 11 may be detrimental to human health. Water with pH levels lower than 6.5, as in the case of the rainwater samples, can also be corrosive (NHMRC and NRMMC, 2011). Studies investigating the chemical quality of harvested rainwater in different regions in the world have recorded an average pH of between 6 and 8.5 (Nevondo and Cloete, 1999; Simmons et al., 2001; Handia et al., 2003; Zhu et al., 2004; Kim et al., 2005; Sazakli et al., 2007; Chapman et al., 2008; Peters et al., 2008; Areeracha-kul et al., 2009; Lee et al., 2010c; Adler et al., 2011; Al-Salaymeh et al., 2011; Huston et al., 2012).

The temperature and pH levels of the rainwater samples decreased slightly with each sampling, when compared to the previous sampling's data, although this difference was not significant ($p > 0.05$). There was also no significant difference ($p > 0.05$, Table 2.12) observed between the temperature and pH levels of the rainwater collected from the 29 tanks during sampling two to eight. Temperature and pH readings could not be recorded for the first sampling as the electrode on the portable thermometer and pH meter was not functioning optimally.

Table 2.12: The mean and range for the temperatures and pH levels recorded during sampling 2 to 8.

Sampling	Temperature		pH	
	Mean	Range	Mean	Range
2	23.97±0.74	22 – 25	6.02±0.09	6 – 6.5
3	19.66±0.94	18 – 22	5.49±0.05	5.25 – 5.5
4	14.79±1.52	13 – 18	5.38±0.32	5 – 6
5	14.86±1.48	13 – 18	4.18±0.23	4.5 – 5
6	14.28±0.84	13 – 16	5.16±0.42	4.5 – 6
7	12.59±0.68	11 – 14	5.00±0.00	5 – 6
8	13.59±1.09	11 – 15	5.14±0.30	5 – 6

2.3.5 Correlations between indicators and physico-chemical properties of rainwater

A significant correlation ($p = 0.00$) could be established between total coliforms (utilising membrane filtration) and the following parameters; *E. coli* counts (utilising membrane filtration) ($R = 0.30$), *E. coli* counts (utilising the spread plate technique) ($R = -0.15$), faecal coliforms ($R = 0.29$), temperature ($R = -0.19$), pH ($R = -0.40$), and rainfall ($R = -41$).

After analysing all the data the REML and the Fixed Effect test yielded significant variation ($p = 0.00$, $F = 16.83$) amongst the eight sampling sessions for total coliforms (utilising membrane filtration). For this reason an LSD test was performed that showed that the same highest mean differences (-1089.28±133.9726) ($p = 0.00$) were observed between sampling sessions one and five, and two and five, while the lowest mean difference with the least amount of variation for total coliforms was recorded between sampling sessions seven and eight (15.31± 133.97) ($p = 0.909$) as indicated in Figure 2.13.

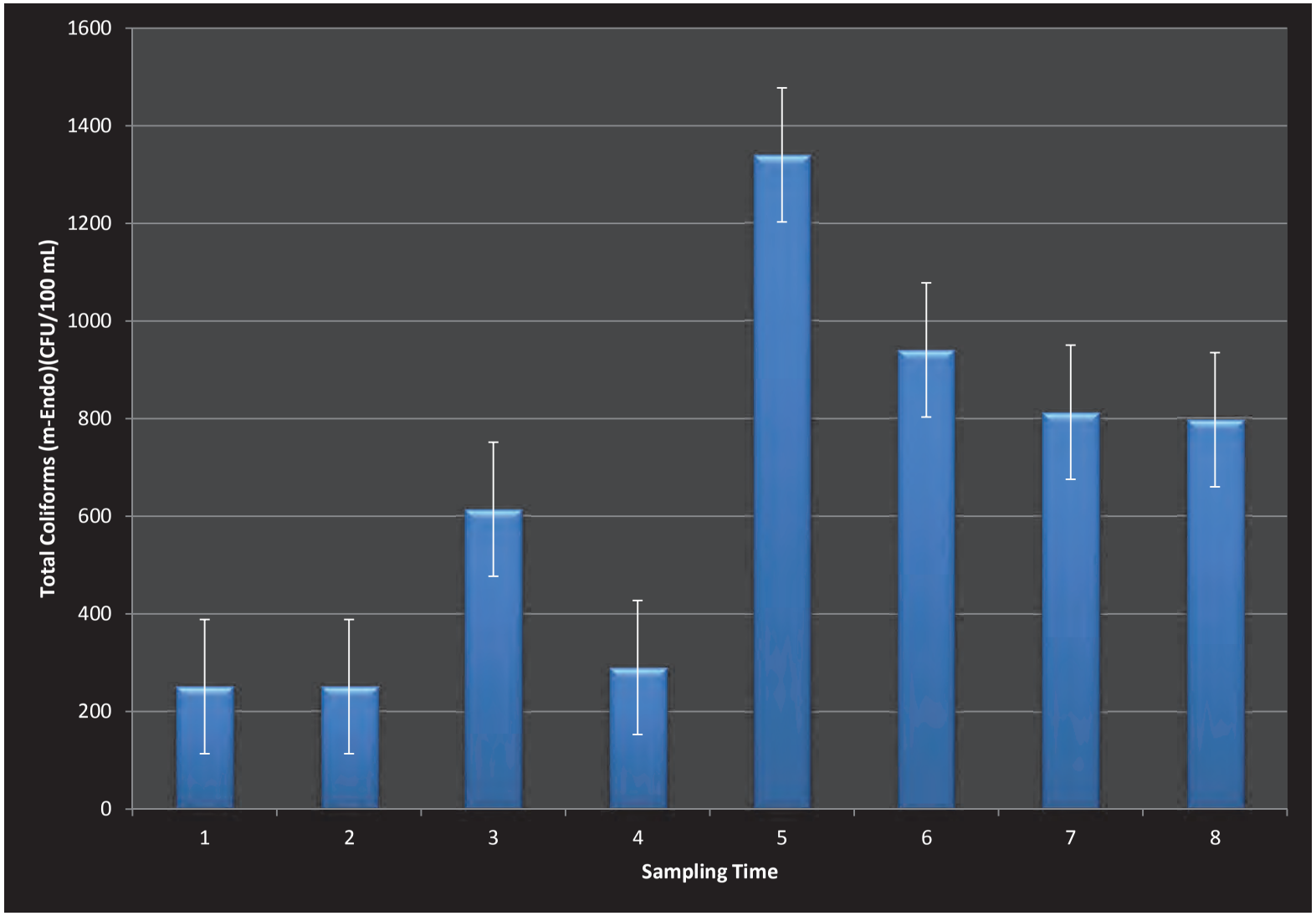

Figure 2.13: The results of an LSD test indicating the significant differences between mean total coliform counts (m-Endo Agar) over a period of eight sampling events

Significant correlations ($p < 0.00$) were also noted between *E. coli* counts, utilising the spread plate technique and the following parameters; *E. coli* counts, utilising membrane filtration ($R = -0.21$), enterococci ($R = 0.15$) and rainfall ($R = -0.36$).

According to the Spearman Rank Order Correlations, no significant correlation could be determined between COD and the microbiological indices ($p>0.05$). Table 2.13 shows all the data sets with significant correlation between the microbiological indicators and various metals and anions in the rainwater samples. The most significant inverse correlation was established between selenium and *E. coli* counts obtained from membrane filtration ($p = 0.01$), with a negative Spearman's correlation coefficient of -0.437. Selenite, an oxyanion of selenium, can influence *E. coli* counts in the rainwater by inactivating proteins, blocking DNA repair and interfering with cellular respiration (Turner et al., 1998). It has also been shown that selenium can be utilised in many metabolic pathways, for example the synthesis of macromolecules such as tRNA, formate dehydrogenase enzymes and many other proteins (Böck et al., 1991; Burk, 1991; Pinsent, 1954). Significant negative correlations were also observed between total coliform counts and the presence of the ions, silicon, vanadium, chromium and sulphate in the harvested rainwater samples. Significant positive correlations were observed between the total coliforms counts and the concentrations of magnesium and nitrite present in the harvested rainwater samples. Douagui et al. (2012) and Nola et al. (2002) also observed significant positive correlations between coliform bacteria in groundwater and nitrite and magnesium, respectively.

Table 2.13: Major correlations between microbiological indicators and various metals and anions in rainwater samples ($p<0.05$).

Variables	Spearman's r	p - value
V and Total Coliforms (SP*)	-0.407	0.02
Cr and Total Coliforms (SP*)	-0.370	0.04
Se and *E. coli* (MF*)	-0.437	0.01
Mg and Total Coliforms (SP*)	0.393	0.03
Si and Total Coliforms (SP*)	-0.415	0.02
NO_2 and Total Coliforms (SP*)	0.393	0.03
SO_4 and Total Coliforms (SP*)	-0.415	0.02

After analysing all the data the REML and the Fixed Effect test showed significant variation ($p = 0.00$, $F = 8.968$) for *E. coli* counts (utilising the spread plate technique) amongst the eight sampling sessions. The LSD test then showed that the highest mean difference (2465.52±449.86) ($p = 0.00$) was recorded between sampling sessions one and seven, while the lowest mean difference, with the least amount of variation, was recorded between sampling sessions two and six (68.97± 449.8644) ($p = 0.878$).

Enterococci ($R = - 0.194$), temperature ($R = - 0.313$, $p = 0.00$), pH ($R = - 0.424$, $p = 0.00$) and rainfall ($R = 0.61$, $p = 0.00$) also exhibited a significant correlation to *E. coli* counts (utilising membrane filtration). Over time, using the REML and the Fixed Effect test, the data exhibited significant variation ($p = 0.00$, $F = 17.005$) amongst the eight sampling sessions. An LSD test showed that the highest mean difference (-73.103 ±9.40) ($p = 0.00$) was recorded between sampling sessions three and seven, while the lowest mean difference with the least amount of variation was recorded between sampling sessions one and two (1.86± 9.4)($p = 0.843$).

Statistical analysis also revealed significant correlations ($p = 0.00$) between faecal coliforms and the parameters total coliforms (utilising membrane filtration) ($R = 0.29$), temperature ($R = - 0.25$) and pH ($R = -0.236$). After analysing all the data the Repeated Measures ANOVA and the Bonferroni test showed no significant variation for faecal coliforms ($p = 0.06$, $F = 2.94$) amongst sampling sessions four, seven and eight. The rest of the sampling sessions were not analysed in this manner due to a lack of variation within

the data. As mentioned previously, enterococci showed significant correlations to *E. coli* counts utilising membrane filtration and the spread plate technique, pH ($R = 0.15$, $p = 0.04$), and rainfall ($R = -0.19$, $p = 0.01$). Due to the lack of variation within the data, no further analysis was performed.

2.4 CONCLUSIONS

Overall the results obtained for the chemical analysis section for the 29 rainwater tanks in the Kleinmond Housing Scheme, for the sampling period March to August 2012, indicated that the rainwater quality was within potable chemical standards. Metals and anions that were analysed for in the harvested rainwater samples, collected during the first sampling, were all below the recommended guidelines according to the WHO (2011), DWAF (1996), the SANS 241 (SABS, 2005) and the Australian government guidelines (ADWG) (NHMRC and NRMMC, 2011). The harvested rainwater samples also had a total hardness of 35.50 and according to the classification by Kunin (1972) the rainwater can be described as soft. Soft water has the potential to be corrosive if it is in contact for extended periods of time with metal surfaces such as plumbing, tanks and geysers (DWAF, 1996). However, the 2000 L rainwater tanks in the Kleinmond Housing Scheme are constructed from polyethylene which significantly reduced the threat of corrosion. The chemical oxygen demand (COD), which refers to the amount of oxygen required to oxidise all the organic matter present (SABS, 2005), for the harvested rainwater samples collected during sampling eight, fell within the range of 4 - 9.5 mg/L, which indicated that the concentration of organic matter in the rainwater was low. However no recommended value for COD is stipulated in the SANS 241 (SABS, 2005), ADWG (NHMRC and NRMMC, 2011) and DWAF (1996) guidelines. An average pH value of 5.28 was also recorded for the duration of this study. The pH levels fell within the SANS 241 (SABS, 2005) standards, but not the guidelines stipulated by DWAF and the ADWG. However, there is insufficient data to stipulate a health guideline for pH levels, although drinking water with a pH of less than 4 and higher than 11 may be detrimental to human health.

While the chemical quality of the rainwater was generally lower than the stipulated drinking water guidelines, in contrast the results obtained for microbial analysis was often significantly higher ($p < 0.05$) than the guidelines. The results for total coliforms, *Escherichia coli* and the heterotrophic bacteria fluctuated throughout the study period and with the exception of a few samples collected during the sampling period (March to August 2012), overall the results recorded significantly exceeded ($p < 0.05$) the stipulated guidelines (DWAF, 1996; NHMRC and NRMMC, 2011). For the duration of this study, only 10% and 36% of the samples did not adhere to the guidelines for enterococci and faecal coliforms, respectively (DWAF, 1996; NHMRC and NRMMC, 2011; WHO, 2011).

In addition, the total coliform counts obtained by the spread plate technique (ChromoCult® Coliform Agar) yielded higher counts in the first four sampling sessions than the last four sessions (five to eight). These results correlated to the counts obtained for *E. coli* and enterococci, where higher counts were also observed during sampling one to four. The temperature average recorded during the first four sampling sessions (19.5ºC) was higher than the temperature average recorded in samplings five to eight (13.8ºC), which could account for the results obtained. The total rainfall measured in sampling five to eight (ranging from 74.7 mm/month in June to 198.1 mm/month in August) was also higher than the total rainfall measured in samplings one to four (ranging from 16.8 mm/month in March to 30.6 mm/month in May). This correlates to results obtained in a previous study where higher total coliform counts were observed during the autumn season (lower total rainfall) than in the winter season (higher total rainfall) (Sazakli et al., 2007). The bacterial numbers obtained in the first four sampling sessions could also have been higher initially due to contaminants, such as debris, bird droppings etc. (that would have collected over the summer months), being washed into the tanks during the first rainfall events in the autumn months, as no first flush diverters were installed. As the rain continued to fall during the autumn and winter months, the bacterial numbers could have decreased due to the inflow of rain diluting the water in the tanks, which does not support the proliferation of microorganisms. The lower temperatures observed during the last four sampling sessions

could also have contributed to the lower total coliform, *E. coli* and enterococci counts obtained during this sampling period (Sazakli et al., 2007).

Isolates (cultured from various media including *Salmonella Shigella* Agar, *Campylobacter* Blood-free Selective Medium and *Legionella* CYE Agar base) were identified by amplifying the 16S rRNA region through PCR, with the subsequent sequencing of the PCR products, which were then compared to sequences on the NCBI database. During the first sampling period (one to four) the species diversity of Gram negative bacteria was slightly lower on average than the species diversity obtained during the last four sampling sessions. Sampling five also yielded the highest species diversity overall, with 11 different genera being identified. In comparison, sampling two yielded the lowest species diversity, with only two genera being identified. According to literature, many opportunistic pathogens and human pathogenic species are associated with rainwater and in the current study species associated with the genera, *Aeromonas*, *Klebsiella*, *Enterobacter*, *Salmonella, Yersinia,* amongst many others, were identified in the harvested rainwater at the Kleinmond Housing Scheme.

As indicated, various methods were also employed to select for *E. coli* isolates from 10 different domestic rainwater tanks (within the cluster of 29 tanks routinely monitored) at the Kleinmond Housing Scheme during the sampling period of 5 March to 7 August 2012 (sampling sessions one to seven). Phylogenetic analysis of the representative organisms isolated in GenBank for samplings one, three and six (majority of the *E. coli* strains were isolated during these sampling times) were then analysed using the Neighbour-joining algorithm in ClustalX. Of particular concern was the fact that presumptive positive enterotoxigenic *E. coli* (ETEC) strains, which contain the heat stable toxin (ST1), were identified predominantly in sampling one and three. Six percent of the total *E. coli* isolates were also identified as *E. coli* 0157:H7 (sampling one, two and three). *Escherichia coli* 0157:H7 is the predominant serotype of enterohaemorrhagic *E. coli* (EHEC) that produces the Shiga-like toxin that is responsible for causing watery and subsequent bloody diarrhoea in humans. The presence of *E. coli* strains in the rainwater samples thus also implies that the tanks do not comply with the guidelines stipulated by DWAF (1996), the WHO and the ADWG (NHMRC and NRMMC, 2011), which indicate that *E. coli* should be absent from water sources utilised for drinking purposes.

CHAPTER 3: FILTER ASSESSMENT – THE EFFICIENCY OF FILTRATION SYSTEMS IN REMOVING CHEMICAL AND MICROBIAL CONTAMINANTS FROM RAINWATER

3.1 INTRODUCTION

The Millennium Development Goals (MDGs) were officially established by the United Nations (UN) in 2000. One of the main aims of the MDG was to improve infrastructure and significantly increase access to safe drinking water by halving the proportion of people without access to potable water and sanitation by 2015. In 2008 the UN published the progress of the MDG, with the report highlighting that from 1990 to 2008, internationally, 1.1 billion people residing in urban areas had gained access to an improved water source, and that during the same time frame, 723 million people living in rural areas achieved the same goal. In addition the number of people using an improved drinking water source in Sub-Saharan Africa increased from 252 million in 1990 to 492 million people in 2008. However, 11% of the world's population still remains without access to an improved drinking water source and it was therefore predicted that, at the current rate, 605 million people would still lack access to safe drinking water by 2015 (UN, 2012).

In Southern Africa, in addition to point and non-point sources of pollution, climate change has also contributed to the long- terms stresses on already compromised hydrological systems and water resources (Mwenge Kahinda et al., 2010). This extensive effect of climate change can impact not only freshwater resources but in turn have detrimental effects on sustainable- and economic development, poverty reduction strategies, child mortality reduction programs, production and availability of food and the well-being of people and ecosystems (Rutashobya, 2008). As Southern Africa has become vulnerable to the impact of climate change, a few adaptive measures have been set in place in an attempt to alleviate the pressures that water security faces. Moreover, countries around the world are exploring alternative water sources due to factors such as climate change and increasing population growth that are having a negative impact on current water sources.

Domestic rainwater harvesting (DRWH) has gained interest as an alternative water source as it has allowed countries in the semi-arid areas of the world to provide local settlements with a water source. For example, it has previously been noted that 50% of the Tanzanian area rely solely on rainwater as a primary water source (Mbilinyi et al., 2005). Technologies such as rainwater harvesting are thus gaining much interest amongst governmental organisations. However, numerous studies have detected microbial contaminants, such as total and faecal coliforms and *Escherichia coli (E. coli)*, in harvested rainwater samples above the drinking water standards of the respective countries guidelines (Uba and Aghogho, 2000; Despins et al., 2009; Ahmed et al., 2010). It is thus strongly recommended that harvested rainwater be treated before it is utilised for drinking and certain domestic purposes.

Slow sand filtration is a water treatment method that has been implemented for centuries and although its usage has been declining in the past decades in favour of treatments such as chlorination and UV inactivation, it is starting to receive renewed attention (McConnell et al., 1984; Haig et al., 2011). This renewed attention is fuelled by the fact that it can serve as a very useful water treatment method in rural communities. Slow sand filtration systems are effective in the removal of even small contaminants such as viruses and its simplistic design eliminates the need for costly hardware such as valves and pumps (McConnell et al., 1984; Burch and Thomas, 1998). The contaminated water filters through approximately 100 cm of fine silica sand at slow enough retention rates to allow for the formation of a biofilm on the top layer of the sand. This biofilm serves as a biological filter that effectively removes over 99% of pathogens

such as bacteria, viruses, *Giardia* cysts, and *Cryptosporidium* oocysts (Burch and Thomas, 1998; Kohne and Logsdon, 2004). McConnell et al. (1984) also found that slow sand filtration is capable of absorbing 95% of viruses from the contaminated water and that the greatest removal took place in the top few centimetres of the system.

Scientists are also becoming increasingly attentive towards polymer nanofibres, a class of nano-material, because of its high surface-to-mass volume and other special characteristics that make it attractive for advanced applications. Electrospun nanofibre membranes can be useful for membrane distillation because of its high porosity, interconnected open pore structure, tailored membrane thickness and high surface hydrophobicity (Feng et al., 2013). Studies have also shown that electrospun nanofibre membranes can be used for desalination and pre-treatment of water to be purified through ultrafiltration or reverse osmosis (Feng et al., 2013). Activated carbon can then be used in conjunction with nanofibres as a very effective point-of-use treatment method for the removal of contaminants that are usually more resistant towards traditional disinfection methods. Research has also shown that it has the ability to remove dangerous and resilient viruses by adsorption of the viruses to the carbon granules (Gerba et al., 1975; Powell et al., 2000).

The aim of this study was to assess the efficiency of a polyvinyl (alcohol) (PVA) nanofibre membrane and activated carbon filtration system, in the treatment of rainwater collected directly from domestically harvested rainwater (DRWH) tanks. The efficiency of slow sand filtration systems and activated carbon systems in the treatment of harvested rainwater were also evaluated in this study. The microbial parameters that were investigated included faecal and total coliforms, *E. coli* and heterotrophic bacteria. In addition, the treated and untreated rainwater samples were screened for the presence of selected pathogenic bacteria and well as selected enteric viruses using molecular techniques. The chemical parameters that were investigated included the concentration of metal ions, anions and cations present in the treated and untreated harvested rainwater samples.

3.2 MATERIALS AND METHODS

3.2.1 Sample Site and Collection

Three polyethylene domestic rainwater harvesting (DRWH) tanks (2000 L) were installed at the Welgevallen Experimental farm, Stellenbosch University, South Africa (Figure 3.1). The solar pasteurization system was connected to tank A (discussed in Chapter 5), while the middle tank (tank B) and the right end tank (tank C) were used to test the filtering systems utilised in the present study. The farm is situated on the periphery of the town of Stellenbosch. As indicated in Figure 3.1, the sampling site was surrounded by trees; however no tree branches obstructed the catchment area. The farm was also surrounded by dirt roads that were continuously used by motor vehicles and the farm workers to herd cattle twice a day as the tanks were situated on the northern side of a well-established building that neighboured the farms' dairy. In addition, due to damage, a section of the guttering lengths on the building where the DRWH tanks were installed had to be replaced, and new down pipes were installed in order to link the tanks to the old gutter system surrounding the catchment area. The older gutter system was constructed from white asbestos (Chrysotile) and the roofing was constructed from corrugated galvanised iron.

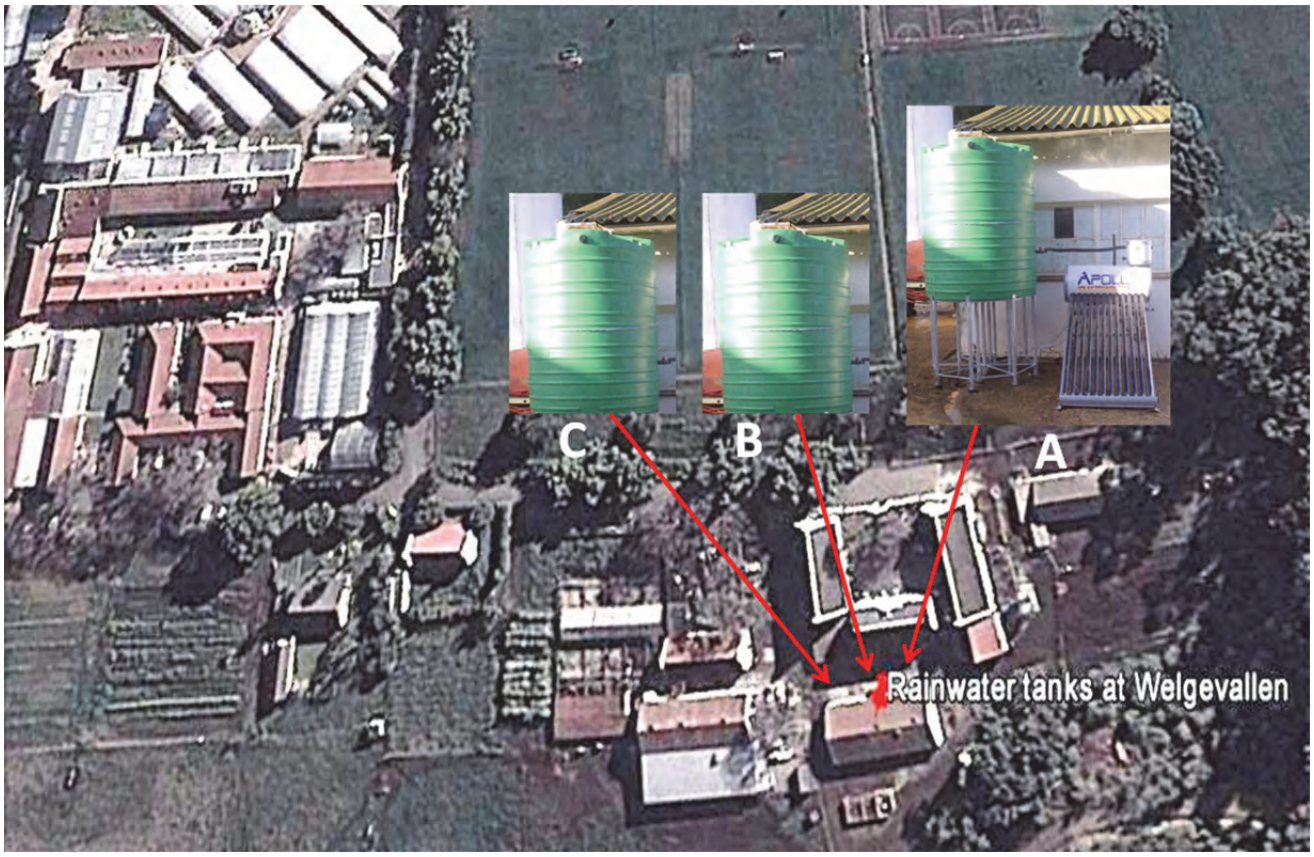

Figure 3.1: The sampling site (33°56'36.19"S, 18°52'6.08"E) used in the study was located at Welgevallen Experimental farm, Stellenbosch, South Africa. Three domestic rainwater harvesting tanks were installed: (A) Solar pasteurization system and (B) and (C) where various treatment systems were intermittently connected.

For microbial and chemical analysis, before and after treatment rainwater samples were collected in 1 L sterile Schott bottles. The temperature and pH of the rainwater at the sampling locations were measured using a hand-held mercury thermometer and colour-fixed indicator sticks with a pH range of 0 - 14 (ALBET®, Barcelona, Spain). Rainfall and temperature patterns were obtained from the South African Weather Services (SAWS, 2013), with samples collected from June to September 2013.

3.2.2 Point of use filtering systems employed for the treatment of rainwater

3.2.2.1 *Biological Filtration: Activated Carbon*

Two activated carbon filtration systems were set up directly next to rainwater tanks B and tank C (Figure 3.1). The filtering systems consisted of a smaller container (20 L) containing holes at the bottom, which fitted into a larger container (25 L) with a tap connected for effluent collection (Figure 3.2). The smaller container was filled with a bottom layer of 5 cm marine pebbles and then a top layer of approximately 17 cm of activated carbon (Aquasorb Udectrading Pty. Ltd) which represented the filtering material. A garden hose was then connected to the tap of a DRWH tank on the one end, and then to a shower head (20 cm diameter) on the other end. A shower head was fitted to the lid of the smaller container to allow the harvested rainwater to trickle through the shower head and over the activated carbon filtering medium. A biofilm was allowed to establish in the slow activated carbon filtration system by filling the system with rainwater and then allowing the system to remain closed for one week. The system was then drained and in a continuous flow arrangement, the rainwater was allowed to flow through the system for the duration of sampling and subsequent filtrate analyses.

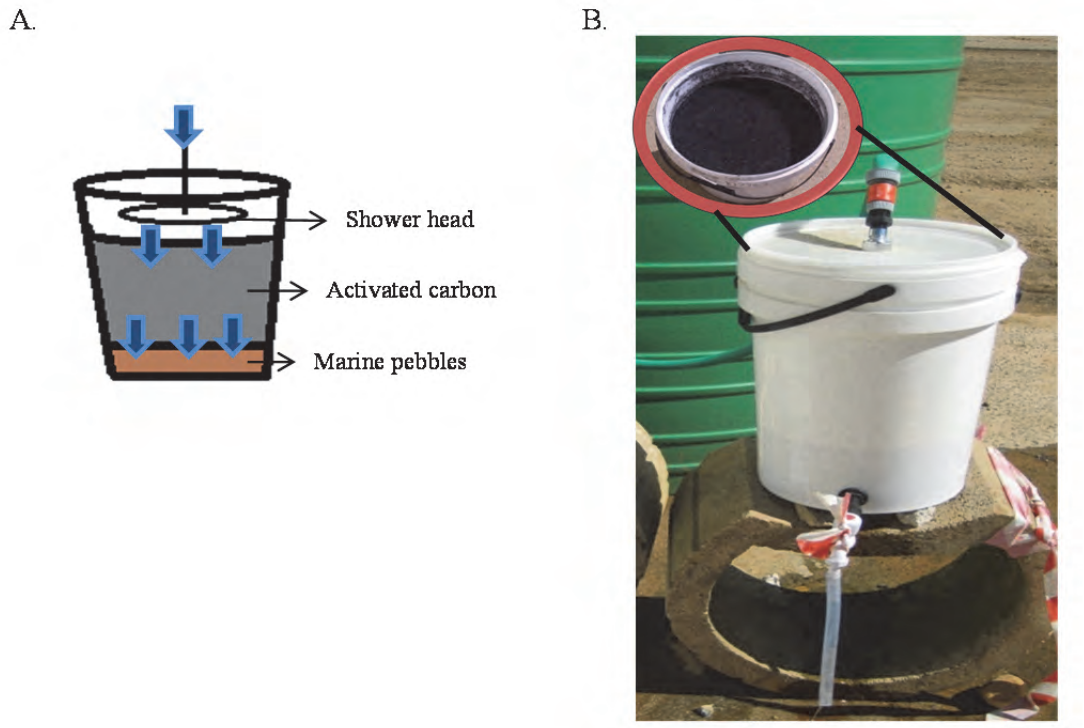

Figure 3.2: (A) Schematic diagram of the smaller container containing marine pebbles and activated carbon which fit into a larger container allowing for a slow activated carbon filtration system. (B) Photograph of the slow activated carbon system.

To determine the degree of chemical and bacterial pollution, samples were collected for five consecutive days. On each sampling day a 1 L water sample was collected directly from rainwater tanks B and C (before sample), respectively, and a 1 L rainwater sample was collected from the filtrate of the activated carbon filtration systems (an after sample) connected to tanks B and C, respectively.

3.2.2.2 *Biological Filtration: Slow Sand Filtration*

Two slow sand filtration systems were set up directly next to rainwater tanks B and tank C (Figure 3.1). The same system utilised for the activated carbon filtration (section 3.2.2.1 and Figure 3.2) was constructed with the exception that the slow sand filtration system consisted of a 5 cm layer of marine pebbles, placed in the bottom of the smaller container and approximately 22 cm of 0.61 mm silica sand (Cape Silica Suppliers CC, Cape Town, South Africa) placed on top of the pebbles. A biofilm was also allowed to establish for two weeks in a closed system before sampling took place by allowing water from the respective DRWH tanks to flow through the system. After the biofilm had formed, samples were collected every second day, for a total of six sampling events over a two week period. The water was allowed to continuously flow through the system between samplings. On each sampling day a 1 L water sample was collected directly from rainwater tanks B and C (before sample), respectively and a 1 L rainwater sample was collected from the filtrate of the slow sand filtration systems (an after sample) connected to tanks B and C, respectively.

3.2.2.3 *Polyvinyl (alcohol) (PVA) nanofibre membrane filtration system*

Polyvinyl (alcohol) nanofibres were produced by a process of needleless electrospinning utilising a Nanospider 200 Lab (Elmarco, s.r.o., Czech Republic). The substrate material onto which the nanofibres were deposited was a Tyvek material (Marshall Hinds, Johannesburg, South Africa) which was wound onto a core. A PVA polymer solution was made up by dissolving a PVA powder (Nippongohsei, Japan) in distilled water at 80˚C. The PVA polymer solution was modified by adding a cross-linker, acid and $CuCl_2$ (proprietary

information). The PVA polymer solution was then poured into a polypropylene tub containing a stainless steel spinning electrode which was then partially submerged in the polymer solution. In order to create an electric field, a high voltage was connected to the spinning electrode with the collecting wire electrode grounded to create a potential difference. The spinning conditions were as follows; spinning distance was 100 mm, rotation speed of electrode was 3.2 rpm, high voltage was 80 kV, relative humidity was below 40% and speed of fabric was 0.1 m/min. Once the nanofibres were spun onto the Tyvek material, the newly synthesised membrane was cross-linked at 140°C for 15 min. A section of the membrane was analysed using scanning electron microscopy (SEM) at the Central Analytical Facility (CAF), Stellenbosch University. Microscopy was performed using a LEO 1450VP SEM (Zeiss, Germany). The final product, a PVA nanofibre membrane was then used in a column flow through system.

The column system that was directly attached to tanks B and C is indicated in Figure 3.3. A schematic diagram of the PVA nanofibre membrane column is represented in Figure 3.3A, where unfiltered rainwater (red arrows) was allowed to flow through the PVA nanofibre membrane to the centre of the column and then filtered rainwater (blue arrows) was collected. The column systems were designed as follows, an inner cylinder containing holes (Figure 3.3B) was fitted inside a larger column (Figure 3.3C, D). A PVA nanofibre membrane was then wrapped around the inner cylinder twice which was then covered with a red netting (Figure 3.3E). This PVA nanofibre membrane system was assessed for bacterial removal efficiency only (Figure 3.3F).

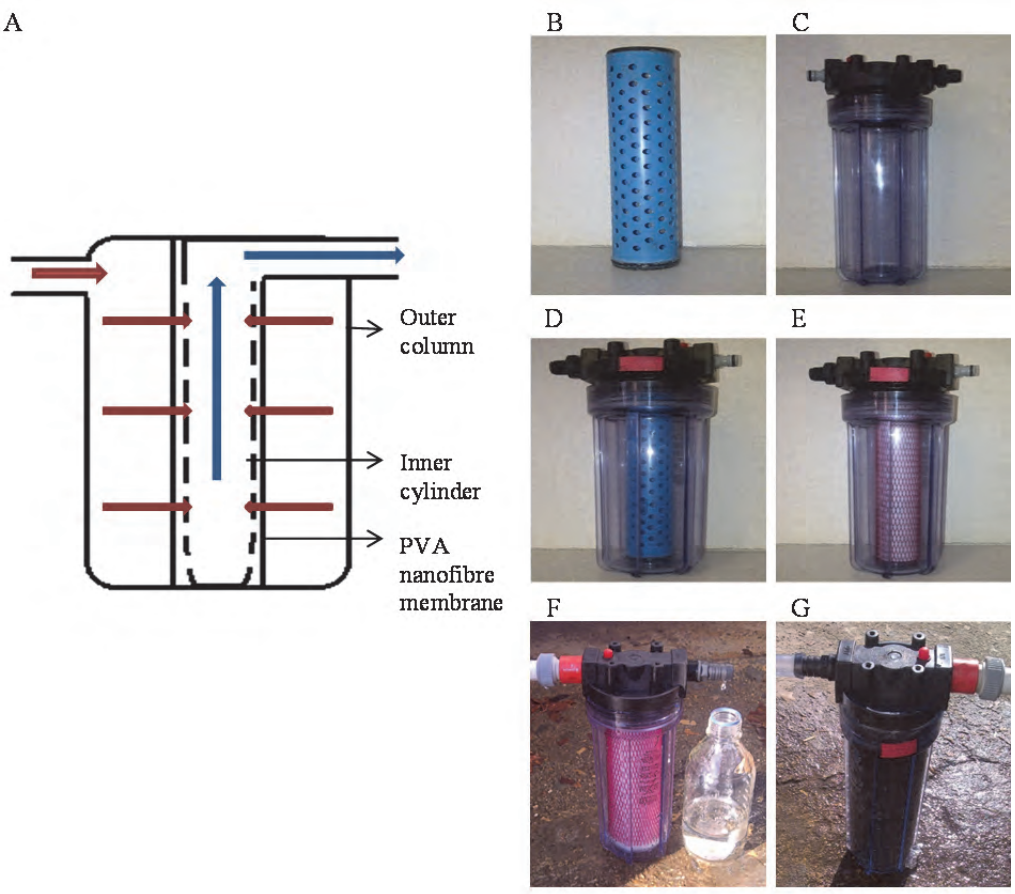

Figure 3.3: A) A schematic diagram of the PVA nanofibre membrane column, where unfiltered rainwater (red arrows) was allowed to flow through the PVA nanofibre membrane to the centre of the column and then filtered rainwater (blue arrows) was collected. B – F) A column system containing a PVA nanofibre membrane. G) Activated carbon was then layered around the PVA nanofibre membrane.

To determine the bacterial and chemical contamination removal efficiency, activated carbon (Aquasorb udectrading Pty. Ltd) was then layered around the PVA nanofibre membrane in order to exclude larger contaminants before passing through the PVA nanofibre membrane (Figure 3.3G). An initial 1 L rainwater sample was collected directly from tanks B and C. Five 1 L samples were then individually collected after the water had passed through the PVA nanofibre membrane/activated carbon filtration system. The PVA nanofibre membrane and activated carbon was then replaced and another sampling was repeated with the system connected to each tank.

3.2.3 Chemical Analysis

Filtered samples collected from the slow sand and activated carbon biological filtration systems (before and after biofilm formation) and the PVA activated carbon column filtration system were analysed for the following chemical parameters. For the determination of the metal concentrations, Falcon™ 50 mL high-clarity polypropylene tubes containing polyethylene caps, were pre-treated with 1% nitric acid before sampling. The concentrations of metals such as aluminium (Al), vanadium (V), chromium (Cr), manganese (Mn), iron (Fe), copper (Cu) and zinc (Zn), amongst others, were then determined using inductively coupled plasma atomic emission spectrometry (ICP-AES) according to Saleh et al. (2000) and nitric acid digestion. All samples were analysed for the presence of metals at the Central Analytical Facility (CAF), Stellenbosch University. The filtered rainwater samples were also sent to the Centre for Scientific and Industrial Research (CSIR) Stellenbosch for anion analyses. The anions detected along with the corresponding detection method are summarised in Table 3.1.

Table 3.1: Methods used in the detection of anions performed by the CSIR, Stellenbosch.

Anion	Method
Nitrate and Nitrite	SALM 7.0 Automated Colorimetry
Soluble phosphate	SALM 9.0 Automated Colorimetry
Sulphate	MALS 6.5 ICP OES Detection
Chloride	SALM 1.0 Automated Colorimetry
Fluoride	SALM 11 Potentiometric measurement

3.2.4 Recovery of Indicator Organisms

To enumerate the heterotrophic plate count (HPC), a serial dilution was prepared for each sample (10^{-1}–10^{-2}) and by use of the spread plate method 100 µl of each dilution was cultured onto R2A agar (Difco) in triplicate, with the plates incubated at 37°C for up to four days. Total coliforms (TC) and *E. coli* were then enumerated simultaneously by filtering a total volume of 100 mL (undiluted and 10-1) 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. The filtration flow rate was approximately ≥ 65 mL/min/cm^2 at 0.7 bar (10 kPa, 10 psi). The filters were then incubated on Membrane Lactose Glucuronide Agar (MLGA) (Oxoid, Hampshire, England) at 35 ± 2°C for 18 - 24 hours (U.S. Environmental Protection Agency, 2009). All analysis was performed in triplicate.

3.2.5 The Bacterial Removal Efficiency of the Filtration Systems and the Hydraulic Retention Time

The bacterial removal efficiency of each treatment system was obtained by comparing the CFU numbers obtained from the samples taken before filtration and the average CFU numbers obtained from samples taken after filtration. The log reduction in bacteria numbers was calculated using equation 1 and the percentage reduction using equation 2 (Brözel and Cloete, 1991).

Equation 1:
Log reduction = (Log10 bacterial count before filtration – Log 10 bacterial count after filtration)

Equation 2:
Percentage reduction = 100 - (Survivor count)/(Initial count) × 100

The hydraulic retention time (HRT) is a measurement of the average length of time that a soluble compound remains in a constructed bioreactor. It can be calculated by dividing the total volume of the tank by the flow rate of the influent. The total volume is measured in litres and the flow rate of influent in litres per hour. Hydraulic retention time is then expressed in hours. Equation 3 was used to calculate hydraulic retention time for each filtration system.

Equation 3:
Hydraulic Retention Time (h) = (Volume of tank (L))/(Flow rate of influent ($L/h^{(-1)}$))

3.2.6 Recovery and Assay of Coliphages

Coliphages were enumerated according to Baker et al. (2003). Briefly, 30 µL of chloroform (BDH AnalaR®) was added to 2 mL of an untreated rainwater sample as well as a treated rainwater sample collected from the different filtration systems. Each sample was then centrifuged at 13 200 × g for five minutes. To ensure that no chloroform was transferred, 1 mL of each sample was subsequently added to 100 µL *E. coli* ATCC 13706 (Microbiologics®) which had been grown to stationary phase in Luria-Bertani Broth (Merck). After each sample had been briefly vortexed, the samples were then incubated at 25˚C for five minutes. The mixture was transferred to a test tube containing 5 mL of melted top agar (7% Luria-Bertani w/v), mixed gently and poured onto a plate containing Luria-Bertani Agar. Once the plates had set, the samples were incubated at 37˚C for 18 hours. Each assay was performed in triplicate for each sample. Rainwater samples that were spiked with *E. coli* ATCC 13706 were also analysed in the same manner and for this, 10 mL of *E. coli* DH5α that had been cultured to the stationery phase was added to 500 mL rainwater, the mixture was then allowed to stand at room temperature for approximately 6 hours before samples were collected to be analysed for the presence of coliphages.

3.2.7 Extraction of Total DNA from Rainwater Samples

Total DNA extractions were performed for each of the 45 rainwater samples collected before and after filtration. In order to extract total genomic DNA from the rainwater samples a modified version of the boiling method was utilised (Watterworth et al., 2005). Each rainwater sample (500 mL) was filtered 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. The filtration flow rate was approximately ≥ 65 $mL/min/cm^2$ at 0.7 bar (10 kPa, 10 psi). The filters for each rainwater sample were then incubated in 2 mL Luria Bertani (LB) (Merck) broth for 5 hours at 37°C. The samples were vortexed for 15 min to detach the cells from the filters and cells were harvested from 2 mL of the cell suspension through centrifugation at 6 000 rpm for 10 min. The supernatant was discarded and the pellet was re-suspended in 100 µL sterile MilliQ water. The re-suspended cells were then boiled at 95°C for 15 min, followed by cooling on ice for 10 min. The sample was subjected to centrifuging at 13 000 rpm for 5 min and the supernatant transferred to a sterile eppendorf tube. In each instance genomic DNA and total DNA was visualised on a 0.8% agarose gel stained with 0.5 µg/mL ethidium bromide. Electrophoresis was conducted at 80 volts for approximately one hour with the use of 1X Tris/Borate/EDTA (TBE) buffer (Sambrook et al., 1989).

3.2.8 Bacterial Genus Specific PCRs

Primers and PCR conditions as outlined in Table 3.2 were utilised in the current study for the identification of documented pathogenic and opportunistic bacterial pathogens. Each PCR mix was performed in a final volume of 50 µL. For the detection of *Shigella* spp., *Salmonella* spp. and *Aeromonas* spp. the PCR mix consisted of 10 µL of 5X Green GoTaq® Flexi Buffer (1X) (Promega), 4 µL $MgCl_2$ (2.0 mM) (Promega), 0.5 µL of each dNTP (0.1 mM) (Thermo Scientific), 0.5 µL of the PCR primer (0.1 µM), 0.3 µL of GoTaq® Flexi DNA Polymerase (Promega) (1.5U) and 10 µL of template DNA. For *Yersinia* spp. and *Klebsiella* spp. the same PCR mix was used with the exception that 1.5 µL of the respective forward and reverse PCR primers (0.3 µM) were used. For *Pseudomonas* spp. and *Legionella* spp., again, the same reaction mixture was used, however 2.0 and 2.5 µL of each PCR primer (0.4 and 0.5 µM, respectively) was used, respectively.

Table 3.2: Primers and PCR cycling parameters for the detection of various potential bacterial pathogens.

Organism	Primer name	Primer sequence (5'-3')	PCR Cycling Parameters	Gene (Size bp)	References
Legionella spp.	JFP	AGGGTTGATAGGTTAAGAGC	5 min at 95°C; 40 cycles of 94°C for 1 min, 57°C for 1.5 min, 72°C for 1 min	Attachment invasion locus gene (386)	Jonas et al. (1995)
	JRP	CCAACAGCTAGTTGACATCG			
Aeromonas spp.	Aero-F	TGTCGGSGATGACATGGAYGTG	2 min at 95°C; 35 cycles of 94°C for 1 min, 62°C for 1 min, 72°C for 2.5 min	Aerolysin (720)	Kong et al. (2002)
	Aero-R	CCAGTTCCAGTCCCACCACTTCA			
Shigella spp.	IpaH-F	CCTTGACCGCCTTTCCGATA	2 min at 95°C; 35 cycles of 94°C for 1 min, 62°C for 1 min, 72°C for 2.5 min	Invasion plasmid Antigen H (606)	Kong et al. (2002)
	IpaH-R	CAGCCACCCTCTGAGGTACT			
Salmonella spp.	IpaB-F	GGACTTTTTAAAAGCGGCGG	2 min at 95°C; 35 cycles of 94°C for 1 min, 62°C for 1 min, 72°C for 2.5 min	Invasion plasmid Antigen B (314)	Kong et al. (2002)
	IpaB-R	GCCTCTCCCAGAGCCGTCTGG			
Pseudomonas spp.	PA-GS-F	GACGGGTGAGTAATGCCTA	2 min at 95°C; 25 cycles of 94°C for 20 s, 54°C for 20 s, 72°C for 40 s	16S rRNA (618)	Spilker et al. (2004)
	PA-GS-R	CACTGGTGTTCCTTCCTATA			
Yersinia spp.	227Fmod	GTCTGGGCTTTGCTGGTC	5 min at 95°C; 40 cycles of 94°C for 20 s, 60°C for 20 s, 72°C for 15 s	*omp*F (428 - 465)	Stenkova et al. (2008)
	669R	GCGTCGTATTTAGCACCAACG			
Klebsiella spp.	gryA-F	CGCGTACTATACGCCATGAACGTA	3 min at 95°C; 35 cycles of 94°C for 1 min, 50°C for 30 s, 72°C for 30 s	Gyrase A gene (383)	Brisse and Verhoef (2001)
	gyrA-C	ACCGTTGATCACTTCGGTCAGG			

The following strains were cultured as positive controls after which, genomic DNA was extracted; *Legionella pneumophila* ATCC 33152, *Shigella sonnei* ATCC 25931, *Salmonella typhimurium* ATCC 14028, *Pseudomonas aeruginosa* ATCC 27853, *Aeromonas hydrophila* (environmental strain), *Klebsiella*

pneumoniae ATCC 13385 and *Yersinia enterocolitica* ATCC 27729. All positive control organisms were obtained from Microbiologics®, unless indicated otherwise. The specificity of each primer set was confirmed by using non target DNA extracted from all the above mentioned positive controls and a negative control (sterile distilled H_2O) was also included.

All PCR products were analysed by gel electrophoresis in 1.5% agarose (Bio- Rad) containing 0.5 µg/mL ethidium bromide in 1X TBE buffer. Deoxyribonucleic acid bands were confirmed by UV illumination and photographed using the Gel Doc 1000 documentation system (Bio-Rad). Once the size and the concentration of the PCR products had been confirmed, products of representatives of the samples were purified and concentrated using the DNA Clean & Concentrator™-5 Kit (Zymo Research) as per manufacturer's instructions. The cleaned products were then sent to the Central Analytical Facility at Stellenbosch University for sequencing. Chromatograms of each sequence were examined using FinchTV v. 1.4.0 software and were aligned using DNAmanTM version 4.1.2.1 software. Sequence identification was completed using the National Centre for Biotechnology Information (NCBI) and The Basic Local Alignment Search Tool (BLAST) (http://blast.ncbi.nlm.nih.gov/Blast.cgi) to find the closest match of local similarity between isolates and the international database in GenBank, EMBL, DDBJ and PDB sequence data (Altschul et al., 1990). The sequences of representative isolates, that showed > 97% similarity (<3% diversity) to organisms was recorded.

3.2.9 Detection of Adenovirus and Rotavirus in Rainwater and Filtered Rainwater Samples

Adeno-and rotavirus in the untreated rainwater as well as the filtered samples were detected as outlined in sections 3.2.9.1 to 3.2.9.4 below.

3.2.9.1 *Concentration of Viruses from Rainwater*

The concentration of viruses from the rainwater samples was carried out as described by Saayman et al. (2012). One millilitre of 1 M $CaCl_2$ and 1 mL of Na_2HPO_4 were added to a 500 mL sample. The mixture was stirred for 5 minutes using a magnetic stirrer to allow for flocculation where after it was filtered through a 47 mm, 0.45 µm pore size non-charged mixed-ester membrane filter (Whatman GmbH, Germany) at a flow rate of approximately ≥ 65 mL/min/cm^2 at 0.7 bar (10 kPa, 10 psi). The membrane was then transferred to a 9 cm petri dish containing 4 mL of 0.3 M citric acid (pH 3.5) and soaked for 3 minutes. The membrane was discarded and the citric acid solution containing the virus was transferred to an Amicon Ultra centrifugal device (Millipore) for concentration. The device was centrifuged (Heraeus, Biofuge Stratos) at 1500 × g for 2 minutes, where after the volume was adjusted to 1 mL with 1X phosphate buffered saline.

3.2.9.2 *Extraction of Virus DNA/RNA from Rainwater*

Deoxyribonucleic acid and RNA were simultaneously extracted from 200 µL of concentrated sample (section 3.2.9.1) using the QIAmp Ultrasens Virus Kit (Qiagen GmbH, Hilden, Germany) according to the manufacturer's instructions. The final volume was approximately 56 µL, of which 30 µL was stored at 4˚C for adenovirus identification and five aliquots containing 4 µL each were stored at -80˚C for rotavirus detection. The presence of genomic DNA/RNA was confirmed by gel electrophoresis in a 0.8% 1X Tris-Borate-EDTA (TBE) agarose gel containing 5 µg/mL ethidium bromide solution for 1 hr at 80 V.

3.2.9.3 *Rotavirus cDNA Synthesis*

For rotavirus detection, complementary DNA (cDNA) from double stranded RNA was synthesised using the Improm-II reverse transcription system (Promega Corp, USA) according to the manufacturer's instructions. A gene specific primer (RV3, Table 3.3) was employed for first strand cDNA synthesis in a final volume of

20 µL. One microlitre RV3 primer (10.0 µM) was added to 4 µL RNA and denatured at 97˚C for five minutes, where after it was immediately placed on ice for five minutes. In a separate tube, a mixture containing 5 mM $MgCl_2$, 0.5 µM dNTP mix, 0.2x Improm-II reaction buffer, 20 units RNasin ribonuclease inhibitor and 1 µL Improm-II reverse transcriptase was added according to the manufacturer's instructions. The mixture from the first tube was then added to the second tube. Reverse transcription was completed at 50˚C for 60 minutes with the reverse transcriptase enzyme heat inactivated at 70˚C for 15 minutes (Saayman et al., 2012). Complementary DNA was used immediately or stored at 4˚C for later use.

3.2.9.4 *Polymerase Chain Reaction for the Detection of Adeno- and Rota-virus*

The PCR mixture for rotavirus was made up to a final volume of 50 µL. The mixture contained 5 µL cDNA as template (prepared as in section 3.2.9.3), a final concentration of 1X Green Go Taq Flexi buffer, 0.2 mM dNTP mix, 1.5 mM $MgCl_2$ and 0.3 µM RV3 and RV4 primers (Table 3.3), respectively. All PCR's were performed with 1.25 units of Go Taq Flexi (Promega Corp. USA) DNA polymerase. The amplification was performed with an initial denaturing step at 95˚C for 3 minutes after which 35 cycles of denaturation at 95˚C for 1 minute, annealing at 45˚C for 1 minute and elongation at 72˚C for 1 minute, were completed with a final elongation step at 72˚C for 7 minutes (Gilgen et al. 1997; Saayman et al., 2012). A rotavirus positive control (Coris BioConcept, Belgium) and a negative control (sterile distilled H_2O) were also included.

Table 3.3: Primer sequences used in the study for the identification of Adeno- and Rotaviruses.

Virus and oligonucleotide	**Region**	**Sequence 5' → 3'**	**Product size**	**Reference**
Rotavirus group RV3 RV4	VP7 gene	 TGTATGGTATTGAATATACCAC ACTGATCCTGTTGGCCAWCC	346 bp	(Gilgen et al. 1997)
Adenovirus group AQ1 AQ2	Hexon gene	 GCCACGGTGGGGTTTCTA AACTTGCCCCAGTGGTCTTACATGCACATC	110 bp	(Heim et al. 2003)

The PCR mixture for adenovirus was made up to a final volume of 50 µL. The mixture contained 6 µL DNA as template (prepared from section 3.2.9.2), a final concentration of 1X Green Go *Taq* Flexi buffer, 0.2 mM dNTP mix, 2 mM $MgCl_2$ and 0.3 µM AQ1 and AQ2 primers (Table 3.3). All PCR's were performed with 1.25 units of *Go Taq* Flexi (Promega Corp. USA) DNA polymerase.

The amplification was performed with an initial denaturing step at 94˚C for 3 minutes, after which 35 cycles of denaturation at 94˚C for 30 seconds, annealing at 55˚C for 1 minute and elongation at 72˚C for 1 minute, was completed with a final elongation step at 72˚C for 7 minutes (Heim et al. 2003; Saayman et al., 2012). An adenovirus positive control (Coris BioConcept, Belgium) and a negative control (sterile distilled H_2O) were also included.

After the PCR analysis of a sample was completed, 20 µL of the product was analysed in a 2% TBE agarose gel containing 0.5 µg/mL ethidium bromide solution submerged in 1X TBE buffer and electrophoresed for 1 hr at 90 V. The product was then visualised using a UV-dock to confirm the presence of the amplicon.

For all PCR analyses, the PCR products were cleaned and concentrated using the Qiagen QIAquick Gel Extraction Kit (Qiagen GmbH, Hilden, Germany) and sent for sequencing at the Stellenbosch University Central Analytical Facility. Chromatograms of each sequence were examined using Finch TV v. 1.4.0 software and were aligned using DNAman™ version 4.1.2.1 software. Sequence identification was completed using the National Centre for Biotechnology Information (NCBI) and The Basic Local Alignment Search Tool (BLAST) (http://blast.ncbi.nlm.nih.gov/Blast.cgi) to find the closest match of local similarity

between isolates and the international database in GenBank, EMBL, DDBJ and PDB sequence data (Altschul et al., 1997). The sequences of representative isolates, that showed > 97% similarity (<3% diversity) to organisms was recorded.

3.3 RESULTS AND DISCUSSION

3.3.1 Temperature, pH, Ambient Temperature and Rainfall

3.3.1.1 *Biological Filtration: Activated Carbon and Slow Sand Filtration*

The slow sand and activated carbon filtration systems were analysed during August and September, 2013. Overall an average pH of 6 was measured for all rainwater samples, collected before and after filtration. For all unfiltered and filtered rainwater samples collected for the duration of the biological filtration systems studies (both activated carbon and slow sand filtration), the temperature of the rainwater samples ranged from a low of 18˚C (02.09.2013) to the highest recorded temperature of 24˚C on two respective days (26.08.2013 and 02.09.2013). For the slow activated carbon filtration system, the average daily ambient temperature ranged from 21.3˚C (19.08.2013) (before the growth of the biofilm) to 19.7˚C (26.08.2013) on the first day of sampling to 11˚C on the last day of sampling (30.08.2013). For the slow sand filtration system, the average daily ambient temperature ranged from 21.3˚C (19.08.2013) (before the growth of the biofilm) to 21.1˚C (26.08.2013) on the first day of sampling to 14.3˚C on the last day of sampling (13.09.2013). The highest total rainfall was recorded during August 2013 (371.6 mm/month) which then decreased in September 2013 (177.2 mm/month).

3.3.1.2 *Activated Carbon and Nanofibre Membrane System*

Columns containing two PVA membrane layers surrounded by activated carbon were connected directly to rainwater tanks B and C during June and July 2013. Overall an average pH of 6 was measured for all rainwater samples collected before and after filtration. The temperature of the rainwater samples ranged from a low of 14˚C (26.06.2013) to the highest recorded temperature of 20˚C (30.07.2013). From the start of the implementation of the microfiltration system, the average daily ambient temperature ranged from 15.6˚C (26.06.2013) on the first day of sampling to 20˚C on the last day of sampling (30.07.2013). The highest total rainfall was recorded during June 2013 (227.4 mm/month) which then decreased in July 2013 (169.6 mm/month).

In addition to the PVA/activated carbon filtration system, a column unit containing only two layers of the PVA membrane was also connected to tanks B and C during July and August 2013. From the start of microfiltration the average daily ambient temperature ranged from 21.3˚C (30.07.2013) on the first day of sampling to 20.2˚C (05.08.2013) on the last day of sampling. During the high rainfall period the PVA membrane system was analysed and rainfall recorded ranged from 169.6 mm/month (July 2013) to 371.6 mm/month (August 2013).

In order to determine the pore sizes of the PVA membrane filters, scanning electron microscopy (SEM) was performed (Figure 3.4) and the average pore size was calculated at 0.9 µm using the Digimizer Software version 4.25, however it should be noted that the pore size of the membrane was not uniform throughout and ranged from 0.32 µm to 1.9 µm.

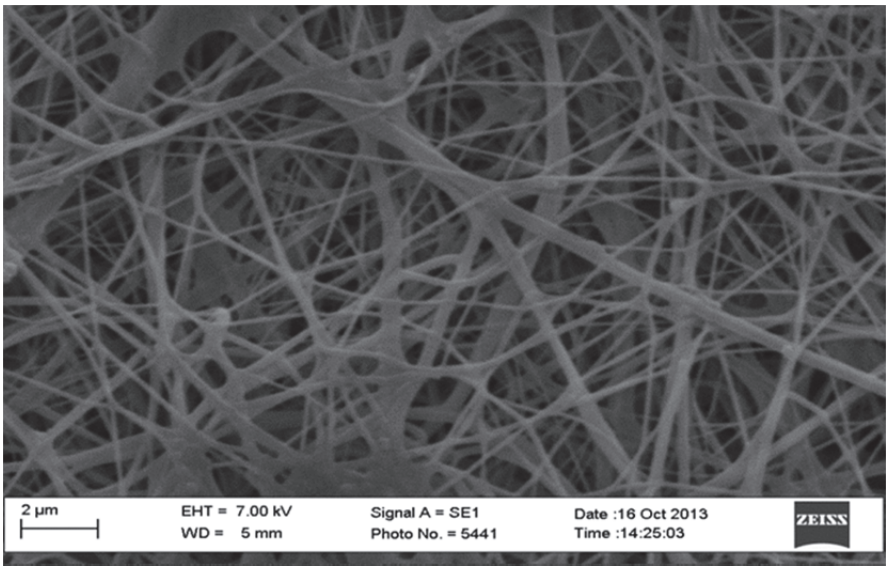

Figure 3.4: A scanning electron microscope image of a polyvinyl (alcohol) (PVA) nanofibre membrane before filtration of rainwater.

Once double layers of the PVA membrane were placed in the centre of the column with the activated carbon layered on the outer section, rainwater was allowed to pass through the activated carbon in order to remove larger contaminating particles, and then through the double layer of PVA fibres to remove smaller particles. Once rainwater samples were filtered (5 x 1 L), the PVA membrane filters were observed to be covered with particles varying in size (Figure 3.5).

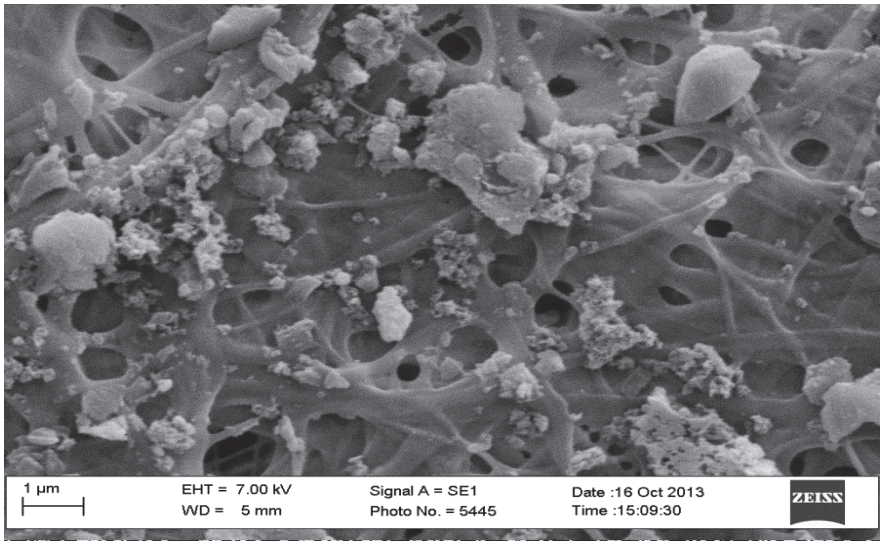

Figure 3.5: A scanning electron microscope image of a polyvinyl (alcohol) (PVA) nanofibre membrane examined after rainwater was filtered through the activated carbon and nanofibre membrane system.

Cocci shaped particles, rod shaped particles, as well as other debris and possibly granules of activated carbon were observed. The average time recorded to filter 1 L of rainwater was recorded as 31.02 minutes for the first litre, 39.57 minutes for the second litre, 38.27 minutes for the third litre, 52.92 minutes for the fourth litre and 61 minutes for the fifth litre. In comparison, the filtering time for the fifth litre (61 minutes) was approximately double that of the filtering time for the first litre (31.02 minutes) as it is hypothesised that over time the membrane had become saturated and the pores had clogged (Figure 3.5).

3.3.2 Chemical analysis

3.3.2.1 *Biological Filtration: Activated Carbon*

A slow activated carbon filtration system was connected to rainwater tanks B and C. Before the efficiency of the system was monitored a biofilm was allowed to establish for one week. The presence of cations was assessed in unfiltered and filtered rainwater samples collected on the first and the fifth day of sampling after the biofilm had formed within the slow activated carbon filtration system. All cations monitored were within the drinking water guidelines according to SANS 241 (SABS, 2005), DWAF (1996), ADWG (NHMRC and NRMMC, 2011) and WHO (2011), with the exception of aluminium and antimony, as represented in Table 3.4.

Table 3.4: Average cation concentrations obtained from rainwater samples collected before and after filtration through the activated carbon on the respective days. These values are compared to the recommended concentrations as stipulated by the respective drinking water guidelines (n = 8, average of each duplicate sample).

Metal	Before Day 1	After Day 1	Before Day 5	After Day 5	SANS 241	DWAF	ADWG	WHO
Boron as B (mg/L)	0.01	0.01	0.01	0.00	-	-	4	2.4
Calcium as Ca (mg/L)	2.76	5.07	2.40	3.02	150	200	200	-
Potassium as K (mg/L)	0.29	1.00	0.21	0.19	50	50	-	-
Magnesium as Mg (mg/L)	0.40	0.82	0.28	0.37	70	30	200	-
Sodium as Na (mg/L)	3.09	4.96	1.86	1.91	200	100	180	-
Phosphorus as P (mg/L)	0.09	0.11	0.04	0.02	-	-	-	-
Silicon as Si (mg/L)	0.20	2.58	0.17	0.41	-	-	-	-
Aluminium as Al (µg/L)	5.96	443.29	7.29	15.15	300	150	100	-
Chromium as Cr (µg/L)	0.06	0.09	<0.303	<0.303	100	50	50	50
Manganese as Mn (µg/L)	4.76	1.61	0.83	0.68	100	50	500	-
Iron as Fe (µg/L)	94.02	30.59	21.23	10.61	200	100	300	-
Cobalt as Co (µg/L)	0.06	0.10	0.02	0.09	500	-	-	-
Nickel as Ni (µg/L)	0.15	1.11	0.64	4.76	150	-	20	70
Copper as Cu (µg/L)	1.06	0.49	1.99	1.53	1000	1000	2000	2000
Zinc as Zn (µg/L)	24.94	1.96	22.46	5.73	5000	3000	3000	-
Arsenic as As (µg/L)	0.24	7.41	0.23	0.77	10	10	10	10
Selenium as Se (µg/L)	2.36	1.18	1.88	0.68	20	20	10	40
Strontium as Sr (mg/L)	21.76	160.29	18.14	52.56	-	-	-	-
Molybdenum as Mo (µg/L)	<0.000	<0.00	0.03	0.06	-	-	50	-
Cadmium as Cd (µg/L)	0.02	0.01	<0.019	<0.019	5	5	2	3
Tin as Sn (µg/L)	0.01	0.02	<0.016	<0.016	-	-	-	-
Antimony as Sb µg/L	0.08	10.57	0.08	0.24	-	-	3	20
Barium as Ba (µg/L)	21.06	20.37	17.20	31.76	-	-	2000	700
Mercury as Hg (µg/L)	0.01	0.03	0.02	<0.021	1	1	1	6
Lead as Pb (µg/L)	0.18	0.17	0.13	0.05	20	10	10	10

The average concentration of aluminium detected for the filtered rainwater samples (443.29 µg/L) collected on day one of sampling exceeded the recommended guidelines of 300 µg/L stipulated by SANS 241 (SABS, 2005), 150 µg/L as stipulated by DWAF (1996) and 100 µg/L as stipulated by the ADWG (NHMRC and NRMMC, 2011). In addition, the average concentration of antimony in the rainwater sample collected on day one after filtration through the activated carbon system, exceeded the ADWG (NHMRC and NRMMC, 2011) guideline of 3 µg/L, with an average concentration of 10.57 µg/L detected. However, the concentrations of aluminium and antimony in the remaining unfiltered and filtered rainwater samples collected on day 5 were within the respective guidelines.

Although aluminium has been associated with Alzheimer's disease, Parkinsonism dementia and amyotrophic lateral sclerosis, it has been concluded that there is insufficient information to link these diseases with the consumption of aluminium through drinking water sources (DWAF, 1996; NHMRC and NRMMC, 2011). In studies performed on rats, the consumption of antimony was also linked to fertility and it was demonstrated that antimony accumulates in the heart, spleen, liver and kidney (NHMRC and NRMMC, 2011). While it was noted that all other cations detected were within the respective guidelines, the significant variations in the concentrations of the cations as well as aluminium and antimony (that were not within guidelines) will be discussed.

The concentration of silicon, aluminium, arsenic, antimony and strontium present in the filtered rainwater samples collected on day one after the formation of the biofilm increased significantly (more than a 10 fold increase) from an average of 0.2 µg/L (unfiltered) to an average of 2.58 µg/L (filtered), from an average of 5.96 µg/L (unfiltered) to an average of 443.29 µg/L (filtered), from an average of 0.24 µg/L (unfiltered) to an average of 7.41 µg/L (filtered), from an average of 0.08 µg/L (unfiltered) to an average of 10.57 µg/L (filtered), and from an average of 21.76 µg/L (unfiltered) to an average of 160.29 µg/L (filtered) respectively. In contrast, in the same rainwater samples the concentrations of manganese, iron, copper and zinc were observed to have decreased on day one after filtration through the activated carbon from an average of 4.76 µg/L (unfiltered) to an average of 1.61 µg/L (filtered), from an average of 94.02 µg/L (unfiltered) to an average of 30.59 µg/L (filtered), from an average of 1.06 µg/L (unfiltered) to an average of 0.49 µg/L (filtered) and from an average of 24.94 µg/L (unfiltered) to an average of 1.96 µg/L (filtered), respectively. On the fifth day after the formation of the biofilm, no significant increases were observed for any of the cations present in the rainwater samples, however potassium, phosphorous, manganese, iron, copper, zinc, selenium and lead exhibited a negligible decrease after filtration through the activated carbon system.

The raw materials used in the manufacturing of commercially available activated carbon contains ppm levels of arsenic and antimony and have been shown to leach small fractions of these elements when in direct contact with water (Vaughn and Distefano, 2013). This could account for the increased levels of antimony and arsenic detected in the rainwater samples that have been filtered through the activated carbon. Moreover ash, used in the manufacturing of activated carbon, contains aluminium and silicon (Block and Dams, 2010). Therefore aluminium and silicon could also have leached from the activated carbon particles into the rainwater during the filtration process.

All anions present in the unfiltered and filtered rainwater samples collected on day one and day five after the formation of the biofilm on activated carbon were within drinking water guidelines according to SANS 241 (SABS, 2005), DWAF (1996), ADWG (NHMRC and NRMMC, 2011) and WHO (2011) and are represented in Table 3.5.

Table 3.5: Average anion concentrations obtained from rainwater samples collected before and after filtration through the activated carbon on the respective days. These values are compared to the recommended concentrations as stipulated by the respective drinking water guidelines (n = 8, average of each duplicate sample).

Anions (mg/L)	Before Day 1	After Day 1	Before Day 5	After Day 5	SANS 241	DWAF	ADWG	WHO
Sulphate as SO_4	1.15	1.7	1.4	1.55	200	100	250	-
Chloride as Cl^-	6.65	2.9	6.55	4.9	400	200	250	-
Nitrate and Nitrite as NO_3 and NO_2	0.2	0.1	0.05	0.05	10	6	50	50
Phosphate as PO_4	0.095	0.085	0.025	0.025	-	-	-	-
Fluoride as F	<0.01	<0.01	0.1	0.1	-	-	-	-

On the first day of the trial chloride and nitrite and nitrate concentrations decreased from an average of 6.65 mg/L (unfiltered) to an average of 2.9 mg/L (filtered) and from an average of 0.2 mg/L (unfiltered) to an average of 0.1 mg/L (filtered), respectively. On the fifth day of filtration only the concentration of chloride was shown to have decreased from an average of 6.55 mg/L (unfiltered) to an average of 4.9 mg/L (filtered).

The formation of a biofilm layer on granular activated carbon (GAC) is referred to as biological activated carbon (BAC) and has gained interest as a water treatment technology. Biological activated carbon has been shown to remove chemical pollutants, disinfection by-product precursors and organic matter (Simpson, 2008). In this study the biofilm was allowed to establish for a week before the rainwater was filtered through the activated carbon. The efficiency of this system could therefore be increased by lengthening the time of biofilm growth before using the system for the filtering of rainwater.

3.3.2.2 *Biological Filtration: Slow Sand Filtration*

A slow sand filtration system was installed on rainwater tanks B and C. Before the efficiency of the system was monitored a biofilm was allowed to establish for two weeks. The presence of cations was assessed in unfiltered and filtered rainwater samples collected on the first and the twelfth day after the formation of the biofilm within the slow sand filtration system. All cations present in the rainwater samples collected before and after slow sand filtration were within the drinking water guidelines according to SANS 241 (SABS, 2005), DWAF (1996), ADWG (NHMRC and NRMMC, 2011) and WHO (2011), with the exception of aluminium, manganese and iron as represented in Table 3.6.

The aluminium concentration of the filtered rainwater sample (average 1601.43 µg/L) collected on day one exceeded the recommended guidelines as stipulated by SANS 241 (SABS, 2005), DWAF (1996), ADWG (NHMRC and NRMMC, 2011) and WHO (2011). However, the concentrations of aluminium in both unfiltered rainwater samples and the filtered rainwater sample collected on day twelve were within the recommended guidelines. The concentration of manganese in the filtered rainwater sample collected on day one had an average concentration of 53.45 µg/L which exceeded the recommended guideline of 50 µg/L stipulated by DWAF (1996). However, the concentrations of manganese in both unfiltered rainwater samples and the filtered rainwater sample collected on day twelve also adhered to recommended guidelines as stipulated by SANS 241 (2005), DWAF (1996), ADWG (NHMRC and NRMMC, 2011) and WHO (2011).

Table 3.6: Average cation concentrations obtained from rainwater samples collected before and after filtration through the slow sand filter on day one and twelve, respectively. These values are compared to the recommended concentrations as stipulated by the respective drinking water guidelines (n = 8, average of each duplicate sample).

Metal	Before Day 1	After Day 1	Before Day 12	After Day 12	SANS 241	DWAF	ADWG	WHO
Boron as B (mg/L)	0.01	0.02	0.01	0.01	-	-	4	2.4
Calcium as Ca (mg/L)	2.95	5.32	2.87	3.04	150	200	200	-
Potassium as K (mg/L)	0.20	0.51	0.27	0.33	50	50	-	-
Magnesium as Mg (mg/L)	0.33	0.73	0.38	0.44	70	30	200	-
Sodium as Na (mg/L)	2.33	3.63	2.67	2.77	200	100	180	-
Phosphorus as P (mg/L)	0.01	0.01	0.02	0.03	-	-	-	-
Silicon as Si (mg/L)	0.21	0.99	0.30	0.54	-	-	-	-
Aluminium as Al (µg/L)	6.17	1601.43	5.42	6.73	300	150	100	-
Chromium as Cr (µg/L)	<0.303	1.98	<0.303	<0.303	100	50	50	50
Manganese as Mn (µg/L)	1.39	53.45	2.92	2.19	100	50	500	-
Iron as Fe (µg/L)	23.04	4083.45	143.99	108.31	200	100	300	-
Cobalt as Co (µg/L)	0.15	1.92	0.08	0.10	500	-	-	-
Nickel as Ni (µg/L)	11.77	16.74	2.62	5.48	150	-	20	70
Copper as Cu (µg/L)	11.61	7.31	6.19	7.99	1000	1000	2000	2000
Zinc as Zn (µg/L)	21.95	67.69	20.96	24.05	5000	3000	3000	-
Arsenic as As (µg/L)	0.26	0.61	0.31	0.69	10	10	10	10
Selenium as Se (µg/L)	1.02	1.54	0.85	1.20	20	20	10	40
Strontium as Sr (mg/L)	20.35	28.96	24.42	25.68	-	-	-	-
Molybdenum as Mo (µg/L)	0.06	0.03	0.03	0.09	-	-	50	-
Cadmium as Cd (µg/L)	<0.019	0.09	0.03	<0.019	5	5	2	3
Tin as Sn (µg/L)	<0.016	0.03	<0.016	<0.016	-	-	-	-
Antimony as Sb µg/L	0.10	0.12	0.12	0.23	-	-	3	20
Barium as Ba (µg/L)	20.78	3.86	32.12	19.15	-	-	2000	700
Mercury as Hg (µg/L)	0.06	<0.021	0.04	0.04	1	1	1	6
Lead as Pb (µg/L)	0.15	0.24	0.16	0.10	20	10	10	10

Only the unfiltered rainwater sample, collected on day one, adhered to the recommended guidelines for iron as stipulated by SANS 241 (SABS, 2005), DWAF (1996), ADWG (NHMRC and NRMMC, 2011) and WHO (2011) with an average concentration of 23.04 µg/L detected. However, the average concentrations of iron in the unfiltered rainwater samples collected on day twelve (143.99 µg/L) and the filtered rainwater sample collected on day twelve (108.31 µg/L) were not within the recommended DWAF (1996) guideline of 100 µg/L. In addition, the concentration of iron in the filtered rainwater sample collected on day one (4083.45 µg/L) was not within the guidelines as stipulated by SANS 241 (SABS, 2005), DWAF (1996), ADWG (NHMRC and NRMMC, 2011) and WHO (2011).

As mentioned previously (Section 3.3.2.1), aluminium present in drinking water has been linked to certain neurodegenerative diseases. Manganese is however, considered to have very low health risks if present in water, but can have aesthetic and taste effects if present at concentrations exceeding the respective guidelines (DWAF, 1996; NHMRC and NRMMC, 2011).

The noteworthy changes in the concentrations of the cations present in the rainwater samples before and after slow sand filtration will be discussed first. A significant increase (more than 10 fold increase) in the concentrations of aluminium, manganese, iron and cobalt was observed after slow sand filtration on day one with concentrations before and after filtration ranging from an average of 6.17 µg/L (unfiltered) to an average of 1601.43 µg/L (filtered), from an average of 1.39 µg/L (unfiltered) to an average of 53.45 µg/L (filtered), from an average of 23.04 µg/L (unfiltered) to an average of 4083.45 µg/L (filtered), and from an average of 0.15 µg/L (unfiltered) to an average of 1.92 µg/L (filtered), respectively. Copper, molybdedum, mercury and barium were the only cations that decreased after slow sand filtration on day one with concentrations before and after filtration ranging from an average of 11.61 µg/L (unfiltered) to an average of 7.31 µg/L (filtered), from an average of 0.06 µg/L (unfiltered) to an average of 0.03 µg/L (filtered), from an average of 0.06 µg/L (unfiltered) to an average of <0.021 µg/L (filtered) and from an average of 20.78 µg/L (unfiltered) to an average of 3.86 µg/L, respectively. All the other cations detected after slow sand filtration on day one also increased, however the increase was negligible (Table 3.6).

The concentrations of manganese, iron, barium, lead and cadmium decreased after slow sand filtration on day twelve with concentrations before and after filtration ranging from an average of 2.92 µg/L (unfiltered) to an average of 2.19 µg/L (filtered), from an average of 143.99 µg/L (unfiltered) to an average of 108.31 µg/L (filtered), from an average of 32.12 µg/L (unfiltered) to an average of 19.15 µg/L (filtered), from an average of 0.16 µg/L (unfiltered) to an average of 0.10 µg/L (filtered) and from an average of 0.03 µg/L (unfiltered) to an average of <0.019 µg/L (filtered), respectively. All the cations detected after slow sand filtration on day twelve increased, however the observed increases were not significant (Table 3.6).

A study by Mwabi et al. (2011) investigated the efficiency of a bucket filter (BF), consisting of a layer of gravel and sand, and a biosand filter (BSF), consisting of a layer of zeolites, sand and a biological layer. The BF and BSF were shown, in both cases, to reduce the concentration of iron before filtration from 0.03 mg/L to < 0.01 mg/L after filtration. The concentration of iron present in the rainwater, in this study, was shown to increase after the first day of the study significantly (more than a 10 fold increase) from an average of 23.04 µg/L (unfiltered) to an average of 4083.45 µg/L (filtered), and to decrease after filtration on day twelve from an average of 143.99 µg/L (unfiltered) to an average of 108.31 µg/L (filtered). It could be hypothesised that in the current study a longer time period was required to form an effective biological layer as, rainwater, is generally less contaminated than wastewater, which was used in the study by Mwabi et al. (2011). Mwabi et al. (2011) also incorporated a layer of zeolites into the biosand filter which is known to have high removal efficiencies for chemical contaminants.

All anions present in the rainwater samples collected before and after filtration through the slow sand system were within drinking water guidelines according to SANS 241 (SABS, 2005), DWAF (1996), ADWG (NHMRC and NRMMC, 2011) and WHO (2011) and are represented in Table 3.7.

Table 3.7: Average anion concentrations obtained from rainwater samples collected before and after filtration through the slow sand filter on day one and twelve, respectively. These values are compared to the recommended concentrations as stipulated by the respective drinking water guidelines (n = 8, average of each duplicate sample).

Anions (mg/L)	Before Day 1	After Day 1	Before Day 12	After Day 12	SANS 241	DWAF	ADWG	WHO
Sulphate as SO_4	1.1	41.5	1.45	4.15	200	100	250	-
Chloride as Cl^-	5.45	5.65	5.95	5.1	400	200	250	-
Fluoride as F	<0.1	<0.1	<0.1	<0.1	-	-	-	-

A significant increase in the concentration of sulphate on day one after slow sand filtration was observed with concentrations before and after filtration ranging from an average of 1.1 mg/L (unfiltered) to an average of 41.5 mg/L (filtered). The increase in sulphate concentrations after slow sand filtration on day twelve was however, less than on the first day with average concentrations ranging from 1.45 mg/L (unfiltered) to an average of 4.15 mg/L (filtered). The concentration of chloride was observed to have decreased slightly on day one and twelve with concentrations before and after filtration ranging from an average of 5.45 mg/L (unfiltered) to an average of 5.65 mg/L (filtered) and from an average of 5.95 mg/L (unfiltered) to an average of 5.1 mg/L (filtered), respectively. As previously noted the bacterial population within the biofilm layer degrades organic matter present in water samples into carbon dioxide, and inorganic salts such as sulphates. Therefore the presence of sulphur oxidizing bacteria within a biofilm layer that might have developed on the sand medium could have contributed to the increased concentrations of sulphate in the filtered rainwater samples (WHO, 1974). Previous studies have shown that inorganic compounds can accumulate in the biofilm of the slow sand filter (Hijnen et al., 2004). This could thus explain the decrease in the concentration of chloride present in the rainwater after filtration through the slow sand filter.

3.3.2.3 *Activated Carbon and Nanofibre Membrane System*

All cations monitored in the rainwater samples collected before and after (first and fifth litre only analysed) filtration through the activated carbon and nanofibre membrane were within the drinking water guidelines according to SANS 241 (SABS, 2005), DWAF (1996), ADWG (NHMRC and NRMMC, 2011) and WHO (2011) as represented in Table 3.8. However, the concentrations of copper, antimony, lead, aluminium, arsenic and strontium increased in the first litre of rainwater filtered through the activated carbon and nanofibre membrane system from an average of 0.89 µg/L (unfiltered) to an average of 33.40 µg/L (filtered), from an average of 0.14 µg/L (unfiltered) to an average of 1.66 µg/L (filtered) from an average of 0.02 µg/L (unfiltered) to an average of 5.28 µg/L (filtered), and from an average of 9.42 µg/L (unfiltered) to an average of 54.49 µg/L (filtered), from an average of 0.34 µg/L (unfiltered) to an average of 3.28 µg/L (filtered) and from an average of 24.69 µg/L (unfiltered) to an average of 61.31 µg/L (filtered), respectively. In addition, in the fifth litre of filtered rainwater the concentrations of copper, antimony, lead, aluminium, nickel, arsenic and strontium increased from an average of 0.89 µg/L (unfiltered) to an average of 1.35 µg/L (filtered), from an average of 0.14 µg/L (unfiltered) to an average of 1.75 µg/L (filtered), from an average of 0.02 µg/L (unfiltered) to an average of 0.32 µg/L (filtered), from an average of 9.42 µg/L (unfiltered) to an average of 65.63 µg/L (filtered), from an average of 0.32 µg/L (unfiltered) to an average of 1.22 µg/L (filtered), from an average of 0.34 µg/L (unfiltered) to an average of 2.36 µg/L (filtered) and from an average of 24.69 µg/L (unfiltered) to an average of 81.07 µg/L (filtered) respectively.

Table 3.8: Cation concentrations obtained from rainwater samples collected before filtration through activated carbon and the nanofibre layers and well as the cation concentrations present in the 1st and 5th litre of rainwater filtered through the activated carbon and nanofibre layer. These values are compared to the recommended concentrations as stipulated by the respective drinking water guidelines (n = 3).

Metal	Before activated carbon and nanofibres	After activated carbon and nanofibres (1ST L)	After activated carbon and nanofibres (5th L)	SANS 241	DWAF	ADWG	WHO
Calcium as Ca (mg/L)	3.53	4.15	4.49	150	200	200	-
Potassium as K (mg/L)	0.22	0.59	0.28	50	50	-	-
Magnesium as Mg (mg/L)	0.43	0.45	0.60	70	30	200	-
Sodium as Na (mg/L)	3.00	2.81	3.15	200	100	180	-
Phosphorus as P (mg/L)	0.02	1.13	0.03	-	-	-	-
Silicon as Si (mg/L)	0.32	0.85	1.13	-	-	-	-
Aluminium as Al (µg/L)	9.42	54.49	65.63	300	150	100	-
Chromium as Cr (µg/L)	0.21	0.11	0.04	100	50	50	50
Manganese as Mn (µg/L)	2.78	1.92	3.35	100	50	500	-
Iron as Fe (µg/L)	90.20	21.83	5.81	200	100	300	-
Cobalt as Co (µg/L)	0.04	0.05	0.07	500	-	-	-
Nickel as Ni (µg/L)	0.32	0.32	1.22	150	-	20	70
Copper as Cu (µg/L)	0.89	33.40	1.35	1000	1000	2000	2000
Zinc as Zn (µg/L)	14.95	17.88	13.01	5000	3000	3000	-
Arsenic as As (µg/L)	0.34	3.28	2.36	10	10	10	10
Selenium as Se (µg/L)	1.38	1.04	1.05	20	20	10	40
Strontium as Sr (mg/L)	24.69	61.31	81.07	-	-	-	-
Molybdenum as Mo (µg/L)	<0.000	0.05	0.01	-	-	50	-
Cadmium as Cd (µg/L)	0.01	0.01	0.01	5	5	2	3
Tin as Sn (µg/L)	<0.000	0.03	0.00	-	-	-	-
Antimony as Sb µg/L	0.14	1.66	1.75	-	-	3	20
Barium as Ba (µg/L)	17.11	16.31	28.38	-	-	2000	700
Mercury as Hg (µg/L)	0.11	0.12	0.12	1	1	1	6
Lead as Pb (µg/L)	0.02	5.28	0.32	20	10	10	10

All anions present in the rainwater samples collected before and after (first and fifth litre only analysed) filtration through the activated carbon and nanofibre membrane were within drinking water guidelines according to SANS 241 (SABS, 2005), DWAF (1996), ADWG (NHMRC and NRMMC, 2011) and WHO (2011) and are represented in Table 3.9.

Table 3.9: Anion concentrations obtained from rainwater collected before filtration through activated carbon and the nanofibre layers and well as the cation concentrations present in the 1st and 5th litre of rainwater filtered through the activated carbon and nanofibre layer. These values are compared to the recommended concentrations as stipulated by the respective drinking water guidelines (n = 3).

Anions (mg/L)	Before activated carbon and nanofibres	After activated carbon and nanofibres (1ST L)	After activated carbon and nanofibres (5th L)	SANS 241	DWAF	ADWG	WHO
Sulphate as SO_4	1.5	14	1	200	100	250	-
Chloride as Cl^-	5.9	2.6	3.2	400	200	250	-
Nitrate and Nitrite as NO_3 and NO_2	0.3	0.2	0.2	10	6	50	50
Phosphate as PO_4	ND	<0.05	ND	-	-	-	-
Fluoride as F	<0.1	<0.1	<0.1	-	-	-	-

ND Not Detected

3.3.3 The Bacterial Removal Efficiency of the Filtration Systems

3.3.3.1 *Biological Filtration: Activated Carbon*

Total coliforms were enumerated before and after biofilm formation on the activated carbon in a slow filtration system (Figure 3.6). Before a biofilm was allowed to establish for one week, average total coliform counts were determined for unfiltered (6.2×10^2 CFU/100 mL) and initial filtered (4.5×10^2 CFU/100 mL) rainwater samples. Although no log reduction was observed, a reduction of 27% was recorded for the average total coliforms after the once off filtration through the slow activated carbon system prior to the formation of a biofilm.

Once the biofilm was established in the slow activated carbon filtration system total coliforms detected in the unfiltered rainwater samples ranged from an average of 3.9×10^2 CFU/100 mL recorded for day one to an average of 4.5×10^2 CFU/100 mL recorded on day five (Figure 3.6). The average total coliforms in filtered rainwater samples ranged from 4.5×10^2 CFU/100 mL on day one to 2.7×10^2 CFU/100 mL on day five. On day one a 13.7% increase in average total coliform numbers was observed for the filtered (4.5×10^2 CFU/100 mL) rainwater samples in comparison to the unfiltered (3.9×10^2 CFU/100 mL) rainwater samples. However, on day two no change was observed between total coliforms detected in the unfiltered rainwater samples in comparison to the filtered rainwater samples. In contrast, on days three, four and five, reductions of 39%, 46% and 41% were observed in average total coliform numbers, respectively, for the rainwater samples filtered through the activated carbon filtrations system in comparison to the unfiltered rainwater samples.

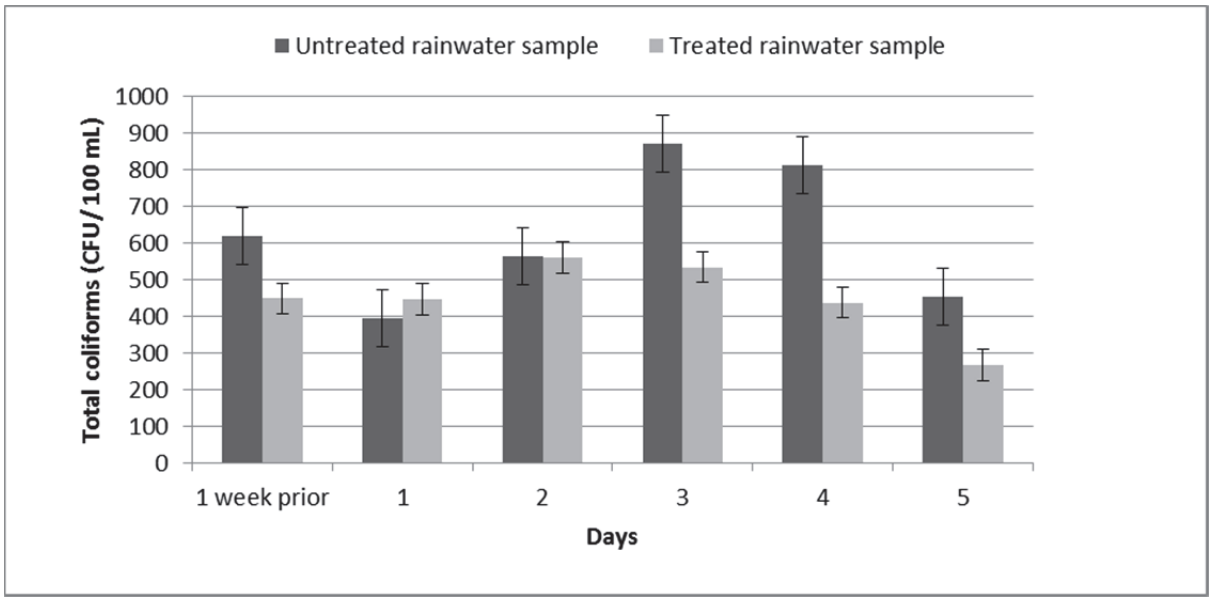

Figure 3.6: Total coliforms enumerated before the growth of a biofilm (one week prior) on activated carbon used in a slow filtration system followed by five days of monitoring the efficacy of the slow activated carbon filtration system.

No significant correlation could be established between total coliforms detected in filtered rainwater samples and an increase over time for the five day period ($R = -0.7$, $p < 0.05$). Therefore it is hypothesised that fluctuations in the filtered and unfiltered rainwater total coliform results influenced the efficiency of the activated carbon system over time. In addition, although reductions in total coliforms numbers were observed for filtered rainwater samples in comparison to unfiltered rainwater samples for days three till five, the reduction was not significant ($p = 0.16$).

Escherichia coli were enumerated before and after biofilm formation on activated carbon in a slow filtration system (Figure 3.7). Before a biofilm was allowed to establish for one week, average *E. coli* counts were determined for unfiltered (9 CFU/100 mL) and initial filtered (3 CFU/100 mL) rainwater samples. Although no log reduction was observed, a reduction of 65% in the average *E. coli* numbers was recorded after slow activated carbon filtration prior to the formation of a biofilm.

After a biofilm had established in the slow activated carbon filtration system, average *E. coli* numbers detected in the unfiltered rainwater samples ranged from 4.9×10^1 CFU/100 mL on day one to 2.1×10^1 CFU/100 mL on day five (Figure 3.7). *Escherichia coli* detected in filtered rainwater samples ranged from 6.3 CFU/100 mL on day one to 2.3 CFU/100 mL on day five of slow activated carbon filtration. On days one, two, three, four and five reductions of 87%, 64%, 85%, 22% and 89% were observed for average *E. coli* numbers for filtered rainwater samples in comparison to unfiltered rainwater samples, respectively.

No significant correlation could be established between *E. coli* numbers detected in filtered rainwater samples and an increase over time for the five day period ($R = -0.1$, $p < 0.05$). As for the removal of total coliforms, fluctuations in the filtered and unfiltered rainwater *E. coli* counts influenced the efficiency of the activated carbon system over time. However a significant decrease ($p < 0.003$) in *E. coli* numbers was recorded for filtered rainwater samples compared to unfiltered rainwater samples.

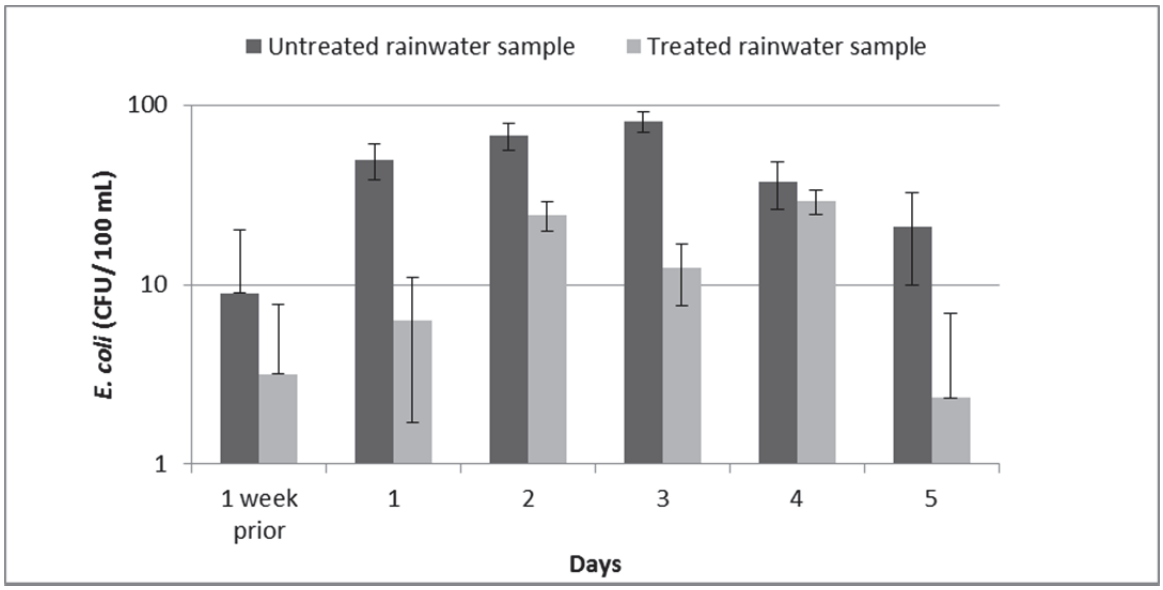

Figure 3.7: *Escherichia coli* numbers enumerated before the growth of a biofilm (one week prior) on activated carbon used in a slow filtration system followed by five days of monitoring the efficacy of the slow activated carbon filtration system.

Heterotrophic plate counts were enumerated before and after biofilm formation on activated carbon in a slow filtration system (Figure 3.8). Before a biofilm was allowed to establish for one week, average heterotrophic bacteria were determined for unfiltered (1.3×10^3 CFU/mL) and initial filtered (1.29×10^3 CFU/mL) rainwater samples. No significant reduction was however, observed in the average HPC counts obtained after slow activated carbon filtration prior to the formation of a biofilm.

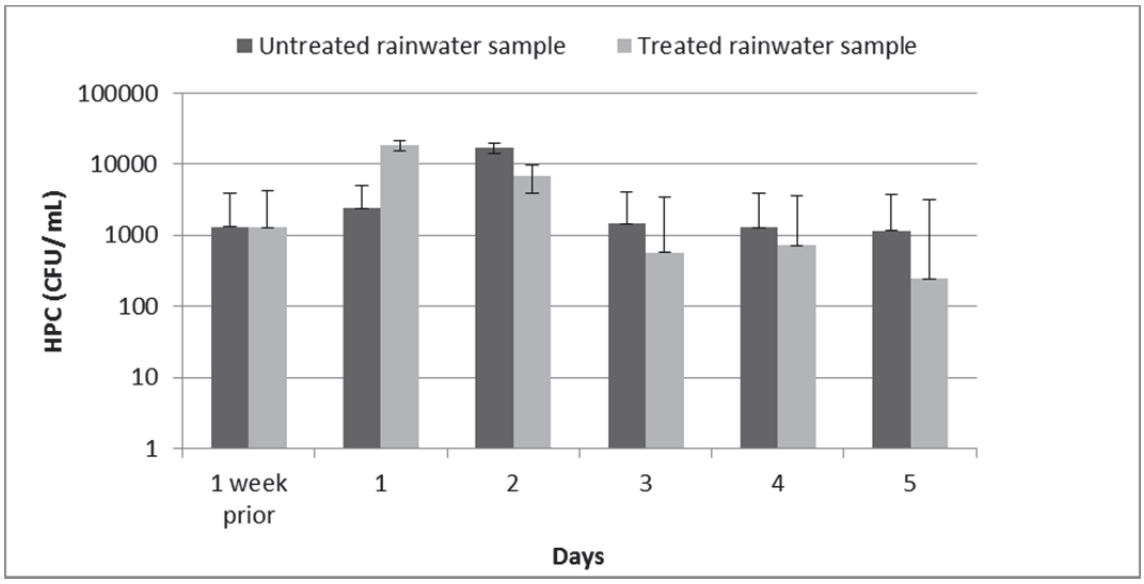

Figure 3.8: The HPC numbers enumerated before the growth of a biofilm (one week prior) on activated carbon used in a slow filtration system followed by five days of monitoring the efficacy of the slow activated carbon filtration system.

After a biofilm had established in the slow activated carbon system, average HPC numbers detected in the unfiltered rainwater samples ranged from 2.4×10^3 CFU/mL on day one to 1.2×10^3 CFU/mL on day five. Heterotrophic bacteria detected in filtered rainwater samples ranged from 1.9×10^4 CFU/mL on day one to 2.3×10^2 CFU/mL on day five of slow activated carbon filtration. On day one a 7 -fold increase in average HPC numbers was observed for filtered (1.9×10^4 CFU/mL) rainwater samples compared to the unfiltered (2.4×10^3 CFU/mL) rainwater samples. However, on days two, three, four and five, reductions of 59%, 60%,

45% and 79% were observed for the average HPC counts for filtered rainwater samples compared to unfiltered rainwater samples, respectively.

A significant negative correlation was established between HPC detected in filtered rainwater samples and an increase over time for the five day period ($R = -0.9$, $p < 0.05$). It is thus hypothesised, that with the exception of day one where heterotrophic bacteria could have sloughed off the biofilm, more heterotrophic bacteria were removed by the activated carbon biofilm layer over time. However, although reductions in HPC numbers were observed for filtered rainwater samples in comparison to unfiltered rainwater samples for days two till five, the overall reduction was not significant ($p = 0.84$).

According to guidelines stipulated by SANS 241 (SABS, 2005), the Australian Drinking Water Guidelines (ADWG) (NHMRC and NRMMC, 2011) and the Department of Water Affairs and Forestry (DWAF, 1996), no *E. coli* should be present in a water sample. No guideline values for total coliforms and HPC have been stipulated by the SANS 241 (SABS, 2005) and the ADWG (NHMRC and NRMMC, 2011), however, DWAF (1996) has stipulated that total coliform and HPC should not exceed 5 CFU/ 100 mL and 100 CFU/ mL, respectively.

Escherichia coli values for all unfiltered and filtered rainwater samples using the slow activated carbon filtration systems did not adhere to the guidelines stipulated by SANS 241 (SABS, 2005), the ADWG (NHMRC and NRMMC, 2011) and DWAF (1996). Moreover, total coliform and HPC values for all unfiltered and filtered rainwater samples using the slow activated carbon filtration systems did also not adhere to the guidelines stipulated by DWAF, (1996).

The hydraulic retention time for the activated carbon filtration system was calculated as follows:

$$HRT = \frac{15\ L}{\frac{0.5\ L}{5\ min}} = \frac{15\ L}{6\ L.h^{-1}} = 2.5\ h$$

A soluble compound would thus remain in the constructed slow activated carbon filter for approximately 2.5 hours.

Before the biofilm was allowed to establish in the slow activated carbon filtration system, total coliforms, *E. coli* and HPC numbers were reduced in the rainwater samples by 39%, 65% and 2% after an initial filtration through the activated carbon system, respectively. Once the biofilm had been established for a week the average total coliforms, *E. coli* and HPC for the whole sampling period (days one to five) were reduced by 27%, 71%, and no reduction, after filtration of rainwater through the activated carbon system, respectively. Therefore, no significant reduction ($p = 0.93$) could be established for total coliforms, *E. coli* and HPC percentages recorded before and after the growth of the biofilm in the slow activated carbon system. It could be hypothesised that the ineffectiveness of the slow activated carbon filtration system in removing indicator bacteria could be due to the growth of an immature biofilm, as the biofilm was only established for approximately one week. Many studies have shown that biological activated carbon, which is granular activated carbon on which bacteria have been immobilised, requires an extended time period before adequate amounts of biomass are attained (LeChevallier et al., 1984; Gao et al., 2010; Zhang et al., 2013). Many studies have also indicated that biological activated carbon has proven effective in removing for example, dissolved organic matter and ammonium (Andersson et al., 2001; Tian et al., 2009). Lately, a bio–enhanced approach has been shown to be more effective in removing pollutants (Gao et al., 2010; Zhang et al., 2013). This approach entails adding a consortium of competent microorganisms, for example Zhang et al. (2013) added bacteria that were able to biodegrade high concentrations of total organic carbon and exhibited high dehydrogenase activity. These bacteria included *Pseudomonas putita*, *Pseudomonas pertucinigena*, *Pseudomonas balearica* and *Bacillus subtilis*, which resulted in a bio-enhanced activated carbon (BEAC). To date no studies have indicated the efficiency of granular activated carbon filtration systems in removing indicator bacteria from rainwater. The focus is generally on the functionality of the

biological biomass found within the granular activated carbon particles and the ability of the activated carbon systems to remove chemical particle and other contaminants such as total organic carbon (TOC) (Lehtola et al., 2002; Mohan and Pittman, 2006; Gibert et al., 2012). However, some studies have shown that cells attached to activated carbon particles show resistance to disinfection with chlorine. LeChevallier et al. (1984) showed that disinfecting HPC bacteria, coliform organisms and other pathogenic microorganisms attached to the activated carbon with chlorine (2.0 mg/L) for one hour, showed no significant decrease in viable counts, with similar results observed when the bacteria were washed from the activated carbon.

3.3.3.2 *Slow Sand Filtration*

Total coliforms were enumerated before and after biofilm formation on sand in a slow filtration system (Figure 3.9). Before a biofilm was allowed to establish for two weeks, average total coliforms were determined from unfiltered (6.9×10^2 CFU/100 mL) and initial filtered rainwater samples, with no total coliforms recovered from the filtered rainwater samples and a three log reduction thus recorded for the average total coliforms after slow sand filtration prior to the formation of a biofilm.

After a biofilm had established in the slow sand filtration system, average total coliform numbers detected in the unfiltered rainwater samples ranged from 1.5×10^2 CFU/100 mL on day one to 1.38×10^3 CFU/100 mL on day twelve. Total coliforms detected in filtered rainwater samples ranged from 7.7×10^1 CFU/100 mL on day one to 1.37×10^3 CFU/100 mL on day twelve of the slow sand filtration system. On days one, three, five, eight, ten and twelve, reductions of 49.8%, 72.9%, 82.8%, 67.4%, 89.4% and 0.4% were thus observed in average total coliform numbers, respectively.

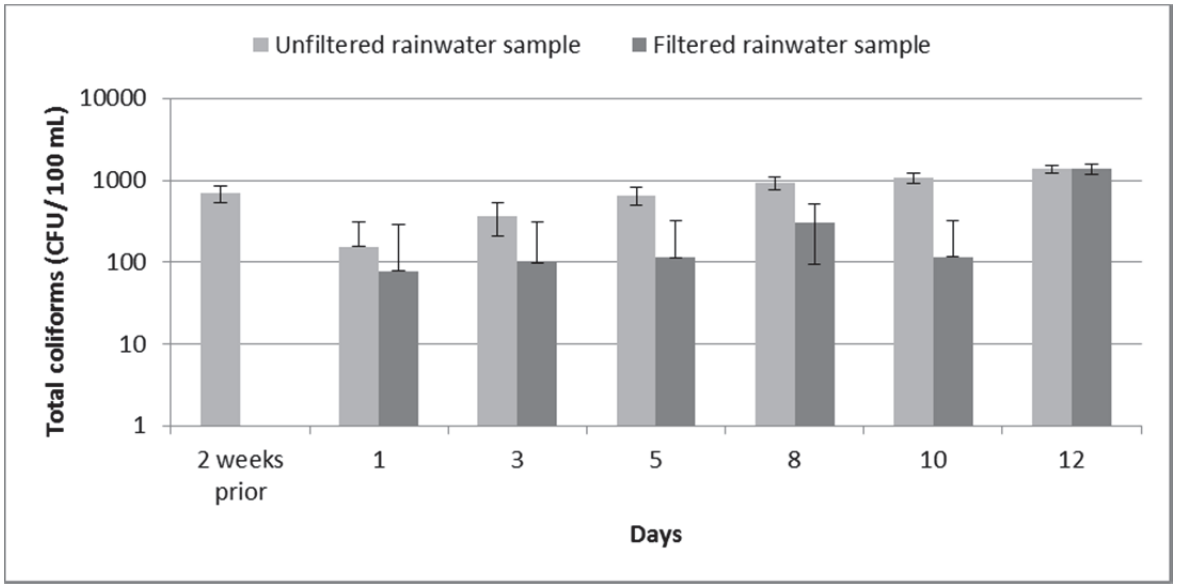

Figure 3.9: Total coliforms enumerated before the growth of a biofilm (two weeks prior) on sand used in a slow filtration system followed by six days (every second day) of monitoring the efficacy of the slow sand filtration system.

Overall, a significant positive correlation ($R = 0.94$, $p<0.05$) was established between average total coliforms recorded for the filtered rainwater samples and an increase in time (days one to twelve). It is thus confirmed that as the total coliform counts in the unfiltered rainwater increased over time, similarly, while reductions were observed, increased total coliform counts were observed in the filtered rainwater samples. However, reductions in total coliform numbers observed for filtered rainwater samples for days one to twelve were not significant ($p = 0.084$).

Escherichia coli counts enumerated before and after biofilm formation on sand in a slow filtration system are indicated in Figure 3.10. During the establishment of a biofilm (two weeks), average *E. coli* numbers were determined for unfiltered (9 CFU/100 mL) and initial filtered rainwater samples, with no *E. coli* recovered for the filtered rainwater samples and a reduction in average *E. coli* numbers recorded after slow sand prior to the formation of a biofilm.

After a biofilm had established in the slow sand filtration system, average *E. coli* numbers detected in the unfiltered rainwater samples ranged from 2 CFU/100 mL on day one to 7 CFU/100 mL on day twelve. *E. coli* detected in filtered rainwater samples ranged from no *E. coli* detected on day one to 1.2×10^1 CFU/100 mL on day twelve of the slow sand filtration system. On days one, three, five and eight reductions of 91.7%, 71.4%, 66.7% and 100% were observed in average *E. coli* numbers for filtered rainwater samples compared to unfiltered rainwater samples, respectively. On days ten and twelve the number of *E. coli* however increased significantly ($p < 0.05$) in comparison to the unfiltered *E. coli* count obtained. No significant corresponding increases in total coliforms and heterotrophic bacterial counts were however, observed on the same sampling days. It has also previously been noted that *E. coli* is regarded as the most specific indicator of faecal contamination. The filtering system could thus have possibly been contaminated with faecal matter passing through the tank and lodging in the sand particles.

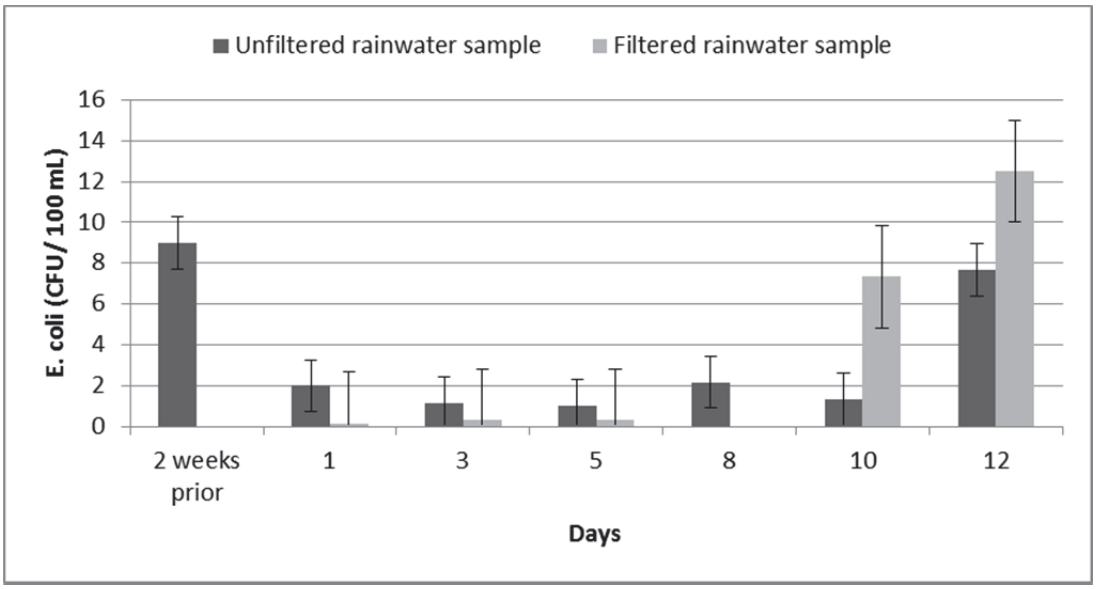

Figure 3.10: *Escherichia coli* numbers enumerated before the growth of a biofilm (two weeks prior) on sand used in a slow filtration system followed by six days (every second day) of monitoring the efficacy of the slow sand filtration system.

No significant correlation ($R = 0.64$, $p<0.05$) was established between average *E. coli* numbers recorded for the filtered rainwater samples and an increase in time (days one to twelve). Therefore it is hypothesised that fluctuations in the filtered and unfiltered rainwater *E. coli* results influenced the efficiency of the sand filtration system over time. Although reductions in *E. coli* numbers were observed for filtered rainwater samples for days one, three, five and eight, the reduction was not significant ($p = 0.82$).

Heterotrophic plate counts enumerated before and after biofilm formation on silica sand in a slow filtration system are indicated in Figure 3.11. Before a biofilm was allowed to establish for two weeks, average HPC numbers were determined from unfiltered (1.3×10^3 CFU/mL) and initial filtered (1.3×10^1 CFU/mL) rainwater samples with a two log reduction observed.

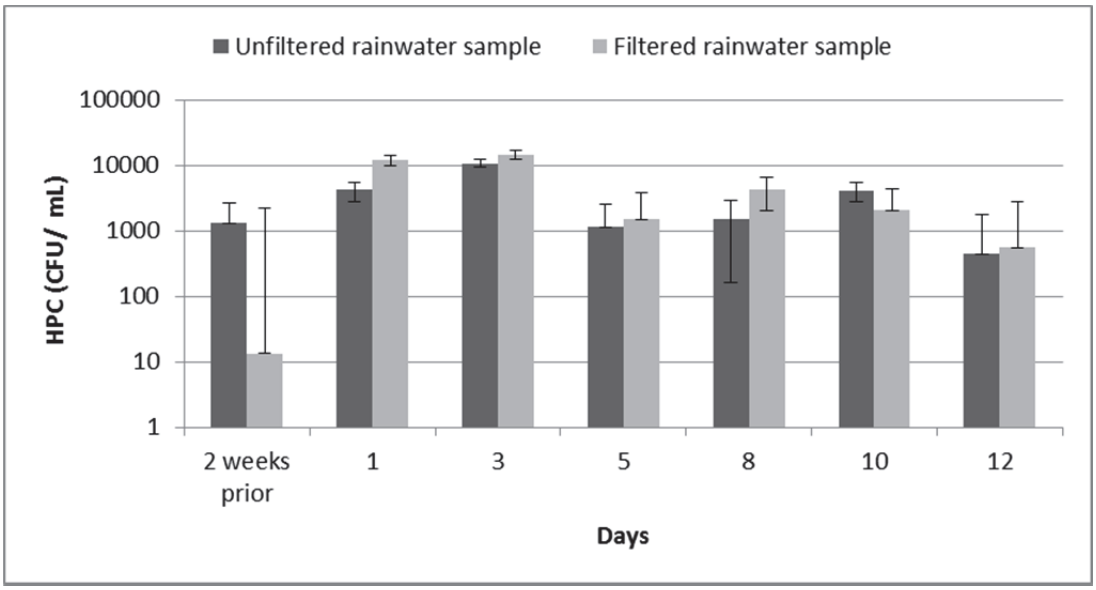

Figure 3.11: The HPC numbers enumerated before the growth of a biofilm (two weeks prior) on sand used in a slow filtration system followed by six days of monitoring the efficacy of the slow sand filtration system.

After a biofilm had established in the slow sand filtration system, average HPC numbers detected in the unfiltered rainwater samples ranged from 4.2×10^3 CFU/ mL on day one to 4.5×10^2 CFU/mL on day twelve. Heterotrophic bacteria detected in filtered rainwater samples ranged from 1.2×10^4 CFU/mL detected on day one to 5.5×10^2 CFU/mL on day twelve of slow sand filtration. All HPC numbers increased after filtration with the exception of one filtered rainwater sample collected on day ten which decreased by 50%.

No significant correlation ($R = -0.77$, $p<0.05$) was established between the average HPC recorded for the filtered rainwater samples and an increase in time (days one to twelve); as the results for heterotrophic bacteria in unfiltered rainwater fluctuated over time. Although reductions in HPCs were observed for filtered rainwater samples collected on day ten, the reduction was not significant ($p = 0.54$).

According to guidelines stipulated by SANS 241 (SABS, 2005), the Australian Drinking Water Guidelines (ADWG) (NHMRC and NRMMC, 2011) and the Department of Water Affairs and Forestry (DWAF, 1996), no *E. coli* should be present in a water sample. No guideline values for total coliforms and HPC have been stipulated by the SANS 241 (SABS, 2005) and the ADWG (NHMRC and NRMMC, 2011), however, DWAF (1996) have stipulated that total coliform and HPC should not exceed 5 CFU/ 100 mL and 100 CFU/ mL, respectively.

While, the hydraulic retention time for the slow sand filtration system was calculated as 6.25 hours, *Escherichia coli* values for all unfiltered and filtered rainwater samples using the slow sand filtration systems did not adhere to the guidelines stipulated by SANS 241 (SABS, 2005), the ADWG (NHMRC and NRMMC, 2011) and DWAF (1996), with the exception that on day eight of the slow sand filtration system, untreated rainwater samples exhibited average *E. coli* numbers of 2 CFU/ 100 mL which decreased to no *E. coli* detected after slow sand filtration (which was within the respective drinking water standards). Moreover, HPC and total coliform values for all unfiltered and filtered rainwater samples using the slow sand filtration systems did not adhere to the guidelines stipulated by DWAF (1996). However, total coliform values were reduced to within the DWAF (1996) guidelines by the slow sand filtration system before the biofilm had been allowed to establish for two weeks.

3.3.3.3 *Activated Carbon and Nanofibre Membrane System*

A column containing two PVA membrane layers surrounded by activated carbon was connected to the rainwater tanks B and C, respectively. Total coliforms, *E. coli* and HPC numbers were detected in unfiltered and five litres of filtered rainwater samples, collected litre by litre (Table 3.10).

Total coliform counts in the unfiltered rainwater samples collected from rainwater tanks B and C had an average of 6×10^2 CFU/ 100 mL. After filtration, total coliform numbers were reduced significantly ($p = 0.008$) to no total coliforms detected in the first litre of filtered rainwater, less than 1 CFU/ 100 mL detected for the second and third litres of filtered rainwater, 1 CFU/100 mL for the fourth litre of filtered rainwater and a slight increase to 3 CFU/ 100 mL for the fifth litre of filtered rainwater. A 100% decrease was thus observed for the first litre of filtered rainwater sample in comparison to the unfiltered rainwater samples. From the second till the fifth litre of filtered rainwater a 99.9% to a 99.5% decrease was observed in comparison to the unfiltered rainwater samples.

Table 3.10: Total coliforms, *E. coli* and HPC numbers detected in unfiltered rainwater (n = 4) and five litres of rainwater filtered through a column containing two PVA membrane layers surrounded by activated carbon (n = 20).

Indicator bacteria	Unfiltered rainwater	Filtered litres of rainwater				
		1 L	2 L	3 L	4 L	5 L
Total coliforms (CFU/100 mL)	6×10^2	0	<1	<1	1	3
E. coli (CFU/100 mL)	3	0	0	0	0	0
HPC (CFU/mL)	3×10^4	3.3	1×10^2	7.8×10^1	1.5×10^2	1.6×10^2

An average of 3 CFU/100 mL *E. coli* counts were detected in the unfiltered rainwater samples collected from rainwater tanks B and C. After filtration, *E. coli* numbers were reduced significantly ($p < 0.000006$) to no *E. coli* detected in the subsequent five litres of filtered rainwater. A 100% decrease for all five litres of filtered rainwater samples was observed in comparison to the unfiltered rainwater sample.

An average of 3×10^4 CFU/mL heterotrophic bacterial counts was detected in the unfiltered rainwater samples collected from rainwater tanks B and C. After filtration, heterotrophic bacterial numbers were reduced significantly ($p = 0.008$) to an average of 3 CFU/mL HPC for the first litre of filtered rainwater, 1×10^2 CFU/mL for the second litre of filtered rainwater, 7.8×10^1 CFU/mL for the third litre of filtered rainwater, 1.5×10^2 CFU/mL for the fourth litre of filtered rainwater and 1.6×10^2 CFU/mL for the fifth litre of filtered rainwater. A 99.99% decrease was thus observed for the first litre of filtered rainwater sample in comparison to the unfiltered rainwater samples. From the second till the fifth litre of filtered rainwater a 99.6% to a 99.5% decrease was observed in comparison to the unfiltered rainwater samples.

A column containing two PVA membrane layers, without activated carbon, was also connected to rainwater tanks B and C, respectively. Total coliforms, *E. coli* and HPC numbers were detected in unfiltered and five litres of filtered rainwater samples, collected litre by litre (Table 3.11).

Total coliform numbers with an average of 3.7×10^2 CFU/100 mL were detected in the unfiltered rainwater samples collected from rainwater tanks B and C. After filtration, total coliform numbers were reduced significantly ($p = 0.006$) to an average of 1 CFU/100 mL total coliforms for the first litre of filtered rainwater, 6.3×10^1 CFU/100 mL detected for the second litre of filtered rainwater and 7.8×10^1 CFU/100 mL for the third till the fifth litre of filtered rainwater. A 99.7% decrease was observed for the first litre of filtered rainwater sample in comparison to the unfiltered rainwater samples. From the second till the fifth litre of

filtered rainwater an 82.9% to a 78.9% decrease was observed in comparison to the unfiltered rainwater samples.

Table 3.11: Total coliforms, *E. coli* and HPC numbers detected in unfiltered rainwater (n = 4) and five litres of rainwater (n = 20) filtered through a column containing a two PVA membrane layers (without activated carbon).

Indicator bacteria	Unfiltered rainwater	Filtered rainwater samples				
		1 L	2 L	3 L	4 L	5 L
Total coliforms (CFU/ 100 mL)	3.7×10^2	1	6.3×10^1	7.8×10^1	7.8×10^1	7.8×10^1
E. coli (CFU/ 100 mL)	2.2	0	<1	<1	<1	1.1
HPC (CFU/mL)	1.6×10^4	1.1×10^2	3×10^3	4×10^3	1.3×10^3	3.1×10^3

The number of *E. coli* detected in the unfiltered rainwater decreased to no *E. coli* detected in the first litre of rainwater filtered and then less than 1 CFU/100 mL for the second, third and fourth litres of filtered rainwater. The final litre of filtered rainwater contained 1.1 CFU/100 mL *E. coli*. A 100% decrease was thus observed for the first litre of filtered rainwater sample in comparison to the unfiltered rainwater samples. From the second till the fifth litre of filtered rainwater an 81.1% to a 50.8% decrease was observed in comparison to the unfiltered rainwater samples.

No significant decrease ($p = 0.15$) was however, observed in *E. coli* numbers for filtered rainwater samples with only the first litre of filtered rainwater results within the guidelines stipulated by SANS 241 (SABS, 2005), the ADWG (NHMRC and NRMMC, 2011) and DWAF (1996) standards.

Heterotrophic bacterial numbers with an average of 1.6×10^4 CFU/mL were detected in the unfiltered rainwater samples collected from rainwater tanks B and C. After filtration, heterotrophic bacterial numbers were reduced to an average of 1.1×10^2 CFU/mL HPC for the first litre of filtered rainwater, 3×10^3 CFU/mL for the second litre of filtered rainwater, 4×10^3 CFU/mL for the third litre of filtered rainwater, 1.3×10^3 CFU/mL for the fourth litre of filtered rainwater and 3.1×10^3 CFU/mL for the fifth litre of filtered rainwater. A 99.3% decrease was observed for the first litre of filtered rainwater sample in comparison to the unfiltered rainwater samples. However, from the second till the fifth litre of filtered rainwater an 81.6% to an 81% decrease was observed in comparison to the unfiltered rainwater samples.

Based on the results obtained, the addition of activated carbon to the PVA nanofibre/activated carbon column rendered the system more efficient in the removal of total coliforms, *E. coli* and heterotrophic bacteria compared to the PVA nanofibre column without the activated carbon. For example in the first litre of filtered water the PVA nanofibre/activated carbon column removed 99.99% of the heterotrophic bacteria while the PVA nanofibre column removed 99.5%. In the fifth litre of filtered water the efficiency of PVA nanofibre/activated carbon column was 99.5% while the PVA nanofibre column efficiency was only 81%. It is therefore recommended that the addition of activated carbon serves as a pre-filter for larger particles as it increases the efficiency of the system.

Microfiltration with the use of electrospun nanfibres onto a substrate has been used in many water filtration applications. As noted previously by Bjorge et al. (2010) the increase in porosity and the pore structures formed, offer higher water permeability compared to conventional methods currently used. In this same study, general hospital wastewater, water from a pond and collected rainwater were all filtered. The microfiltration system was designed to include a hydraulic resistance time of 0.5 d. When the efficiency of the nanofibre membrane in removing culturable organisms and coliforms was compared to other microfiltration membranes, a reduction 1.5 $\log_{10}$ was observed. For the other membranes a 2 $\log_{10}$ to a 4 $\log_{10}$ removal was

observed (Gómez et al., 2006; Zodrow et al., 2009). Daels et al. (2011) also noted that after filtering hospital wastewater a 2 to 3 × 10^1 CFU/ 100 mL reduction using a non-functionalised membrane was observed.

3.3.4 The Detection of Coliphages in Harvested Rainwater

The number of coliphages present in the harvested rainwater samples measured throughout this study was below the detection limit. However, when rainwater samples (500 mL) were spiked with 10 mL of *E. coli* ATCC 13706 (that had been incubated at 37˚C until log phase reached) 1 PFU/ mL were detected. These results were thus not significant and overall coliphages could not be detected in any of the rainwater samples before and after filtration. The same observations were made by a previous study aimed at analysing, amongst others, one rainwater source in South Africa, whereby no coliphages were isolated from any of the rainwater samples. The strain of *E. coli* used in the current study, strain WG4, was also reported to be more effective in yielding coliphages counts than *E. coli* strain K12 (Nevondo and Cloete, 1999).

3.3.5 Bacterial Genus Specific PCR Reactions

3.3.5.1 *Biological Filtration: Slow Sand Filtration*

Two slow sand filtrations systems were connected to rainwater tanks B and C. After biofilm formation, the before and after rainwater filtered samples collected on days one, three, five, eight, ten and twelve, were analysed using genus specific PCR. No *Shigella* spp. or *Salmonella* spp. were detected in the unfiltered or the filtered rainwater samples collected throughout the study period (Table 3.12).

In summary *Aeromonas* spp. (GenBank accession no. EF450824.1, CP005966.1) were the least dominant species and were detected in 17% of the unfiltered rainwater samples, with no *Aeromonas* spp. detected in the slow sand filtration samples. Slow sand filtration was however, not effective in removing *Yersinia* spp. as *Yersinia* spp. (GenBank accession no. HM142628.1) were detected in 58% of the filtered rainwater samples. *Pseudomonas* spp. (GenBank accession no. JX279939.1) were one of the more dominant genera detected in this study with the use of genus specific PCRs and were detected in 92% of the unfiltered rainwater samples. Slow sand filtration was however, ineffective in removing *Pseudomonas* spp. as PCR assays confirmed the sporadic presence of *Pseudomonas* spp. in 75% of the filtered rainwater samples. Similarly, *Klebsiella* spp. (GenBank accession no. EU430287.1) were also one of the more dominant species detected in this study with the use of genus specific PCR as *Klebsiella* were detected in all the unfiltered rainwater samples and slow sand filtration was again ineffective in removing *Klebsiella* spp. from rainwater as this genera was sporadically detected in 92% of the filtered rainwater samples. *Legionella* spp. (GenBank accession no. AB638719.1) were the most dominant species detected throughout this study and slow sand filtration was ineffective in removing *Legionella* spp. as *Legionella* spp. were detected in all the filtered and unfiltered rainwater samples collected.

Table 3.12: Bacteria genera detected in unfiltered and slow sand filtered rainwater samples with the use of PCR.

Organism	Unfiltered Rainwater Sample (%)	Filtered Rainwater Sample (%)
Aeromonas spp.	17	0
Klebsiella spp.	100	92
Legionella spp.	100	100
Pseudomonas spp.	92	75
Salmonella spp.	0	0
Shigella spp.	0	0
Yersinia spp.	42	58

With the exception of *Aeromonas* spp., genus specific PCR assays revealed that the slow sand filtration system was ineffective in removing pathogenic bacteria commonly associated with rainwater. The biofilm known as the schmutzdecke is a biologically active layer that forms at the sand water interface (Campos et al., 2002). Joubert and Pillay (2008) suggested that a ripening period of six to eight weeks was required for the schmutzdecke to mature and in shortened time periods the schmutzdecke has been shown to be sub-optimal. As the slow sand filter utilised in the current study was ineffective in removing bacterial indicators it is hypothesised that the biofilm layer had not reached full maturation. Slow sand filters have however, been effective in reducing for example, nitrates (Aslan and Cakici, 2007), antimicrobial contaminants (Rooklidge et al., 2005) as well as pathogenic bacteria such as *E. coli*, *Vibrio cholerae* and *Salmonella typhimurium* (Mwabi et al., 2011). Although many faster and more effective filtration methods exist, slow sand filtration is generally considered cost effective, easy to operate, requires minimal maintenance and has shown to be effective in removing pathogenic bacteria in other studies (Joubert and Pillay, 2008). For these reasons, slow sand filtration is an attractive alternative point of use treatment system in developing countries and rural communities (Logsdon et al., 2002).

3.3.5.2 *Activated Carbon and Nanofibre Membrane System*

A column containing two PVA membrane layers surrounded by activated carbon were connected to rainwater tanks B and C, respectively. Total DNA was extracted from unfiltered and five litres of filtered rainwater samples, collected litre by litre, followed by subsequent genus specific PCR analysis.

Genus specific PCR assay revealed the presence of certain potentially pathogenic bacteria, commonly associated with rainwater (Table 3.13). Throughout this study, no *Salmonella* spp. were detected in any of the filtered and unfiltered rainwater samples, and *Shigella* spp. (GenBank accession no. HE616529.1) were detected in 25% of the third litre of filtered rainwater samples.

Of the reoccurring genera, *Aeromonas* spp. (GenBank accession no. CP005966.1) were detected in all the rainwater samples collected before filtration, and were reduced to no *Aeromonas* spp. detected in the first litre of filtered rainwater, and thereafter were detected in 25% of the remaining filtered rainwater samples, from the second to the fifth litre of filtered rainwater.

Table 3.13: Bacteria genera detected in unfiltered and filtered rainwater samples of filtration through the activated carbon and nanofibre membrane system.

Organism	**Unfiltered rainwater (%)**	**Filtered litres of rainwater (%)**				
		1st	**2nd**	**3rd**	**4th**	**5th**
Aeromonas spp.	100	0	25	25	25	25
Klebsiella spp.	100	25	75	50	50	75
Legionella spp.	100	75	100	75	75	100
Pseudomonas spp.	100	50	50	75	25	25
Salmonella spp.	0	0	0	0	0	0
Shigella spp.	0	0	0	25	0	0
Yersinia spp.	100	75	50	100	100	75

In summary, *Klebsiella* spp. (GenBank accession no. X16817.1) were detected in all the unfiltered rainwater samples and after filtration were detected in 25% after the first litre of rainwater had been filtered and in 75% of the second and fifth litre of filtered rainwater, respectively. During the filtration of the third and fourth litres of rainwater, *Klebsiella* spp. were detected in 50% of the rainwater samples filtered, respectively. Similar to the detection of *Klebsiella* spp., *Pseudomonas* spp. (GenBank accession no. HF952526.1) were detected in all the unfiltered rainwater samples and after filtration were detected in 50% of the first and second litres of filtered rainwater samples and in 75% of the third litre of filtered rainwater sample. *Pseudomonas* spp. were also detected in 25% of the fourth and fifth litres of rainwater samples filtered.

Of the dominant bacteria detected, *Yersinia* spp. (GenBank accession no. HM142628.1) were detected in all the unfiltered rainwater samples as well as all the samples collected for the third and fourth litres of filtered rainwater. *Yersinia* spp. were also detected in 50% (second litre) and in 75% (first and fifth litres) of the respective filtered rainwater samples. *Legionella* spp. (GenBank accession no. JN381009.1, HQ111823.1, HQ711922.1, HQ112142.1) were also dominant and PCR assays confirmed the presence of *Legionella* in all the unfiltered rainwater samples as well as all the second and fifth litres of filtered rainwater. *Legionella* spp. were also detected in 75% of the first, third and fourth rainwater litres that were filtered.

Microfiltration is currently being widely applied in water treatment. Due to size, *Staphylococcus aureus* (0.8 µm x 1 µm) and *E. coli* (2 µm x 1 µm) are presumed not likely to pass through the nanofibre membrane with a mean pore size of 0.20 – 0.45 µm. Daels et al. (2011) however noted that in hospital wastewater that had been spiked with *S. aureus*, a 1.6×10^1 CFU/ 100 mL reduction was observed using a non-functionalised membrane. The average pore size of the membrane used in this study was larger (0.9) µm than 0.2 – 0.45 µm (Daels et al., 2011), and for this reason two layers of the nanofibre membrane was used in this filtration system. It is therefore possible that the pore size did not decrease after doubling the layers of the membrane and even with the addition of activated carbon, the pore size could possibly not have been reduced to 0.2 – 0.45 µm. It should however be noted as indicated by the recovery of indicator bacteria that the first litre of filtered rainwater was within DWAF (1996) standards.

3.3.6 Detection of Adenovirus and Rotavirus in Rainwater

Molecular based methods were utilised for the routine detection of the viral strains as they are considered faster and more reliable than traditional viral culture techniques. Traditional viral culture techniques can be expensive, labour intensive, the sensitivity is low and some enteric viruses such as hepatitis A are difficult to cultivate (Gilgen et al., 1997). In addition, for the detection of the RNA virus, rotavirus, gene specific primers were used instead of oligo(dT)15 primers, because they are more specific when cDNA is synthesised (Saayman et al., 2012; van Pelt-Verkuil et al., 2008). Gene specific primers also target a specific gene sequence of the template whereas oligo(dT)15 primers only target the poly(A) of the template when cDNA is prepared for PCR assays.

Rainwater was collected from four DRWH tanks [tanks A, B and C (on Welgevallen Experimental Farm, Figure 3.1) and tank D (JC Smuts Building)] for the detection of adeno- and rotavirus. The PCR results for the detection of adenovirus are presented in Figure 3.12. The Gene Ruler 1 kb Plus DNA ladder (Thermo Scientific) was loaded into the first lane, with the positive control (Coris BioConcept, Belgium) with the expected band size of 110 bp, loaded into lane two. Subsequently, duplicate samples from the four tanks were loaded into lanes three to ten. The expected PCR product of 110 bp was present in all the lanes where the rainwater tank samples were loaded in duplicate. Representative samples were sent for sequencing and analysed using BLAST analysis where the sequences are compared to similar sequences within the NCBI database. The results confirmed the presence of adenovirus in all four tanks (Appendix B). Subsequent treatment experiments were then performed on the three rainwater tanks at the Welgevallen Experimental farm. It should however, be noted that PCR analysis of RNA and subsequent cDNA extracted from all four tanks did not yield bands (346 bp) correlating to the rotavirus positive control. For the on-site treatment of rainwater, adenovirus only will thus be discussed.

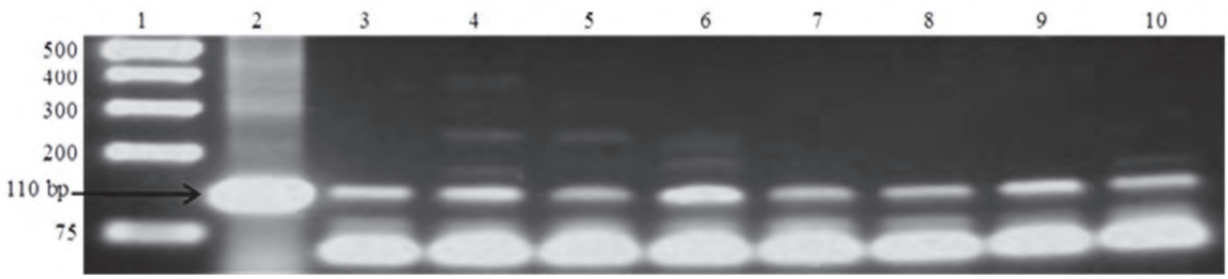

Figure 3.12: Gel electrophoresis (1.2%) of adenovirus PCR products. A 1 kb ladder was used as a molecular size marker (lane 1) with the sizes indicated in base pairs. Lane 2 is the adenovirus positive control while adenovirus PCR reactions for the JC Smuts tank and tanks, A, B and C are loaded in lanes; 3 and 4, 5 and 6, 7 and 8, 9 and 10, respectively.

The frequent detection of bovine adenovirus type 3 strain HLJ0955 in the rainwater could be explained by the tank location (Table 1 in Appendix B). Tanks A, B and C are located on Welgevallen Experimental farm (Stellenbosch University) where experiments with cattle farming, viticulture, wine making, etc. are frequently conducted. Other adenovirus strains detected in the rainwater tanks included simian adenovirus B isolate BaAdV-1 and human adenovirus 40 strain M-364 structural protein gene (Table 1 in Appendix B). The source of the simian strains could be primates that inhabit the environment surrounding the farm. In addition, it is hypothesised that adenovirus from the human faeces, originating from farm employees and settlements where many inhabitants do not have access to flushable toilets, could have been transferred by small animals and rodents and end up on the roof surface (Ahmed et al., 2012).

The virus concentration and detection methods used in this study thus confirmed the presence of adenovirus in all four tanks tested, but the presence of rotavirus could not be confirmed. To the best of our knowledge this is the first study where the enteric virus, adenovirus, was detected in DRWH tanks. This poses a great threat to rural communities that utilise rainwater sources for domestic purposes and rely on DRWH as a potential potable water source. It was thus concluded that further studies are needed to evaluate removal or treatment systems in order to provide water that is free of viral contamination.

3.3.7 Detection of Adenovirus in Filtered and Unfiltered Rainwater

3.3.7.1 *Biological Filtration: Activated Carbon*

Activated carbon filtration systems were connected to the DRWH tanks B and C (Welgevallen Experimental farm) to evaluate their efficiency in removing adenovirus (rotavirus not detected in the rainwater tanks). Once the biofilm had been established, the filtrations systems were sampled for three consecutive days, with an initial untreated rainwater sample collected per tank on each sampling day. In comparison to the positive control, the expected adenovirus PCR product of 110 bp was present in 83% of the before samples as well as 83% of the after samples that were analysed (results not presented). These results imply that the activated carbon filtration system utilised in the current study was ineffective in removing adenovirus from the rainwater system.

The low removal efficiency of the activated carbon filtration system is most probably due to the fact that virus removal is not dependent on biofilm formation (Hijnen et al., 2004), which effectively does not aid in the removal of viruses as for bacteria. Secondly, the adsorption and subsequent removal of the viruses from the contaminated water is shown to be dependent on; the column length, lowered pH of the water (pH below 4.5), low pressure of the influent and the shape of the activated carbon granules (Gerba et al., 1975; Powell et al., 2000). Powell et al. (2000) found that the shape of the activated carbon granule can either inhibit or enhance the adsorption of viruses. Different types of activated carbon materials should thus be evaluated in order to determine all the aspects that may play a role in the adsorption capabilities. According to Powell et

al. (2000) granular activated carbon has not yet been used as an economic or feasible method to remove enteric viruses from water, however new activated carbon materials that has the ability to overcome diffusion limitations should be investigated when they become available.

3.3.7.2 *Biological Filtration: Slow Sand Filtration*

Slow sand filtration systems were connected to the DRWH tanks B and C (Welgevallen Experimental farm, Figure 3.1) to evaluate their efficiency in removing adenovirus. Once the biofilm had been established, the filtrations systems were sampled for three consecutive days, with an initial untreated rainwater sample collected per tank on each sampling day. The results for adenovirus PCR analysis are presented in Figure 3.13. The Gene Ruler 1 kb Plus DNA ladder (Thermo Scientific) was loaded into the first lane, with the positive control (110 bp) loaded into lane two. The PCR analysis of the slow sand filtration treated samples collected from tank B and C are loaded in lanes three to twenty (lanes 3, 9 and 15 are the before slow sand filtration samples of tank B from days 1, 2 and 3, respectively. Lanes 4, 5, 10, 11, 16 and 17 are the duplicate samples taken after slow sand filtration of tank B from days 1, 2 and 3, respectively. Lanes 6, 12 and 18 are the before slow sand filtration samples of tank C from days 1, 2 and 3, respectively. Lanes 7, 8, 13, 14, 19 and 20 are the duplicate samples taken after slow sand filtration of tank C from days 1, 2 and 3, respectively). The expected adenovirus PCR product of 110 bp was present in 100% of the before samples for both tanks B and C, and 83% of the after slow sand filtration treated samples that were analysed. The adenovirus PCR product of 110 bp (lanes 4 and 5, Figure 3.13) was absent after slow sand filtration for the samples collected on the first day from tank B. It is hypothesised that the absence of the adenovirus in the one rainwater treated sample could have been due to a low initial viral concentration. The efficiency of the slow sand filtration system could thus be dependent on the initial viral load in the rainwater sample however, quantitative studies will have to be conducted in order to evaluate the efficiency of filtration system at different viral concentrations (Fong and Lipp, 2005). Representative samples were sent for sequencing and analysed using BLAST analysis where the sequences are compared to similar sequences within the NCBI database. The results confirmed the presence of bovine adenovirus 3 strain HLJ0955 before and after filtration (Table 2 in Appendix B).

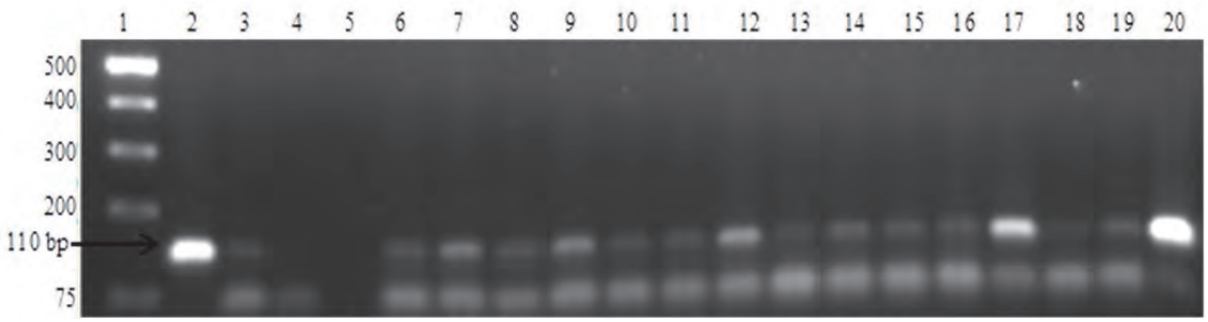

Figure 3.13: Gel electrophoresis (1.2%) of adenovirus PCR products. A 1 kb ladder was used as a molecular size marker (lane 1) with the sizes indicated in base pairs. Lane 2 is the adenovirus positive control. Lanes 3, 9 and 15 are the before slow sand filtration samples of tank B from day 1, 2 and 3, respectively. Lanes 4, 5, 10, 11, 16 and 17 are the duplicate samples after slow sand filtration of tank B from day 1, 2 and 3, respectively. Lanes 6, 12 and 18 are the before slow sand filtration samples of tank C from day 1, 2 and 3, respectively. Lanes 7, 8, 13, 14, 19 and 20 are the duplicate samples after slow sand filtration of tank C from day 1, 2 and 3, respectively.

With the exception of the after sand filtration samples collected on day 1 for tank B (lanes 4 and 5, Figure 3.13), the inability of the slow sand filtration system to effectively remove adenovirus from the majority of the rainwater samples is most probably due to the charge of the silica sand. A study conducted by Hijnen et al. (2004) showed that the removal of viruses is not influenced by biofilm formation or cleaning of the biofilm as

with bacteria, but rather by the charge of the sand itself. The silica sand used in this study did not possess a positive charge that would aid in the adsorption of the viruses to the sand particles. Virus removal by slow sand filtration is also affected by water temperature; filtration rate; sand bed depth; filter maturity and filter cleaning (McConnell et al., 1984; Hijnen et al., 2004).

3.3.7.3 *Activated Carbon and Nanofibre Membrane System*

Activated carbon was then utilised in conjunction with PVA nanofibre membrane filters for the removal of the enteric adenovirus from rainwater. The filtration system was attached to tanks B and C, with an initial sample collected for all analysis. The results for adenovirus PCR analysis are presented in Figure 3.14. The Gene Ruler 1 kb Plus DNA ladder (Thermo Scientific) was loaded into the first lane, with the positive control (110 bp) loaded into lane two. The PCR analysis of the initial and PVA/activated carbon treated samples collected from tank B are loaded in lanes three to six (lanes three and five contain the initial sample, while the after treatment samples were loaded in lanes four and six). Similarly, the PCR analysis of the initial and treated samples collected from tank C are loaded in lanes seven to ten (lanes seven and nine contain the initial sample, while the after treatment samples were loaded in lanes eight and ten). The expected adenovirus PCR product of 110 bp was present in 100% of the before samples for both tanks B and C, and 75% of the after PVA/activated carbon treated samples that were analysed. While the adenovirus PCR product of 110 bp was present in the before treatment sample collected from tank C (lane 9, Figure 3.14), it was however, absent in the second after PVA/activated carbon filtration sample (lane 10, Figure 3.14). Representative samples were sent for sequencing and analysed using BLAST analysis where the sequences are compared to similar sequences within the NCBI database. The results confirmed the presence of bovine adenovirus 3 before and after filtration (Table 2 in Appendix B).

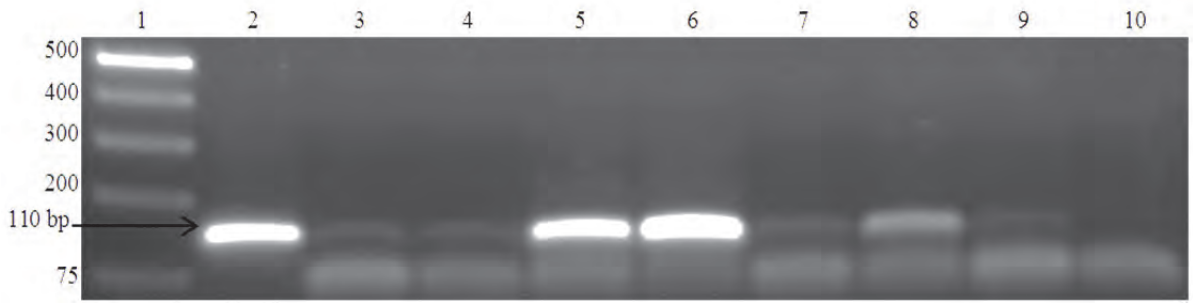

Figure 3.14: Gel electrophoresis (1.2%) of adenovirus PCR products. A 1 kb ladder was used as a molecular size marker (lane 1) with the sizes indicated in base pairs. Lane 2 is the adenovirus positive control. Lanes 3 and 5 are the before PVA and activated carbon samples of tank B; lanes 4 and 6 are the after PVA and activated carbon samples of tank B. Lanes 7 and 9 are the before PVA and activated carbon samples of tank C; lanes 8 and 10 are the after PVA and activated carbon samples of tank C.

Nanofibres can be useful in water treatment processes due to their high porosity, interconnected open pore structure, tailored membrane thickness and high surface hydrophobicity (Feng et al., 2013). However, with the exception of the second rainwater sample collected from tank C (lanes 9 and 10, Figure 3.14), the PVA nanofibre membrane in conjunction with the activated carbon filtration system did not aid in the removal of adenovirus from the rainwater sample, probably due to the fact that the adenovirus is small enough (90-100 nm) to pass through the nanofibre pore size of approximately 0.91 µm. Similar to the results obtained for slow sand filtration, it is hypothesised that the absence of the adenovirus in the one rainwater treated sample (lanes 9 and 10, Figure 3.14) could have been due to a low initial viral concentration. Quantitative studies will however, have to be conducted in order to evaluate the PVA/activated carbon filtration system at different viral concentrations (Fong and Lipp, 2005). Furthermore, a study conducted by

Wang et al. (2013) showed that a novel microfiltration membrane consisting of a two layered nanoscale polyacrylonitrile (PAN)/microscale polyethelyne terephthalate (PET) fibrous scaffold with an ultra-fine functional cellulose nanofibre could simultaneously remove bacteria, viruses and toxic heavy metals. Their PAN/PET scaffold membrane could remove viruses and ions from polluted water with relatively large pore sizes that could originally only be used to remove bacteria by size exclusion. It was thus the electrostatic interactions between the viruses and the membrane that were found to be the reason for removal and not size exclusion as for bacterial removal.

3.4 CONCLUSIONS

The aim of this deliverable was to access the efficiency of four water treatment systems in the removal of rainwater contaminants. Activated carbon based disinfection systems, included a slow activated carbon filtration system and an activated carbon/PVA nanofibre column. Other disinfection systems included a slow sand filtration system and a PVA nanofibre (without activated carbon) column. All systems were analysed for harvested rainwater treatment. The microbial parameters that were investigated for all disinfection systems included the enumeration of total coliforms, *Escherichia coli* and heterotrophic bacteria. In addition, the treated and untreated rainwater samples collected for the slow sand filtration and the activated carbon/PVA nanofibre column were screened for the presence of both selected pathogenic bacteria as well as selected enteric viruses using molecular techniques. However, the treated and untreated rainwater samples collected for the slow activated carbon filtration system was screened solely for the presence selected enteric viruses using molecular techniques. The chemical parameters that were investigated during the pilot scale study included the concentration of metal ions, anions and cations present in the treated and untreated harvested rainwater samples.

Chemical analysis of the activated carbon based systems indicated that all cations and anions present in both the unfiltered and filtered rainwater samples were within the drinking water guidelines according to SANS 241 (2005), DWAF (1996), ADWG (NHMRC and NRMMC, 2011) and WHO (2011), with the exception of aluminium and antimony detected in rainwater samples filtered through the slow activated carbon system. Moreover, all cations and anions present in the rainwater samples collected before and after slow sand filtration were also within the drinking water guidelines according to SANS 241 (SABS, 2005), DWAF (1996), ADWG (NHMRC and NRMMC, 2011) and WHO (2011), with the exception of aluminium, manganese and iron. These four cations were shown to increase significantly ($p< 0.05$) in the filtrate of the biological filtration systems and it is hypothesised that these elements could have leached from the commercially available filtration media of each system. As increased concentrations of aluminium, antimony, magnesium and iron were only detected on the first day of sampling and not on the last day, it is thus recommended that rainwater should be allowed to flow through the biological filtration systems for at least two weeks before use for domestic and irrigation purposes.

While a decrease in heterotrophic bacteria and total coliforms was observed utilising the slow activated carbon filtration system, the decrease was not significant. *Escherichia coli* numbers, however, were shown to have decreased significantly ($p < 0.05$) when utilising these filtration systems, but the reduction was not to within drinking water standards. In addition, heterotrophic bacteria, total coliforms and *E.coli* still persisted in filtered rainwater samples collected after five days of maintaining the filtration systems. Fluctuations in the filtered and unfiltered rainwater total coliform, *E. coli* and heterotrophic bacteria counts thus influenced the efficiency of the activated carbon system. Adenovirus was also detected in the domestic rainwater harvesting tanks, and PCR analyses showed that the slow activated carbon filtration system was not effective in removing this enteric virus (adenovirus) from the filtered rainwater samples.

Heterotrophic bacteria, total coliforms and *E. coli* persisted in filtered rainwater samples collected after twelve days of maintaining the slow sand filtration system and no significant reductions in the indicator organism

numbers was recorded. Based on genus specific PCR analysis, utilised to screen filtered rainwater samples for the presence of pathogenic bacteria that have previously been detected in harvested rainwater (WRC K5_2125; Deliverable 2), no *Salmonella* spp. or *Shigella* spp. were detected in any of the rainwater samples analysed. *Aeromonas* spp. were the least dominant species detected and were only detected in unfiltered rainwater samples. Of the more dominant genera detected, *Yersinia* spp., *Klebsiella* spp. and *Pseudomonas* spp. were identified in more than half of the filtered rainwater samples analysed, including the last filtered sample collected after twelve days. Moreover the slow sand filtration system had no effect on the removal of *Legionella* spp. as PCR assays confirmed the presence of *Legionella* in all unfiltered and filtered rainwater samples. Adenovirus was also detected in all the unfiltered rainwater samples and PCR analyses showed that slow sand filtration system was not effective in removing this enteric virus from the filtered rainwater samples.

It is therefore recommended that the biological filtration systems utilising activated carbon and silica sand, be analysed for a longer time period to monitor the effectiveness of a mature biofilm in the removal of pathogenic bacteria. The biological filtration systems have practical limitations such as the extended time required for the maturation of the biofilm, sporadic sloughing off of the biofilm during filtration and the need for a continuous flow of rainwater. However, these systems are cost-effective and can be easily maintained.

While the chemical quality of the rainwater was generally lower than the stipulated drinking water guidelines, the microbial quality of rainwater filtered through the activated carbon/PVA nanofibre column indicated that heterotrophic bacteria persisted in five litres of filtered rainwater. However, total coliforms were reduced to zero (100%) for the first litre of filtered rainwater and *E. coli* were reduced to zero (100%) in each of the five filtered rainwater samples. Based on genus specific PCR analysis, utilised to screen the five litres of filtered rainwater samples for the presence of pathogenic bacteria, no *Salmonella* spp. were detected in any of the rainwater samples analysed. *Shigella* spp. were detected only in the third litre of filtered rainwater, while *Aeromonas* spp. were removed in the first litre of filtered rainwater and were detected in all the unfiltered rainwater samples. However, *Klebsiella* spp. *Legionella* spp., *Pseudomonas* spp. and *Yersinia* spp. were not removed by the activated carbon/PVA nanofibre column, as these organisms where detected in all five litres of the filtered rainwater and all the unfiltered rainwater samples. The PVA nanofibre membrane system with the addition of activated carbon thus needs to be further optimised, as only one litre of potable water can be produced before the components of the system need to be replaced. Moreover, the average time required to filter one litre of rainwater is approximately 37.48 minutes, rendering this system impractical for use at a household level.

Furthermore, the activated carbon/PVA nanofibre column was not effective in removing the enteric virus (adenovirus) from the filtered rainwater samples, as adenovirus was detected in all the unfiltered rainwater samples and 75% of the filtered rainwater samples. It is however hypothesised that a correlation exists between the concentration of the viruses present in the water samples before treatment and the efficiency of that specific treatment system. Further studies will thus include the quantification of the enteric viruses in the respective water samples with the use of real time quantitative PCR reactions. A positively charged SMI-Q10 nanofibre is also being optimised to determine whether this nanofibre will be effective in adsorption of negatively charged viruses.

Results from this study also show that the addition of activated carbon to the PVA system may have provided an additional filtration barrier, as based on the microbial analysis performed to monitor the PVA nanofibre (without activated carbon) column, the five filtered litres could not be utilised for potable purposes. Heterotrophic bacteria and total coliforms persisted in all five litres of filtered rainwater, and while *E. coli* numbers were reduced (100%) in the first litre of filtered rainwater, *E. coli* were not removed in the remaining four litres of filtered rainwater. The number of coliphages present in the unfiltered and filtered harvested rainwater samples, were however below the detection limit throughout the study period.

CHAPTER 4: SOLAR PASTEURIZATION SYSTEM – THE EFFICIENCY OF A SOLAR WATER PASTEURIZATION SYSTEM IN DISINFECTING WATER FROM DOMESTIC RAINWATER HARVESTING TANKS

4.1 INTRODUCTION

Water resources are becoming increasingly scarce throughout the world due to population increases, climate change and contamination caused by point- and non-point source pollution (Alam Imteaz et al., 2011). While access to safe water and sanitation services were identified as part of the Millennium Development Goals, currently it is estimated that approximately 9% of the population in South Africa do not have access to a sufficient water supply infrastructure, while approximately 22 million (43%) people have insufficient sanitation services (Census, 2011). In addition, the South African government struggles to meet the water requirements of the large number of inhabitants in rural and many peri-urban and urban areas. Alternative and sustainable sources of water must thus be considered to supply freshwater for domestic and potable purposes (Lévesque et al., 2008).

Alternative water resources include storm water harvesting, grey water and wastewater reuse as well as desalination. Storm water harvesting is utilised most often and includes rainwater harvesting and river water utilisation (Alam Imteaz et al., 2011). While these alternate water sources are routinely utilised in many international countries (Nevondo and Cloete, 1991), in South Africa water sources such as rainwater and river water are severely contaminated and it is therefore not advisable to use this water as a primary potable water source (Mwenge Kahinda et al., 2007). In addition, untreated water could cause diseases, which poses a threat to new-borns, young children, the elderly, immuno-compromised people and people living in unsanitary conditions (Mwenge Kahinda et al., 2007). It is also estimated that approximately 38 000 South Africans die each year from diarrhoeal diseases associated with contaminated water (Sciencescope, 2009). Domestically harvested rainwater may thus provide an alternative source of drinking water, but only if the water meets the international standards of drinking water as stipulated by the World Health Organisation (WHO, 2008). In order to provide clean and safe drinking water to rural communities and informal settlements in urban areas, the development of effective water treatment methods are required.

Solar pasteurization can be utilised as a possible treatment for contaminated water sources as microorganisms are susceptible to heat (pasteurization) and ultraviolet-A radiation. The sun is a free, natural source of energy and its full potential remains untapped. Solar pasteurization (SOPAS) differs from solar disinfection (SODIS) in that the SOPAS reactor inactivates microorganisms by only using the thermal effect at a temperature of at least 70°C without radiation, whereas the SODIS reactor uses both the thermal effect and UV-A radiation (Sommer et al., 1997).

According to Nieuwoudt and Mathews (2005) the technology of heating water to below boiling temperature has gained much interest and for this reason the design and implementation of the heat based disinfection systems is fairly advanced. Currently, there are three types of water heating systems that are manufactured predominantly for domestic use (Solar Energy Equipment, 2000; SANS 1307, 2003). The most expensive system is a split system of two components, a collector and a storage tank, where water is heated directly or indirectly. The collector is usually installed on the north-facing area of the roof and the storage tank inside the roof. This allows for a thermo-siphon effect whereby water is able to circulate through the collector due to the ranges of temperatures. This is a passive system and for this reason an electric pump for circulating water is not required. The second closed coupled system is comprised of a flat plate collector, that can heat

water directly or indirectly, and a separate elevated storage tank attached to the end of the collector. It has been noted that these systems are less expensive and installation is easier than the split systems. Lastly, a less efficient closed system is the integrated collector storage, or the integral collector (ICS) system comprising of a collector that is used to heat and store the water. These systems are the most cost effective. Close-coupled systems are also usually placed on the north-facing section of pitched roofs. For this system, both the flat plate collector and the storage tanks are exposed. Being a passive system, again the water moves via the thermo-siphon effect (Nieuwoudt and Mathews, 2005).

The aims of this study were to assess the efficiency of different pasteurization temperatures (laboratory scale treatment of rainwater samples) and a heating based disinfection system, namely a closed coupled system [pilot scale treatment of rainwater samples directly from a domestic rainwater harvesting (DRWH) tank] in treating harvested rainwater. The microbial parameters that were investigated during the laboratory scale experiments included the enumeration of heterotrophic bacteria as well as the identification of microbial isolates. The microbial parameters that were investigated during the pilot scale study included the enumeration of faecal and total coliforms, *Escherichia coli* and heterotrophic bacteria. In addition, the treated and untreated rainwater samples of the pilot scale study were screened for the presence of selected pathogenic bacteria and well as selected enteric viruses using molecular techniques. The chemical parameters that were investigated during the pilot scale study included the concentration of metal ions, anions and cations present in the treated and untreated harvested rainwater samples.

4.2 MATERIALS AND METHODS

4.2.1 Preliminary Pasteurization Study

A preliminary pasteurization study was conducted in the Environmental Microbiology laboratory of the Department of Microbiology at Stellenbosch University.

4.2.1.1 *Sample Site and Collection*

Rainwater samples were collected from the rainwater harvesting tank (2000 L) installed outside the JC Smuts building of Stellenbosch University. Samples were then incubated at various temperature and time intervals in order to determine the optimum range at which the heterotrophic plate counts (HPC), which serves as an indicator for disinfection, were reduced to within drinking water standards as stipulated by South African Water Quality Guidelines for Domestic Water Use of the Department of Water Affairs and Forestry (DWAF, 1996) and the Australian Drinking Water Guidelines (NHMRC and NRMMC, 2011). Re-growth of the heterotrophic bacteria was also monitored by incubating the respective plates at different temperatures for varying time periods. In addition, the bacterial groups that were able to withstand extreme temperature conditions were isolated and identified.

4.2.1.2 *Laboratory-scale Pasteurization Experiments and Recovery of Heterotrophic Bacteria*

In order to analyse the efficiency of thermal heat inactivation and the corresponding time interval in reducing bacterial numbers, rainwater samples were pasteurized as follows. Erlenmeyer flasks containing 100 mL rainwater samples, collected from the DRWH tank installed outside the JC Smuts building, Stellenbosch University, were incubated in a water bath at 50˚C, 55˚C, 60˚C, 65˚C and 72˚C, respectively for five minute intervals (0 min, 5 min, 10 min, 15 min, 20 min, 25 min and 30 min). Note: the rainwater samples were placed in the water baths and were heated to the respective temperatures, once the rainwater sample had reached the same temperature as the water bath, this was designated as time zero (t = 0). An undiluted and a 10-fold dilution of the untreated and treated rainwater was then prepared for each temperature at the different time intervals and spread plated (100 µL) onto Nutrient Agar (NA) and R2A agar (Difco). To ensure

that there was no cross contamination of samples and to eliminate false positive results, a control sample of sterile water that had been autoclaved at 121˚C, 100 kPa (15 psi) for 20 minutes was also included and plated out each time a treated rainwater sample was taken. The various incubation temperatures and incubation periods are summarised in Table 4.1. According to La Duc et al. (2007) for the recovery of psychrophiles, mesophiles and thermophiles, incubation should be conducted at the following temperatures ≤15˚C, 20 - 45˚C, and 55 - 65˚C, respectively. Moreover, in order to recover slow growing bacteria from environmental samples, incubation time periods will also vary, for example for the enumeration of psychrophiles it is suggested that the incubation period be up to 10 days in order to recover all bacteria able to grow in colder temperatures.

Table 4.1: Classification of bacteria based on a physiological characteristic (cardinal temperature) (La Duc et al., 2007).

Descriptive Term	Definition	Incubation Temperature	Incubation Time (days)
Psychrophile	Grows at 0˚C, optimum ≤15˚C	4	10
Mesophile	Optimum 20 - 45˚C (25˚C)	37	4
Thermophile	Grows ≥55˚C, optimum 55 and 65˚C	56	4

4.2.1.3 *Genomic DNA Extractions from Isolates*

Isolates were selected based on differences in colour reactions and morphological characteristics and sub-cultured onto NA at least three times. Once pure colonies had been obtained, a single colony was inoculated into 5 mL Nutrient Broth (Merck) and incubated at 37°C overnight. Cells were harvested by centrifuging 2 mL of each culture for 10 min at 6000 × g. The extraction of genomic DNA from each isolate was performed using the ZR^{TM} Soil microbe DNA Miniprep Kit (Zymo Research) according to the manufacturer's instructions. The genomic DNA was then visualised on a 0.8% agarose gel stained with 0.5 µg/mL ethidium bromide. Electrophoresis was conducted at 80 volts for approximately one hour with the use of 1X Tris/Borate/EDTA (TBE) buffer (Sambrook et al., 1989).

4.2.1.4 *Polymerase Chain Reactions Used to Identify Isolates*

Polymerase Chain Reactions (PCR) were used to amplify the 16S rRNA conserved sequence of each isolate using previously described primers (Table 2.5) and cycling parameters and reagents (Section 2.2.7). The PCR products were analysed and sequenced as described in Section 2.2.7.

4.2.2 Phase 2: Pilot Scale Pasteurization System

Based on the results obtained in the laboratory-scale experiments (Section 4.2.1), a solar pasteurization system (Apollo Solar Technology Pty. Ltd., South Africa) was attached directly to the DRWH tank A (Figures 3.1 and 4.1) set up on the Welgevallen Experimental farm, Stellenbosch University.

4.2.2.1 *Sample Site and Collection*

Three polyethylene domestic rainwater harvesting (DRWH) tanks (2000 L) were installed at the Welgevallen Experimental farm, Stellenbosch University, South Africa as indicated in Sections 3.2.1. For ease of sampling tank A was installed on a metal stand so that the rainwater could flow from the tank into the solar pasteurization system in a passive manner. This pasteurization system was donated to Stellenbosch

University by a company in Somerset West, Crest. The middle tank (tank B) and the right end tank (tank C) were used to test the filtering systems (discussed in Chapter 4).

For microbial and chemical analysis before and after solar pasteurization, rainwater samples were collected in 1 L sterile Schott bottles. The temperature and pH of the rainwater at the sampling locations were measured using a hand-held mercury thermometer and colour-fixed indicator sticks with a pH range of 0 - 14 (ALBET®, Barcelona, Spain). A MadgeTech IRTC101A - Infrared thermocouple temperature Data Logger (Madge Tech, Inc) was installed to monitor the temperature of the rainwater inside the storage tank of the solar pasteurization system. To ensure that only the less dense warm water was being monitored, the probe of the logger was passed through the inlet tank and approximately half way into the storage tank (indicated by a black arrow in Figure 4.1). The temperature data obtained from the log tagger was analysed using Data Logger Software version 4.1.5. Rainfall and temperature patterns were obtained from the South African Weather Services (SAWS, 2012), while direct solar radiation data was obtained from Stellenbosch Weather Services, Engineering Faculty (http://weather.sun.ac.za/).

Samples were collected from July 2013 till October 2013 (Table 4.2) at various temperature ranges (55 to 57˚C; 64 to 66˚C; 72 to 74˚C; 78 to 81˚C; 90 to 91˚C). For each temperature range, three sampling events were performed with a total of 15 sampling events conducted. For each temperature a before (untreated) and 1 L of heat treated rainwater was collected in duplicate.

Table 4.2: Sampling dates and temperatures of the solar pasteurized samples analysed.

Sampling Date	Temperature of Pasteurized Water Sample (°C)
11.07.2013	56
22.07.2013	57
30.07.2013	65
19.08.2013	55
20.08.2013	81
26.08.2013	78
02.09.2013	64
06.09.2013	81 (2nd)
06.09.2013	91
10.09.2013	66
10.09.2013	74
11.09.2013	72
11.09.2013	90
12.09.2013	91 (2nd)
09.10.2013	73

4.2.2.2 *Solar Pasteurization System*

The Apollo^{TM} solar pasteurization system was designed and manufactured in China and donated to Stellenbosch University by a company in Somerset West, Crest. The information regarding the solar pasteurization system was obtained from http://www.apollotechnology.co.za/low_pressure_system.php. The water from the rainwater tank A flows through the system components (Figure 4.1 and Table 4.3) as follows; firstly, cold water flows from the rainwater tank through the cold water feed (A) into the cold water stainless steel inlet tank (C). To increase the flow rate into the inlet tank it is suggested that larger, shorter pipes with gentle bends be used in the system. From the inlet tank, cold water flows into the stainless steel main storage tank (D) (capacity: 100 L) then down through the high borosilicate glass collector tubes (E). Through the principle of thermo-siphoning, as the cold water (blue arrow) heats, it loses density, and becomes more buoyant, the heated water is then able to move up (red arrow) into the main storage tank again. In this manner, a natural circulation of cold water and hot water is started. If the process of hot water being replaced

by colder more dense water continues, the whole body of water in the main tank will heat up. Heated water is then harvested from the hot water outlet (F).

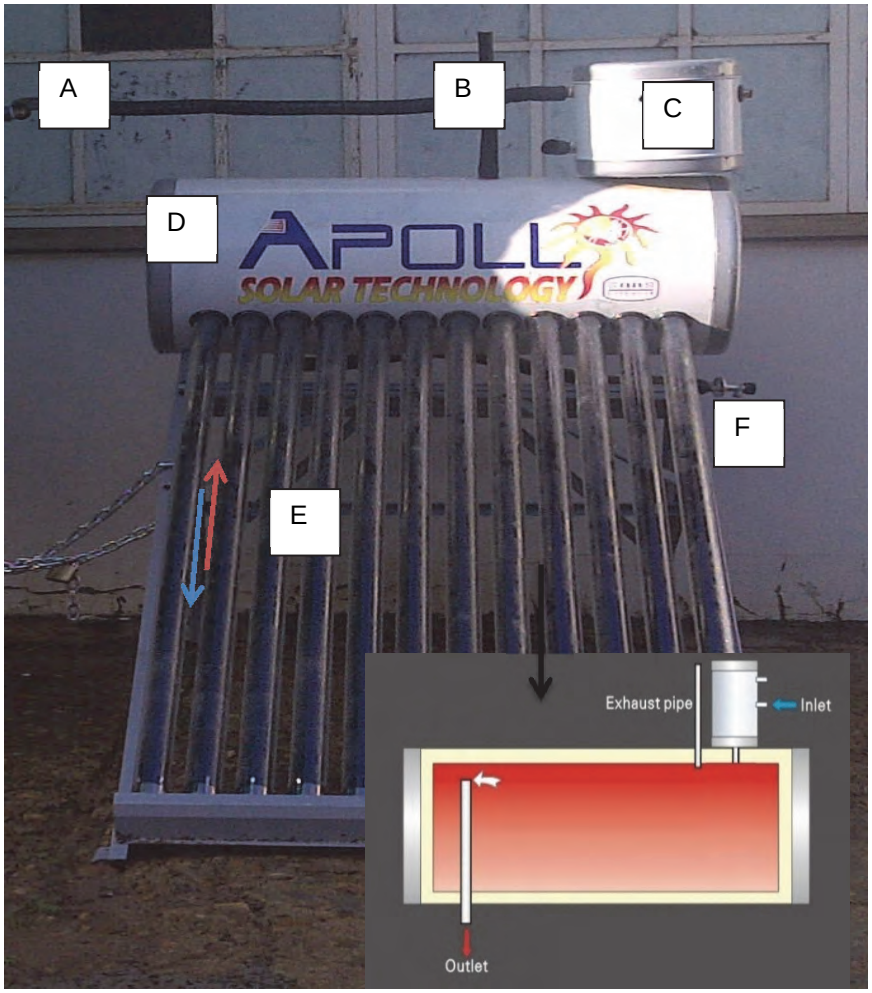

Figure 4.1: The low pressure solar pasteurization system (ASLP-12/1800-58) used in this study was donated to Stellenbosch University by a company in Somerset West, Crest.

Table 4.3: The labels and corresponding components of the solar pasteurization system.

Label	Component
A	Cold water feed into the tank
B	Exhaust pipe
C	Cold water inlet tank
D	Main storage tank
E	12 × Collector tubes
F	Hot water outlet

4.2.2.4 *Chemical Analysis*

Pasteurized and unpasteurized rainwater samples were analysed to determine the concentration of cations and anions present using the methods described in Section 3.2.3.

4.2.2.5 *Recovery of Indicator Organisms*

The number of indicator organisms (HPC, total coliforms and *E. coli*) present in the pasteurized and unpasteurized rainwater samples was enumerated using the methods described in Section 3.2.4.

4.2.2.6 *The Bacterial Removal Efficiency of the Pasteurization System*

The bacterial removal efficiency of the system was obtained by comparing the CFU numbers obtained from the samples taken before pasteurization and the average CFU numbers obtained from samples taken after pasteurization. The log reduction was calculated using equation 1 and the percentage reduction was calculated using equation 2 (Brözel and Cloete, 1991).

Equation 1:

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

Equation 2:

$$\text{Percentage reduction} = 100 - \frac{\text{Survivor count}}{\text{Initial count}} \times 100$$

4.2.2.7 *Recovery and Assay of Coliphages*

Coliphages present in the pasteurized and unpasteurized rainwater samples were enumerated according to Baker et al. (2003) as described in Section 3.2.6.

4.2.2.8 *Extraction of Total DNA from Rainwater Samples*

Total DNA extractions were performed for each of the 45 rainwater samples collected before and after pasteurization. In order to extract total genomic DNA from the rainwater samples a modified version of the boiling method was utilised (Watterworth et al., 2005) as described in Section 3.2.7.

4.2.2.9 *Genus Specific PCR Reactions*

Primers and PCR conditions as outlined in Table 3.2 were utilised in the current study for the detection and identification of documented pathogenic and opportunistic bacterial pathogens present in the pasteurized and unpasteurized rainwater samples. The reagents used for the detection of *Legionella* spp., *Aeromonas* spp., *Shigella* spp., *Salmonella* spp., *Pseudomonas* spp., *Yersinia* spp. and *Klebsiella* spp. are described in Section 3.2.8, as well as the method for the subsequent analyses and sequencing of the PCR products.

4.2.3 Detection of Adenovirus and Rotavirus in Rainwater and Pasteurized Rainwater Samples

Adeno-and rotavirus in the untreated rainwater as well as the pasteurized samples were detected as outlined in Sections 3.2.9.1 to 3.2.9.4.

4.3 RESULTS AND DISCUSSION

4.3.1 Section 1: Preliminary Pasteurization Study

In order to determine which media would be best suited for the monitoring of rainwater treated with various point of use systems, two different media i.e. nutrient agar and R2A agar, generally used in the recovery of heterotrophic bacteria, were compared. Briefly for the laboratory-scale pasteurization experiments, rainwater samples were incubated in a water bath at 50˚C, 55˚C, 60˚C, 65˚C and 72˚C, respectively at five minute intervals (0 min, 5 min, 10 min, 15 min, 20 min, 25 min and 30 min) for each temperature. Untreated and heat treated rainwater samples were then, by means of the spread plate technique, plated onto nutrient agar and R2A agar and incubated at 37˚C (mesophiles) for up to four days. The heat treated samples were also incubated at 10˚C (psychrophiles) and 56˚C (thermophiles) for up to 10 days and four days, respectively, in order to isolate and identify the bacterial groups that were able to withstand extreme temperature conditions.

4.3.1.1 *The Recovery of Heterotrophic Bacteria from Untreated and Treated Rainwater Samples Utilising Two Different Media (Nutrient Agar and R2A Agar)*

In order to determine which media would be better suited to monitor the efficacy of the solar pasteurization system (Figure 4.1), untreated rainwater samples and rainwater samples heat treated at the various temperatures and different time intervals were spread plated onto nutrient agar (Table 4.4) and R2A agar (Table 4.5) with the plates incubated at 37˚C.

As indicated in Table 4.4, significant differences ($p < 0.05$) in heterotrophic bacteria counts (nutrient agar) were recorded between the untreated rainwater samples and the treated rainwater samples, from the 10 minute time interval at the varying temperature ranges. On average the heterotrophic counts in the untreated rainwater ranged from 2×10^3 to 5×10^3 CFU/mL. While fluctuations in the temperature treated rainwater samples at the different time intervals were noted, on average a two log reduction in the heterotrophic bacterial numbers was obtained for all the temperatures analysed from the 10 minute time interval. In addition, for the sample treated at 65°C for 25 min, the heterotrophic bacteria decreased from 2×10^3 CFU/ml (before sample) to zero, with numerous counts decreasing to within the DWAF (1996) standards of < 100 CFU/mL for drinking water.

Table 4.4: The heterotrophic bacteria results for untreated and heat treated rainwater samples spread plated on nutrient agar and incubated at 37°C for 4 days.

Temp (˚C)	Heterotrophic Plate Count (CFU/ mL) at time interval (t=x)							
	Before	**0 min**	**5 min**	**10 min**	**15 min**	**20 min**	**25 min**	**30 min**
50°C	3×10^3	2×10^2	2×10^2	6×10^1	1×10^2	9×10^1	6×10^1	5×10^1
55°C	4×10^3	7×10^1	4×10^1	4×10^1	5	2×10^1	1×10^1	2×10^1
60°C	2×10^3	3×10^1	1×10^1	5	1×10^1	2×10^1	3×10^1	2×10^1
65°C	2×10^3	1×10^1	2×10^1	5	2×10^1	2×10^1	0	5
72°C	5×10^3	3×10^1	3×10^1	3×10^1	3×10^1	2×10^1	1×10^1	2×10^1

Similarly, significant differences ($p < 0.05$) in the enumeration results for heterotrophic bacteria spread plated onto R2A agar at the different temperature and time intervals were also recorded (Table 4.5). On average the results of the heterotrophic bacterial numbers obtained for the untreated rainwater samples ranged from 5×10^4 to 2×10^5 CFU/mL. Significant differences ($p < 0.05$) in the heterotrophic plate count at the different treatment temperatures were then recorded from the 10 minute time interval. As indicated in Table 4.5, treatment at 60°C and 72°C from the 5 minute time interval also significantly decreased ($p < 0.05$) the heterotrophic bacteria count to within the DWAF (1996) standards of < 100 CFU/mL for drinking water.

Table 4.5: The heterotrophic bacteria results for untreated and heat treated rainwater samples spread plated on R2A agar and incubated at 37°C for 4 days.

Temp (˚C)	Heterotrophic Plate Count (CFU/ mL) at time interval (t=x)							
	Before	**0 min**	**5 min**	**10 min**	**15 min**	**20 min**	**25 min**	**30 min**
50°C	1×10^5	2×10^4	6×10^3	9×10^2	7×10^2	3×10^2	6×10^2	4×10^2
55°C	5×10^4	1×10^3	2×10^2	7×10^1	5×10^1	2×10^1	1×10^1	5
60°C	1×10^5	1×10^1	0	0	5	0	5	5
65°C	1×10^5	1×10^1	1×10^1	2×10^1	2×10^1	7×10^1	1×10^1	1×10^1
72°C	2×10^5	2×10^1	0	0	0	5	3×10^1	0

The Thermal Death Time (TDT) is defined as the “shortest time needed to kill all organisms in a microbial suspension at a specific temperature and under defined conditions” (Willey et al., 2008). For the spread plate technique utilising nutrient agar the TDT could not be calculated as growth of heterotrophic bacteria was observed at all temperatures up to t = 30 min, however a count of zero was obtained at the 65°C temperature after the rainwater had been treated for 25 min (Table 4.4). In contrast, while fluctuating results were obtained for the rainwater samples spread plated onto R2A agar after treatment at the varying temperature and time intervals, the TDT was calculated as t = 30 min, at a treatment temperature of 72˚C. In addition, counts of zero were also sporadically obtained at the 60°C and 72°C treatment temperatures from the 5 minute time interval (Table 4.5).

It has previously been noted that the recovering of the level of heterotrophic bacteria gives a good indication of the microbiological quality of water during treatment, storage and distribution of potable water (DWAF, 1996; Carter et al., 2000). Traditionally, to recover heterotrophic bacteria in treated water for potable purposes, plate count agar (PCA) by means of the pour plate method was used (APHA, 1996). It was then proposed that the spread plate method using a low-nutrient media such as Reasoner´s 2A (R2A) agar could be employed (APHA, 1996). For this study a standard minimal media namely, nutrient agar, and a low nutrient media namely, R2A agar were compared for the recovery of heterotrophic bacteria from treated and untreated rainwater.

In the current study the average number of heterotrophic bacteria recovered from the R2A agar (Table 4.5) were significantly higher ($p < 0.05$) when compared to the average HPC CFU/mL recovered from the nutrient agar (Table 4.4). It should be noted that on average a one to two log difference in the heterotrophic bacterial numbers was obtained when the before rainwater samples were plated onto R2A agar (Table 4.5) versus plating on nutrient agar (Table 4.4). Carter et al. (2000) found that when monitoring a drinking water distribution system in Milford, Ohio (about 30 km east of Cincinnati) the average plate counts were much lower using plate count agar (PCA) and tryptic soy agar with 5% sheep’s blood (TSA-SB) compared to average plate counts recovered from R2A agar. Nagarkar et al. (2001) also showed that media modified to enhance the growth of oligotrophs, such as R2A, acquired an increase of about 2 orders of magnitude in the bacteria counts, compared to bacterial counts obtained when samples are cultured with standard minimal media. Moreover, as previously mentioned by Allen et al. (2004), time and temperature of incubation are important variables in determining the HPC bacteria present in an environment. This study therefore, proposes the use of R2A media and incubation at 37˚C for up to four days for the monitoring of microbial contamination of treatment systems in the treatment of rainwater.

4.3.1.2 *Bacteria Isolated from Heat Treated Rainwater Samples Incubated at 10˚C and 56˚C on Nutrient Agar and R2A Agar.*

Dominant isolate representatives obtained from heat treated samples and incubated at 10˚C and 56˚C on nutrient agar and R2A agar, respectively were selected and identified. The identities of all the bacterial isolates, obtained from the Genbank results (>98% identity), along with the treatment temperature, plate incubation temperature and time interval of the heat treatment are recorded in Table 4.6. Of the bacteria that

were isolated from the heat treated rainwater samples and incubated at 10˚C on R2A agar and nutrient agar, *Flectobacillus* spp. and *Acinetobacter baumannii* were able to withstand heat treatment at 50˚C at t = 0 min and t = 30 min, respectively. *Bacillus aryabhattai* was isolated from rainwater that was heat treated at 55˚C for 30 min, while *Bacillus thuringiensis* was able to withstand the heat treatment of 60˚C for 30 minutes. Both *Bacillus cereus* and *Bacillus firmus* were isolated from rainwater samples that had been heat treated at 65˚C for 30 minutes.

Of the bacteria that were isolated from the heat treated rainwater samples and incubated at 56˚C on R2A agar and nutrient agar, *Bacillus licheniformis* and *Oceanobacillus* spp. were able to withstand heat treatment at 50˚C at t = 20 min and t = 30 min, respectively. *Bacillus thermolactis* and *Anoxybacillus rupiensis* were also able to withstand the heat treatment of 55˚C for 20 and 30 minutes, respectively. The majority of the bacteria isolated from the heat treated rainwater samples, such as *B. aryabhattai, B. cereus, B. firmus, B. thuringiensis, B. licheniformis, B. thermolactis, Oceanobacillus* spp. and *A. rupiensis,* belonged to the *Bacillaceae* family. *Flectobacillus* spp. and *A. baumannii*, which belong to the *Flexibacteraceae* and *Moraxellaceae* family respectively, were also isolated.

Table 4.6: Bacteria isolated and identified from heat treated samples at different temperatures and for different time intervals.

Treatment Temperature (Plate incubation) (°C)	Time Interval (min)	Organism	Accession Number
50 (10)	30	*Acinetobacter baumannii*	AP013357.1
55 (10)	25	*Bacillus aryabhattai*	JX524506.1
65 (10)	30	*Bacillus cereus*	KF601958.1
65 (10)	30	*Bacillus firmus*	KF535122.1
60 (10)	30	*Bacillus thuringiensis*	KF151161.1
50 (10)	0	*Flectobacillus* spp.	AJ011917.1
55 (56)	25	*Anoxybacillus rupiensis*	AM988775.1
50 (56)	20	*Bacillus licheniformis*	HM006901.1
55 (56)	5	*Bacillus thermolactis*	FN666256.1
50 (56)	30	*Oceanobacillus* spp.	HQ316193.1

The rainwater samples were subjected to laboratory scale heat treatment experiments in order to determine which media was suitable for pasteurization experiments and to identify the time and temperature range at which the lowest heterotrophic bacterial numbers were recorded. A study conducted by La Duc et al. (2007) found similar results when investigating the microbial contamination in clean room environments. With the use of R2A agar physiologically diverse bacteria of the *Bacillaceae* family were identified. It was also noted that in a near neutral environment halotolerant, alkalophilic species such as *Oceanobacillus* and non-spore forming microbes such as *Actinobacter* were also identified.

4.3.2 Section 2: Pilot Scale Pasteurization System

4.3.2.1 *Physico Chemical Parameters*

The dates the samples were collected from the solar pasteurization system, the temperatures of the untreated (before pasteurization) and treated rainwater samples (after pasteurization) as well as the average ambient temperature are recorded in Table 4.7. Overall an average pH of 6 was measured for all rainwater samples, collected before and after pasteurization. The temperature of the rainwater samples collected from the rainwater harvesting tank ranged from the lowest temperature of 17˚C (11.07.2013) to the highest recorded temperature of 24˚C on two days (26.08.2013 and 02.09.2013). The lowest total rainfall throughout

the sampling period was recorded for October (39.6 mm/month). At the start of the sampling period rainfall was recorded in July 2013 (169.6 mm/month), which then increased in August 2013 (371.6 mm/month) and decreased again in September 2013 (177.2 mm/month).

In order to monitor the temperature fluctuations of the pasteurized rainwater, the probe of a temperature logger was inserted into the storage tank of the solar pasteurization system and the temperature of the pasteurized rainwater (Figure 4.2) as well as the ambient temperature were monitored for approximately one month (26.07.2013 – 24.08.2013). The direct solar radiation (W/m^2) data was obtained for the same time period as for the temperature logger from the Stellenbosch Weather Services, Engineering Faculty. Readings for both the solar radiation and the temperature were recorded for every 30 minutes. An average of 61˚C and a range of 34˚C (lowest) to 98˚C (highest) was obtained for the temperature of the pasteurized rainwater samples monitored by the log tagger for the period monitored (Figure 4.2).

Table 4.7: The ambient temperature and the temperatures of the untreated and treated rainwater samples on the sampling dates.

Sampling Date	Temperature of untreated rainwater (˚C)	Temperature of treated rainwater (˚C)	Ave. daily ambient temperature (˚C)
11.07.2013	17	56	27.1
22.07.2013	18	57	20.8
30.07.2013	20	65	21.3
19.08.2013	19	55	21.3
20.08.2013	23	81	19.09
26.08.2013	24	78	19.7
02.09.2013	24	64	21.1
06.09.2013	20	81	22.9
06.09.2013	22	91	22.9
10.09.2013	22	66	15.8
10.09.2013	22	74	15.8
11.09.2013	19	72	17
11.09.2013	20	90	17
12.09.2013	21	91	20
09.10.2013	22	73	29.4

A significant negative correlation ($R = -0.128$; $p < 0.05$) could be determined between the temperature readings of the stored pasteurized rainwater and the direct solar radiation data obtained, with the direct solar radiation data exhibiting an average of 1.23 W/m^2 and a range of 0 to 741.92 W/m^2. As the direct solar radiation heats the high borosilicate glass collector tubes, the principle of thermo-siphoning heats the rainwater, which loses density, becomes more buoyant, and is then able to move up into the main storage tank. A time delay between the direct solar radiation and final pasteurized water is thus observed, which could explain the significant negative correlation obtained.

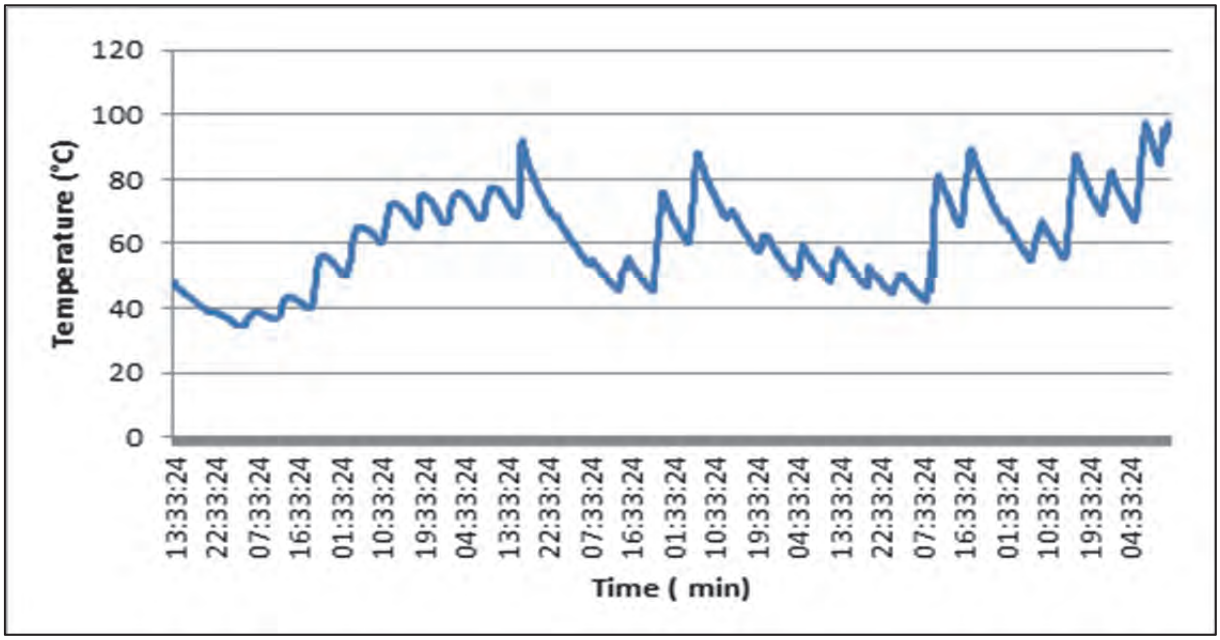

Figure 4.2: Temperatures recorded for the pasteurized rainwater every 30 minutes for approximately one month (26.07.2013 – 24.08.2013).

The ambient temperatures recorded versus the direct solar radiation readings obtained over time are indicated in Figure 4.3. An average of 16˚C and a range of 6˚C (lowest) to 40˚C (highest) was obtained for the ambient temperature using the log tagger (Figure 4.3). A significant positive correlation ($R = 0.74$, $p > 0.05$) was however, observed between the ambient temperature recorded using the log tagger and the direct solar radiation readings obtained from the Stellenbosch Weather Services, which implies that as the direct solar radiation data increased, a similar increase was noted in the ambient temperature.

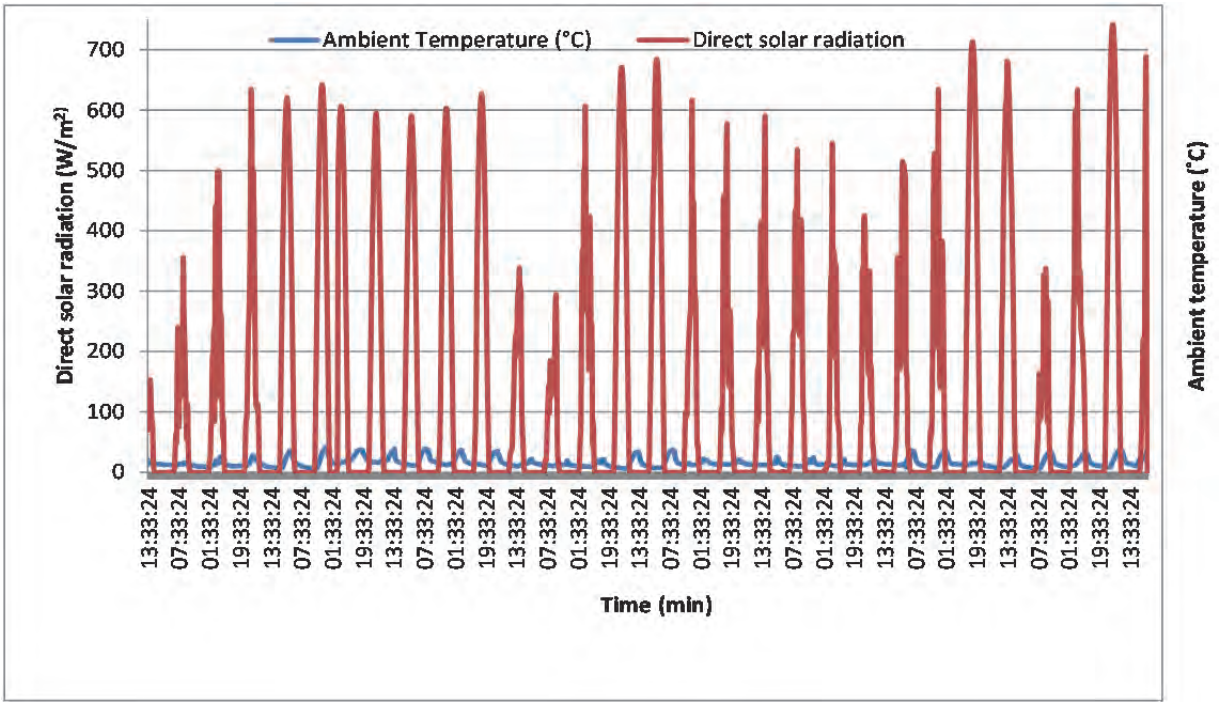

Figure 4.3: Ambient temperatures and the corresponding solar radiation data obtained from the Stellenbosch Weather Services, Engineering Faculty recorded every 30 minutes for approximately one month (26.07.2013 – 24.08.2013).

4.3.2.2 *Chemical Analysis*

All cations were within the drinking water guidelines according to SANS 241 (SABS, 2005), DWAF (1996), ADWG (NHMRC and NRMMC, 2011) and WHO (2011), with the exception of iron, aluminium, lead and nickel as represented in Table 4.8. The DWAF (1996) guidelines stipulate that iron should not exceed 100 µg/L and only one rainwater sample (collected before pasteurization at 55˚C) was within the DWAF (1996) guidelines for iron, with the iron values ranging from the lowest value of 113.4 µg/L (before pasteurization at 65˚C) to the highest value of 441.99 µg/L (before pasteurization at 91˚C) which exceeded the DWAF (1996) guidelines. In addition, two samples were not with the SANS 214 guidelines for iron (200 µg/L), namely the sample taken after pasteurization at 65˚C (218.22 µg/L) and before pasteurization at 91˚C (441.99 µg/L) which was also not within the ADWG of 300 µg/L. No adverse effects among adults have however been noted for consuming high doses of iron. This has been contributed to the physiology of the human body that is able to regulate the absorption of this cation. Iron poisoning has however been observed among young children (NHMRC and NRMMC, 2011).

The ADWG (NHMRC and NRMMC, 2011) guidelines stipulate that nickel should not be above 20 µg/L and while all the samples collected before pasteurization and after pasteurization at 91˚C were within the ADWG, samples collected after pasteurization at 55˚C, 65˚C and 78˚C were not within standards and were recorded at 46.43 µg/L, 22.94 µg/L and 32.82 µg/L, respectively. The concentration of nickel in all the rainwater samples, were however within the stipulated standards of the SANS 241 (SABS, 2005) and WHO (2011) of 150 µg/L and 70 µg/L, respectively. Nickel is distributed by the blood in the body, with the main excretion route via the urinary tract. The toxicity exhibited by nickel compounds is dependent on many factors such as the chemical species, their physical form, their concentration and the whether the individual is exposed to nickel by ingestion, inhalation or dermal interaction (Christensen and Lagesson, 1981).

The aluminium concentrations of all the collected rainwater samples were within the drinking water guidelines as stipulated by SANS 241 (SABS, 2005), DWAF (1996), ADWG (NHMRC and NRMMC, 2011) and WHO (2011). The average concentration of rainwater samples collected after pasteurization at 78˚C, however, exceeded the ADWG (NHMRC and NRMMC, 2011) of 100 µg/L, and with an average concentration of 130.98 µg/L recorded. Although the effect of aluminum on human health needs to be clarified, continuous exposure of humans to high concentrations of aluminium has been implicated in chronic neurological disorders such as Alzheimer's disease, Parkinsonism dementia (PD) and amyotrophic lateral sclerosis (ALS). It is also important to take into consideration that at neutral pH aluminium is non-toxic and is not an essential nutrient for humans (DWAF, 1996; Perl, 1985).

The concentration of lead in drinking water should not exceed 10 µg/L according to DWAF (1996), ADWG (NHMRC and NRMMC, 2011) and WHO (2011). However three samples collected after pasteurization at 55˚C, 78˚C and 91˚C exceeded these guidelines with average concentrations of 12.81, 17.20 and 13.2 µg/L recorded in the respective rainwater samples. These values were however, still within the SANS 241 (SABS, 2005) guideline of 20 µg/L. Lead is a powerful and persistent neurotoxicant and the effects of lead poisoning range from death to impaired cognitive and behavioral development that can have long term detrimental consequences for children (Lidsky and Schneider, 2003).

Table 4.8: Cation concentrations obtained from the unpasteurized and duplicate pasteurized rainwater samples collected at various temperatures that were compared to the recommended concentrations as stipulated by the respective drinking water guidelines (n = 12).

Metal	Before 55˚C	After 55˚C	Before 65˚C	After 65˚C	Before 78˚C	After 78˚C	Before 91˚C	After 91˚C	SANS 241	DWAF	ADWG	WHO
Boron as B (mg/L)	0	0.22	0.00	0.00	0.00	0.26	0.01	0.11	-	-	4	2.4
Calcium as Ca (mg/L)	3.72	6.86	5.03	7.14	4.07	7.00	4.56	4.58	150	200	200	-
Potassium as K (mg/L)	0.28	0.56	0.27	0.49	0.38	0.54	0.31	0.32	50	50	-	-
Magnesium as Mg (mg/L)	0.42	0.95	0.49	0.96	0.44	0.93	0.46	0.50	70	30	200	-
Sodium as Na (mg/L)	3.32	5.58	3.20	5.34	3.33	5.57	2.91	3.14	200	100	180	-
Phosphorus as P (mg/L)	0.03	0.05	0.06	0.05	0.04	0.06	0.03	0.06	-	-	-	-
Silicon as Si (mg/L)	0.32	2.93	0.64	2.58	0.35	3.47	0.55	1.78	-	-	-	-
Aluminium as Al (µg/L)	9.4	61.14	16.0	44.81	15.08	130.98	4.86	48.8	300	150	100	-
Chromium as Cr (µg/L)	0.00	0.39	0.2	0.25	0.18	0.36	0.30	0.3	100	50	50	50
Manganese as Mn (µg/L)	1.20	15.68	0.1	13.67	12.13	13.14	12.77	8.4	100	50	500	-
Iron as Fe (µg/L)	70.59	195.59	113.4	218.22	182.71	179.08	441.99	170.1	200	100	300	-
Cobalt as Co (µg/L)	0.03	0.64	0.0	0.35	0.10	0.50	0.19	0.3	500	-	-	-
Nickel as Ni (µg/L)	0.29	46.43	0.4	22.94	1.04	32.82	1.88	16.0	150	-	20	70
Copper as Cu (µg/L)	4.15	43.57	6.6	19.56	29.34	59.56	23.75	71.7	1000	1000	2000	2000
Zinc as Zn (µg/L)	46.60	338.73	9.1	316.97	57.67	276.62	39.64	171.8	5000	3000	3000	-
Arsenic as As (µg/L)	0.15	0.64	0.4	0.55	0.22	0.41	0.33	0.4	10	10	10	10
Selenium as Se (µg/L)	0.79	1.77	3.7	1.80	0.79	2.36	1.02	0.7	20	20	10	40
Strontium as Sr (mg/L)	31.69	47.16	17.3	43.03	32.12	45.96	39.36	36.7	-	-	-	-
Molybdenum as Mo (µg/L)	0.00	0.00	0.03	0.02	0.00	0.00	0.04	0.0	-	-	50	-
Cadmium as Cd (µg/L)	0.01	0.13	0.0	0.17	0.06	0.19	0.02	0.1	5	5	2	3
Tin as Sn (µg/L)	0.00	0.10	0.1	0.07	0.03	0.07	0.02	0.0	-	-	-	-
Antimony as Sb µg/L	0.02	0.14	0.1	0.20	0.08	0.18	0.14	0.1	-	-	3	20
Barium as Ba (µg/L)	28.93	36.29	11.5	25.93	41.12	35.95	61.79	39.5	-	-	2000	700
Mercury as Hg (µg/L)	0.04	0.03	0.1	0.12	0.01	0.02	0.03	0.0	1	1	1	6
Lead as Pb (µg/L)	0.09	12.81	0.8	3.86	2.46	17.20	1.06	13.2	20	10	10	10

The noteworthy changes in the concentrations of the cations present in the rainwater samples before and after treatment at 55˚C will be discussed first. The increase in manganese, cobalt, nickel, copper, lead and zinc was significant (more than a 10 fold increase) as concentrations before and after pasteurization ranged from 1.20 µg/L to an average of 15.68 µg/L, from 0.03 µg/L to an average of 0.64 µg/L, from 0.29 µg/L to an average of 46.43 µg/L, from 4.15 µg/L to an average of 43.57 µg/L, from 0.09 to an average of 12.81 µg/L and from 46.60 µg/L to an average of 338.73 µg/L, respectively. The concentration of tin increased after treatment from 0 µg/L to an average of 0.1 µg/L and mercury decreased from 0.04 µg/L to an average of 0.03 µg/L, these changes were, however, negligible (0.1 µg/L). All the other cations monitored also increased after treatment at 55˚C, with molybdenum being the only exception as this cation was not detected in either the before or after treatment samples.

While increases for many of the cations monitored before and after treatment at 65˚C were recorded, the only significant increase (more than a 10 fold increase) for the rainwater samples treated at 65˚C was observed for zinc. The concentration of zinc increased from 9.1 µg/L in the before sample to an average of 316.87 µg/L in the after 65˚C treated sample. In addition, decreases were observed after treatment at 65˚C for phosphorous, selenium, molybdenum and tin, with concentrations before and after pasteurization ranging from 0.06 µg/L to an average of 0.05 µg/L, from 3.7 µg/L to an average of 1.8 µg/L, from 0.03 µg/L to an average of 0.02 µg/L and from 0.1 µg/L to an average of 0.07 µg/L, respectively. Boron was the only cation which was not detected in either the before or after treatment samples.

Nickel was the only cation that had a significant increase (more than 10 fold increase) in concentration after treatment at 78˚C with a concentration of 1.04 µg/L detected before treatment and an average concentration of 32.82 µg/L detected after treatment. The concentrations of iron and barium decreased after treatment at 78˚C with before and after concentrations ranging from 182.71 µg/L to an average of 179.08 µg/L and from 41.12 µg/L to an average of 35.95 µg/L, respectively. All the other cations monitored also increased after treatment at 78˚C, however the increase was not significant. Molybdenum was not detected in either the before or after treatment samples.

The concentration of aluminium and lead increased significantly (more than 10 fold increase) after treatment at 91˚C with the before and after concentrations ranging from 4.86 µg/L to an average of 48.8 µg/L and from 1.06 µg/L to an average of 13.20 µg/L, respectively. In contrast, the concentration of manganese, iron, barium and mercury decreased after treatment at 91˚C with before and after concentrations ranging from 12.77 µg/L to an average of 8.4 µg/L, from 441.99 µg/L to an average of 170.1 µg/L, from 61.79 µg/L to an average of 39.5 µg/L and from 0.03 µg/L to an average of 0.0 µg/L, respectively. All the other cations monitored also increased in concentration after treatment at 91˚C, however the increase in concentration was not significant.

Iron, aluminium, lead and nickel were shown to be present at higher concentrations in the rainwater samples collected from the pasteurized solar system (at varying temperatures) than in the unpasteurized samples stored in the rainwater tank. The rainwater is in direct contact with borosilicate glass collector tubes and the main stainless steel storage tank. Researchers have shown that during simulated cooking process, nickel leaches from stainless steel cooking ware into food (Kamerud et al., 2013). In a study conducted by Semwal et al. (2006) aluminium also leached from stainless steel cooking utensils during food preparation. Therefore it is hypothesised that the iron, aluminium, lead and nickel were leached from the stainless steel storage tank into the rainwater during the current study.

All anions were within drinking water guidelines according to SANS 241 (SABS, 2005), DWAF, (1996), ADWG (NHMRC and NRMMC, 2011) and WHO (2011) and are represented in Table 4.9. Sulphate concentrations after pasteurization at 55˚C and 65˚C, increased from 1.1 mg/L to an average of 5.35 mg/L and from1.6 mg/L to an average of 5.7 mg/L, respectively. At higher pasteurization temperatures of 78˚C and 91 ˚C the concentrations of sulphate increased from 1.2 mg/L to an average of 3.5 mg/L and from 1.6 mg/L

to an average of 1.95 mg/L, respectively. Chloride concentrations increased after pasteurization at 55˚C and 65˚C from 7.2 mg/L to an average of 12 mg/L and from not being detected to an average of 11 mg/L, respectively. A decrease in the concentration of chloride was however, observed after pasteurization at 78˚C from 7.2 mg/L to an average of 3.1 mg/L. At 91˚C no changes in the concentration of chloride was observed with 5.9 mg/L of chloride detected in both the before and after pasteurization rainwater sample. In addition, no significant change in concentrations was observed for nitrate and nitrite, phosphate and fluoride in the rainwater samples after pasteurization for all temperatures (55 to 91˚C).

Table 4.9: Anions concentrations obtained from the unpasteurized and duplicate pasteurized rainwater samples collected at each temperature that were compared to the recommended concentrations as stipulated by the respective drinking water guidelines (n = 12).

Anions	Before 55˚C	After 55˚C	Before 65˚C	After 65˚C	Before 78˚C	After 78˚C	Before 91˚C	After 91˚C	SANS 241	DWAF	ADWG	WHO, (2011)
Sulphate as SO_4 (mg/L)	1.1	5.35	1.6	5.7	1.2	3.5	1.6	1.95	200	100	250	-
Chloride as Cl^- (mg/L)	7.2	12	ND	11	7.2	3.1	5.9	5.9	400	200	250	-
Nitrate Nitrite as NO_3 and NO_2 (mg/L)	0.1	0.1	ND	0.1	0.1	0.1	0	0	10	6	50	50
Phosphate as PO_4 (mg/L)	0.05	0.05	ND	ND	0.05	0.1	0	0	-	-	-	-
Fluoride as F (mg/L)	0.1	0.1	0.1	0.1	0.1	2.5	0.1	0.1	-	-	-	-

ND – not detected

4.3.2.3 *Indicator Bacteria Detected in Untreated and Solar Pasteurized Water Samples*

Untreated (1 L) and duplicate treated rainwater samples (2 x 1 L) were collected from a solar pasteurization system at various temperatures. The lowest temperature range included in this study was 55 to 57˚C and the highest range was 90 to 91˚C. Total coliforms, *E. coli* and HPC were enumerated for all treated and untreated rainwater samples at the varying temperature ranges (Table 4.10). In addition, heterotrophic bacteria were enumerated in stored solar pasteurized treated rainwater samples (for every temperature range) every day for a week in order to determine the maximum storage time allowed for the pasteurized rainwater before the recovery of heterotrophic bacteria was observed (Table 4.10).

Table 4.10: Indicator bacteria enumerated from pasteurized rainwater samples at various temperatures and the amount of time allowed for storage of the heat treated rainwater samples.

Pasteurization Temperature (˚C)	Indicator	Untreated Sample (Ave. CFU/100 mL)	First 1 L Treated (Ave. CFU/100 mL)	Second 1 L Treated (Ave. CFU/100 mL)	Log Reduction	Reduction (%)	Storage (Days)
55 -57	Total Coliforms	7×10^2	0	0	2	100	0
	E. coli	4×10^1	0	0	1	100	
	HPC	2×10^7	6×10^5	5×10^5	2	97	
64 - 66	Total Coliforms	7×10^2	0	0	2	100	0
	E. coli	2×10^1	0	0	1	100	
	HPC	3×10^6	4×10^5	5×10^5	1	86	
72 - 74	Total Coliforms	6×10^2	0	0	2	100	2
	E. coli	1	0	0	0	100	
	HPC	3×10^6	0	0	6	100	
78 - 81	Total Coliforms	6×10^2	0	0	2	100	7
	E. coli	4×10^1	0	0	1	100	
	HPC	4×10^6	0	0	6	100	
90 - 91	Total Coliforms	3×10^3	0	0	3	100	7
	E. coli	4×10^1	0	0	1	100	
	HPC	4×10^6	0	0	6	100	

At each pasteurization temperature an untreated and duplicate treated rainwater samples were collected and analysed for the presence of indicator bacteria. On average, in comparison to the untreated rainwater, the rainwater samples pasteurized at 55˚C, 56˚C and 57˚C yielded a two log reduction in the average total coliforms and a one log reduction in the average *E. coli* counts, with no total coliforms and *E. coli* detected after treatment (Table 4.10). Similar results were obtained for the rainwater samples pasteurized at 64˚C, 65˚C and 66˚C; 72˚C, 73˚C and 74˚C; and 78˚C, 81˚C and 81(2^{nd})˚C, with the exception of *E. coli* numbers for the 72 to 74ºC temperature range where no log reduction was observed. In addition, comparison between the before treatment rainwater sample (3×10^3 CFU/mL) and the rainwater solar pasteurized at 90˚C, 91˚C and 91(2^{nd})˚C yielded a three log reduction in the average total

coliforms, with no total coliforms detected in the treated samples. The initial *E. coli* count of 4×10^1 CFU/mL was also decreased to zero after treatment at 90˚C, 91˚C and 91(2nd)˚C.

Comparison between the heterotrophic plate counts on R2A for the untreated rainwater samples (2×10^7 CFU/mL) and the rainwater samples pasteurized at 55˚C, 56˚C and 57˚C showed a 2 log reduction in average heterotrophic bacteria counts. In addition, the average heterotrophic plate count of 3×10^6 CFU/mL recorded in the rainwater sample before treatment at temperatures 64˚C, 65˚C and 66˚C, was reduced by one log to 4×10^5 and 5×10^5 CFU/mL in the duplicate solar pasteurized treated rainwater samples (Table 4.10). Rainwater samples pasteurized at 72˚C, 73˚C and 74˚C; 78˚C, 81˚C and 81(2nd)˚C; and 90˚C, 91˚C and 91(2nd)˚C, showed a significant six log reduction in the average heterotrophic bacterial values, in comparison to the before treatment rainwater heterotrophic counts, with no heterotrophic bacteria detected in the pasteurized rainwater samples (Table 4.10).

Overall, all total coliforms and *E. coli* numbers enumerated after the pasteurization treatment ranging from temperatures of 55 to 57˚C up to 90 to 91˚C were reduced to zero and were within the DWAF (1996) guidelines. However the HPC numbers were above the DWAF (1996) guidelines for the temperature ranges of 55 to 57˚C (average 5.5×10^5 CFU/ mL) and 64 to 66˚C (average 4.5×10^5 CFU/ mL), with no HPC numbers obtained after solar pasteurization at the temperature range of 72˚C to 91˚C.

To monitor the maximum storage time of the rainwater pasteurized at the various temperature ranges as outlined in Table 4.2, Section 4.2.1, samples were stored at room temperature for up to one week. The re-growth of heterotrophic bacteria after pasteurization at the various temperatures was observed and heterotrophic counts were enumerated daily. As HPC numbers were above the DWAF (1996) guidelines for the temperature ranges of 55 – 57˚C (average 5.5×10^5 CFU/mL) and 64 to 66˚C (average 4.5×10^5 CFU/ mL) the regrowth of HPC was observed after one day. In contrast the rainwater treated at higher solar pasteurization temperatures could be stored for approximately two days (72 to 74˚C) and up to one week (78 to 81˚C, 90 to 91˚C). In summary the storage time allowed before HPC numbers grew to above the DWAF (1996) guidelines ranged from no storage allowed, (55 to 57˚C; 64 to 66˚C) to two days of storage (72 to 74˚C) and one week of storage if the water was pasteurized at the higher temperatures (78 to 81˚C, 90 to 91˚C). The *D* value has been defined as the time required in reducing a bacterial population by 90% or a 1 log reduction (Prescott et al., 1993) and is used by a number of studies in determining the time required to reduce bacterial numbers (Juneja et al., 2001; Spinks et al., 2006a). However, *D* values could not be established for the heat treatment of total coliforms, *E. coli* and HPC bacteria found in rainwater in this study as the solar pasteurization is a continuous flow system and the number of inactive bacterial cells does not necessarily represent the number of active bacterial cells entering the storage tank of the pasteurization system. Spinks et al. (2006a) have described the limitations of using the *D* value thermal inactivation data as it is assumed that there is a constant reduction rate over time. It is therefore suggested that in order to evaluate the efficacy of a heat treatment system, conclusions should not be made by relying solely on the *D* value. However, this study showed that heterotrophic bacteria were reduced by an average of 85% at temperatures ranging from 55˚C to 65˚C and from temperatures of 72˚C onward a 100% reduction in heterotrophic bacteria was observed. However, total coliform and *E. coli* numbers were reduced by 100% with no total coliforms or *E. coli* observed from 55˚C and higher. Spinks et al. (2006a) also suggested that water temperatures should reach between 55 and 65˚C in order to eliminate enteric pathogenic bacteria. Other studies have suggested that temperatures below boiling greatly reduce bacterial numbers from rainwater samples resulting in water quality that is within the Australian Drinking Water Guidelines (Lye, 1991; Coombes et al., 1999; 2003;). However, it has been suggested that *Enterococcus faecalis* instead of *E. coli* should be used as an indicator organism for the monitoring of hot water quality as *E. faecalis* demonstrated the greatest heat resistance at 55˚C (Spinks et al., 2006a).

4.3.2.4 *The Detection of Coliphages in Harvested Rainwater*

As indicated in Chapter 4, the number of coliphages present in the harvested rainwater samples (unpasteurized and pasteurized at temperatures mentioned in Section 4.2.1, Table 4.2) were below the detection limit. However, when unpasteurized rainwater (500 mL) was spiked with 10 mL of *E. coli* ATCC 13706 (that had been incubated at 37˚C until log phase reached) 1 PFU/ mL was detected. These results were thus not significant and overall coliphages could not be detected in the rainwater samples before and after pasteurization.

4.3.2.5 *Genus Specific PCR Detection of Bacteria Commonly Identified in Harvested Rainwater*

Untreated rainwater samples as well as the rainwater samples treated at various temperatures (outlined in Section 4.2.1, Table 4.3) by solar pasteurization, were screened using PCR assays for the detection of various pathogenic bacteria commonly associated with harvested rainwater. The results of the PCR assays are presented in Appendix B, Table 3. The percentages of the various bacterial genera present in the untreated rainwater and two pasteurized rainwater samples, along with the highest pasteurization temperature where the PCR assays tested positive, are summarised in Table 4.11.

Table 4.11: The percentage of untreated rainwater and duplicate pasteurized rainwater samples that tested positive for various bacterial genera and the highest pasteurization temperature where the PCR assays tested positive.

Organism	Rainwater Samples (%)		Highest Pasteurization Temperature (˚C)
	Untreated	Pasteurized (n = 30)	
Aeromonas spp.	20	0	56
Klebsiella spp.	47	17	65
Legionella spp.	87	100	91
Pseudomonas spp.	67	47	91
Salmonella spp.	0	0	NA
Shigella spp.	7	3	55
Yersinia spp.	27	13	78

No *Salmonella* spp. were detected with the use of PCR based assays throughout the study in the untreated and pasteurized rainwater samples at the various temperature ranges (Table 4.11). In addition, while PCR assays confirmed the presence of *Aeromonas* spp. (GenBank accession no. CP005966.1) in the unpasteurized rainwater samples collected before treatment at 56˚C, 57°C and 65°C, no *Aeromonas* spp. were detected in the solar pasteurized rainwater samples and throughout the remainder of the study period (Table 3, Appendix B). *Shigella* spp. (GenBank accession no. HE616529.1) were also only detected twice throughout the study period in a duplicate rainwater sample pasteurized at 55°C and in an untreated rainwater sample collected before pasteurization at 56°C. Similarly, *Yersinia* spp. were present in a duplicate rainwater sample pasteurized at 55°C and in both rainwater samples collected after treatment at 78°C (Table 3, Appendix B). For each temperature, samples are collected before treatment from the rainwater tank, with solar treated samples collected directly from the pasteurization unit. This could explain why *Shigella* spp. and *Yersinia* spp., amongst others, were present in the after treatment sample and not in the initial sample collected directly from the rainwater tank. *Yersinia* spp. were also detected sporadically throughout the sampling period in untreated rainwater samples collected before treatment at 57°C, 73°C and 81°C, with no *Yersinia* spp. detected in the samples solar pasteurized at these respective temperatures. However, *Yersinia* spp. were detected in the untreated rainwater sample

collected before solar pasteurization at 65°C as well as in a duplicate rainwater sample pasteurized at this temperature (Table 3, Appendix B).

Klebsiella spp. (GenBank accession no. AF303617.1) were also only detected sporadically throughout the study period at the varying temperature ranges, with this organism present only in the unpasteurized rainwater samples collected for the temperatures 55°C, 56°C, 81°C and 91°C. In addition, *Klebsiella* spp. were detected using PCR assays in the before and after solar pasteurization samples collected at 57°C and 65°C, where it was present in only one after treatment sample, and 64°C, where it was present in the before and both samples analysed after solar pasteurization at this respective temperature. Similar to the results obtained for *Shigella* and *Yersinia* spp., *Klebsiella* spp. were also detected in a duplicate rainwater sample pasteurized at 73°C, while it was not detected in the before treatment rainwater sample collected directly from the rainwater tank.

The frequency of detection for *Pseudomonas* spp. and *Legionella* spp. were highest throughout the study period (Figure 4.4; Table 3, Appendix B), with *Pseudomonas* spp. detected in 67% of the untreated rainwater samples and 47% of the solar pasteurized samples collected at various temperature ranges. Overall *Legionella* spp. were the most persistent organisms and were present in all the rainwater samples, unpasteurized and pasteurized (for all the temperature ranges) collected throughout the study period as indicated in Table 3, Appendix B.

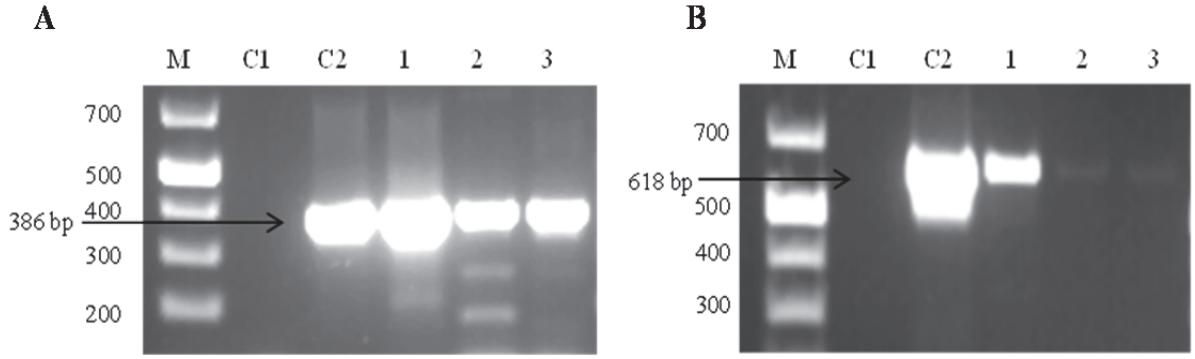

Figure 4.4: Agarose gel electrophoresis (1.2%) of PCR assays conducted to detect the presence of *Legionella* spp. (A) and *Pseudomonas* spp. (B). Lane M represents a 1 kb ladder which was used as a molecular size marker with the sizes indicated in base pairs. Lane C1: the respective negative controls. Lane C2: the respective positive controls. Lane 1: Unpasteurized rainwater sample. Lanes 2 and 3: the duplicate rainwater samples, pasteurized at 55˚C.

The PCR based assays utilising genus specific primers thus detected the presence of *Legionella* spp., *Yersinia* spp., and *Pseudomonas* spp. at the pasteurization temperature greater than 78˚C. No literature has previously detected the presence of viable planktonic *Legionella*, *Pseudomonas*, or *Yersinia* species cable of surviving treatment temperatures of approximately 78˚C and higher. In this study however organisms identified at temperatures greater than 78˚C according to Genbank (>97% identity) included uncultured *Legionella* (GenBank accession no. KC209485.1, AB858005.1, KC209446.1) and *Yersinia enterocolitica* (GenBank accession no. HM142628.1). Results also confirmed the presence of uncultured *Pseudomonas* sp. (GenBank accession no. JX279939.1), *Pseudomonas stutzeri* (GenBank accession no. KF260975.1), *Pseudomonas* sp. (GenBank accession no. KF561877.1) at temperatures greater than 78˚C. The viability of these organisms however, is not verified and of the many pitfalls associated with PCR detection methods, PCR based assays cannot distinguish between viable and non-viable pathogenic organisms (Ahmed et al., 2013). However, it is hypothesised that the presence of these organisms at such high pasteurization temperatures could be due to two possible scenarios, either the PCR based assays were detecting non-viable cells, or the bacteria were ingested by protozoa. Protists,

especially amoebae, have been described by Bichai et al. (2008) as "the Trojan Horse of microorganisms" as protozoa are cable of heterotrophic feeding. Amoebae have two stages of development generally, known as the trophozite and the cyst which, because of the two layers that surround it, is cable of surviving treatments such as chlorination and temperatures of between -20˚C and +42˚C (Greub and Raoult, 2004). *Legionella* spp. and amoeba have also been detected simultaneously in rainwater sources (Lye, 2002). As amoeba, like protists, feed mainly on bacteria, and most bacteria are able to survive after being ingested, studies have shown that various species of *Legionella* are able to remain viable after being ingested by species of *Acanthamoebae*. For example, *Legionella erythra* and *Legionella pneumophila*, ingested by *Acanthamoeba castellanii* and IA (an environmental thermotolerant *Acanthamoebae* isolate) increased by 1 – 2 logs after being treated at temperatures varying from 40 to 80˚C (Storey et al., 2004). Other bacteria that have been seen to be ingested by *A. castellanii* include *Salmonella typhimurium*, *Yersinia enterocolitica*, *Shigella sonnei* and *E. coli* (King et al., 1988). This hypothesis will however, have to be investigated in future research projects. In addition, viability assays will have to be conducted on the solar pasteurized samples.

4.3.2.6 *Determining the Maximum Volume of Rainwater Harvested from the Solar Pasteurization System*

The volume of pasteurized rainwater that was harvested from the 100 L storage tank of the solar pasteurization system at various temperature ranges is summarised in Table 4.12. As the storage tank of the solar pasteurization system was drained, the temperature of the pasteurized rainwater decreased by approximately 4˚C in an average of 21:47 minutes. An average of 64.4 L could also be harvested before the flow rate decreased and pasteurized rainwater could no longer be collected form the solar system.

Table 4.12: The volume of pasteurized rainwater harvested at various temperatures.

Temperature Range (˚C)	Pasteurized Rainwater Harvested (L)	L/m^2 for each kWh of Incident Solar Energy
89 - 93	68	0.99
87 – 90	65	1.2
71 - 73	63	11.9
93 - 97	63	0.6
56 - 58	63	61.8

In addition, on two occasions, the temperature of the pasteurized rainwater in the storage tank of the solar system was monitored while the water was drained from the system. When the temperature of the system reached approximately 93˚C, the storage tank was drained (Figure 4.5, green arrow) and after approximately 4.5 hours the system had stabilised (the storage tank had been re-filled with rainwater) and the temperature of the rainwater started to increase again to approximately 54˚C. On the second occasion, when the temperature of the storage tank had reached approximately 50˚C, the storage tank was again drained, as is indicated in Figure 4.5, red arrow) and approximately 7.75 hours later the system had stabilised (the storage tank had been re-filled with rainwater) and the temperature of the rainwater started to increase again to approximately 30˚C.

The direct solar radiation data showed an average of 1.82 W/m^2, 2.28 W/m^2, 23.79 W/m^2, 1.27 W/m^2 and 122.6 W/m^2 for the corresponding pasteurized water temperature of 89˚C, 87˚C, 71˚C, 93˚C and 56˚C, respectively (see Section 4.3.2.1, Figure 4.5) and it was then determined that the system could produce an average of 61.8 L/m^2 for each kWh of incident solar energy, for lower temperatures ranging from 56 to 58˚C and at higher temperatures (93 to 97˚C), the system could produce approximately 0.6 L/m^2 for each kWh of incident solar. El-Ghetany and Dayem (2010) found that a solar system could produce 171 L/m^2 daily at 60˚C which also decreased to 39 L/m^2 at higher temperatures of 90˚C. Other studies were able to

harvest 3.61 L/m^2 for each kWh of incident solar at 80˚C (Bansal et al., 1988), 2.8 L/m^2 for each kWh of incident solar at 70˚C (El-Ghetany and El-Seesy, 2005) and 1.1 L/m^2 for each kWh of incident solar at 90˚C (El-Ghetany and Dayem, 2010).

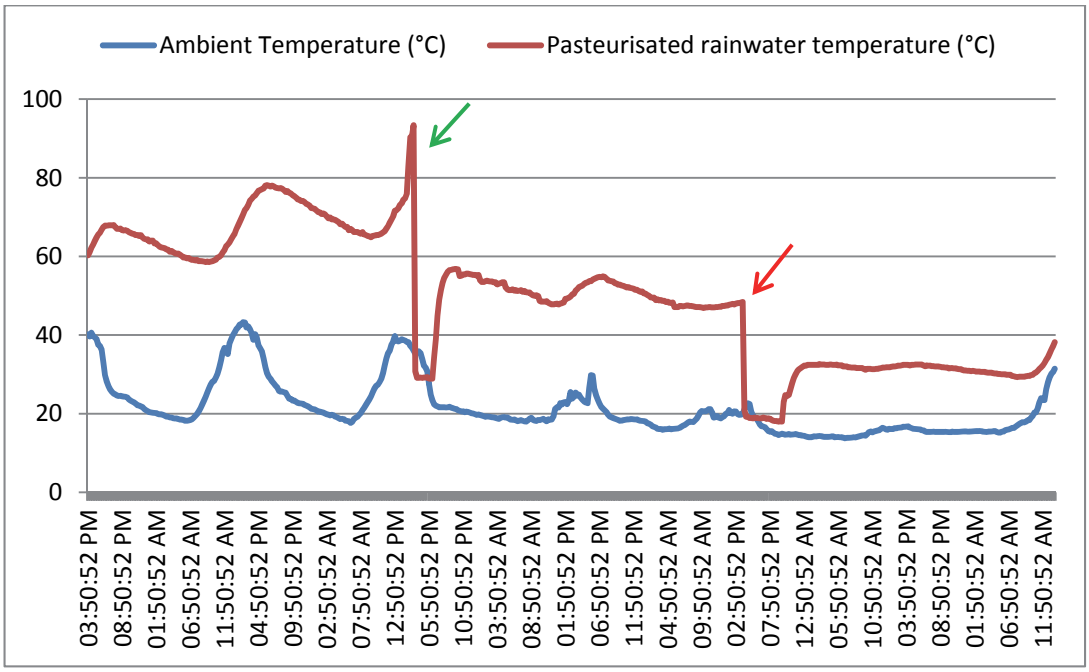

Figure 4.5: The temperature of the rainwater inside the storage tank over a two week period, monitoring the temperature changes due to the system being drained sporadically (five times). The temperature logger was adjusted to record readings every 15 minutes. The green and red arrows indicate where the storage tank was drained of pasteurized rainwater.

It was reported that in Southern Africa, households generally use water for drinking, cooking and hygiene purposes. Studies indicated that the average household requires at least 20 L of potable water per day, of which 50% of the water is utilised for personal hygiene (Nieuwoudt and Mathews, 2005). Reiff et al. (1996) found that in Latin America average families of five members required approximately between 40 L and 60 L of potable water. Taylor (2001) found that approximately 50% of people living around and in Pretoria did not have access to running water in their houses. Le Roux (2003) showed that 60% of the rural community in Mabedlane, KwaZulu-Natal, collected their water from the Umgeni River although central taps connected to the main municipal lines where available. It is thus estimated that the pasteurization system used in the current study should provide an adequate volume of treated rainwater for drinking and domestic purposes at the average sized household level (up to four people).

4.3.3 Detection of Adenovirus and Rotavirus in Rainwater and Pasteurized Rainwater Samples

As indicated in Section 3, this is the first study that detected the enteric virus, adenovirus, in rainwater harvesting tanks. Therefore the removal of this enteric virus from rainwater with the use of solar pasteurization was investigated. The solar pasteurization system was connected to the DRWH tank A (Figure 3.1, Welgevallen Experimental farm) to evaluate its efficiency in removing adenovirus (rotavirus was not detected in the rainwater tanks). The results for adenovirus PCR analysis are presented in Figure 4.6. The Gene Ruler 1 kb Plus DNA ladder (Thermo Scientific) was loaded into the first lane, with the positive control (110 bp) loaded into lane two. The PCR analysis of the solar pasteurization treated samples collected from tank A are loaded in lanes three to ten (lanes 3 and 5 are the before 85˚C

samples; lanes 4 and 6 are the after 85˚C samples; lanes 7 and 9 are the before 90˚C samples; lanes 8 and 10 are the after 90˚C samples). The expected adenovirus PCR product of 110 bp was present in 100% of the before samples, 100% of the after 85°C samples and 50% of the after 90°C samples that were analysed. The absence of the adenovirus PCR product of 110 bp (lane 8) after solar pasteurization is most likely due to the fact that the virus was present in a low concentration in the before sample. These results suggest the efficiency of the solar pasteurization could be dependent on the viral load in the initial water sample; however, quantitative studies will have to be conducted in order to evaluate the solar pasteurization system at different viral concentrations (Fong and Lipp, 2005). Representative samples were sent for sequencing and analysed using BLAST analysis where the sequences were compared to similar sequences within the NCBI database. The results confirmed the presence of bovine adenovirus 3 strain HLJ0955 before and after pasteurization (Table 2 in Appendix B).

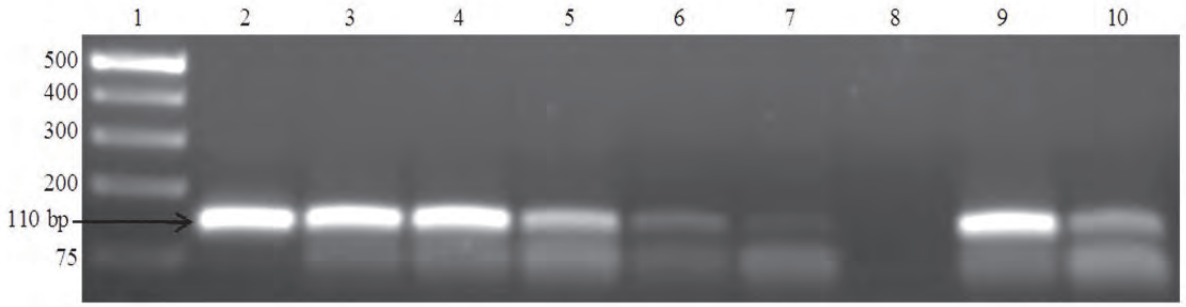

Figure 4.6: Gel electrophoresis (1.2%) of adenovirus PCR products. A 1 kb ladder was used as a molecular size marker (lane 1) with the sizes indicated in base pairs. Lane 2 is the adenovirus positive control while lanes 3 and 5 are the before 85˚C samples and lanes 4 and 6 are the after 85˚C samples. Lanes 7 and 9 are the before 90˚C samples and lanes 8 and 10 are the after 90˚C samples.

The results obtained in this study correlates with previous studies stating that adenovirus is the most persistent non-enveloped enteric virus that can persevere for extended periods of time in aquatic environments and can withstand extreme physico-chemical treatments (Ansari et al., 1991; Gratacap-Cavalier et al., 2000; Villena et al., 2003; Fong and Lipp, 2005; Albinana-Gimenez et al., 2006). Furthermore, research has shown that double-stranded DNA viruses, such as adenoviruses, are extremely stable when exposed to UV (Thurston-Enriquez et al., 2003; Gerba et al., 2007).

4.4 CONCLUSIONS

The first phase of the deliverable was aimed at assessing the efficiency of different pasteurization temperatures (laboratory scale treatment of rainwater samples) and determining the optimum culture media to enumerate heterotrophic bacteria, which served as a measure of disinfection efficiency, at the laboratory scale level. While preliminary pasteurization results indicated that significant differences ($p < 0.05$) in the heterotrophic plate count at the different treatment temperatures were then recorded from the 10 minute time interval on both nutrient agar and R2A (Tables 4.4 and 4.5) and that treatment at 65°C to 72°C also significantly decreased the heterotrophic bacteria count to within the DWAF (1996) standards of < 100 CFU/mL for drinking water, the thermal death time the was calculated as t = 30 min, at a treatment temperature of 72˚C (from results obtained for R2A agar). In addition, based on the results obtained, R2A agar was the most suited growth media for enumerating the recovery of heterotrophic bacteria from heat treated rainwater samples and this media was thus utilised to assess the efficiency of the solar pasteurization system in the on-site pilot scale study. Dominant bacterial isolate representatives obtained from heat treated samples and incubated at 10˚C and 56˚C on nutrient agar and R2A agar, respectively were also selected and identified. The majority of the bacteria isolated from the heat treated

rainwater samples, such as *B. aryabhattai, B. cereus, B. firmus, B. thuringiensis, B. licheniformis, B. thermolactis, Oceanobacillus* spp. and *A. rupiensis,* belonged to the *Bacillaceae* family.

In the second phase of this deliverable a heating based disinfection system, namely a closed coupled system [pilot scale treatment of rainwater samples directly from a domestic rainwater harvesting (DRWH) tank] was analysed for harvested rainwater treatment. The microbial parameters that were investigated during the pilot scale study included the enumeration of faecal and total coliforms, *Escherichia coli* and heterotrophic bacteria. In addition, the treated and untreated rainwater samples of the pilot scale study were screened for the presence of selected pathogenic bacteria and well as selected enteric viruses using molecular techniques. The chemical parameters that were investigated during the pilot scale study included the concentration of metal ions, anions and cations present in the treated and untreated harvested rainwater samples.

Chemical analysis indicated that all cations and anions present in both the unpasteurized and pasteurized water at all the varying temperature ranges were within the drinking water guidelines according to SANS 241 (SABS, 2005), DWAF (1996), ADWG (NHMRC and NRMMC, 2011) and WHO (2011), with the exception of iron, aluminium, lead and nickel. These four cations were shown to increase significantly ($p < 0.05$) after pasteurization at various temperatures and it is hypothesized that these elements could have leached from the stainless steel storage tanks of the pasteurization system. A company in Somerset West, Crest, is currently developing a solar pasteurization system with a storage tank that will be manufactured from Safrene^{TM}, which is a high density based polyethylene. It is therefore recommended that the storage tank of the pasteurization system be manufactured from an alternative material, such as a high grade polymeric material, which is able to withstand the high temperatures yet will not negatively influence the quality of harvested rainwater.

While heterotrophic bacteria still persisted at the 55 to 57ºC and 64 to 66ºC temperature ranges, total coliform, *E. coli* and heterotrophic bacteria counts were reduced to zero (100%) in the rainwater samples pasteurized at the 72 to 74˚C, 78 to 81˚C, and 90˚C to 91˚C temperature ranges (Table 4.10). It is therefore recommended that the minimum temperature required to treat harvested rainwater should be above 72ºC in order to utilise rainwater for drinking and domestic purposes. In addition, at the higher temperature ranges (78 to 81˚C, and 90˚C to 91˚C) pasteurized rainwater could safely be stored for to up to 7 days before bacterial re-growth occurred. The number of coliphages present in the unpasteurized and pasteurized harvested rainwater samples, were also below the detection limit throughout the study period.

Based on genus specific PCR analysis, utilised to screen pasteurized rainwater samples for the presence of pathogenic bacteria that have previously been detected in harvested rainwater (WRC K5_2125; Deliverable 2), no *Salmonella* spp. were detected in any of the rainwater samples analysed. However, while *Aeromonas* spp., *Klebsiella* spp., and *Shigella* spp. were not detected in the rainwater samples solar pasteurized at temperatures of higher than 72ºC, *Yersinia* spp., *Legionella* spp. and *Pseudomonas* spp. were detected in the rainwater samples pasteuriszd at the temperature ranges of 72 to 74˚C and 78 to 81˚C, while *Legionella* spp. and *Pseudomonas* spp. were still detected at the temperature range of 90 to 91ºC. In addition, adenovirus was detected in the domestic rainwater harvesting tank (WRC K5_2125; Deliverable 4), and PCR analyses clearly showed that solar pasteurization system was also not effective in removing this enteric virus (Adenovirus) from the pasteurized rainwater samples. It is however possible that the PCR assays used in this study detected the DNA of non-viable cells (both bacterial and virus PCR analysis), which is a major drawback when utilising PCR analysis. As noted previously by Ahmed at al. (2013), the consequences of insufficient and misleading analysis for the detection of bacterial pathogens could firstly lead to expensive treatment methods, that may not be assessed efficiently and render the quality of the water inadequate, and secondly, the water could wrongfully be deemed

inadequate which allows for overly restricted access to the water source. For this reason future studies will incorporate whole sample quantitative analysis, such as quantitative PCR (qPCR) and flow cytometry analysis (bacteria), in order to confirm the presence of viable pathogenic organisms within rainwater samples pasteurized at temperatures of greater than 72ºC.

The average temperature readings recorded during July to August (winter period) for the rainwater in the pasteurization system was 61ºC with a maximum temperature of 98ºC and a minimum temperature of 34ºC recorded. This data is important when determining the volume of rainwater that can be treated with the pasteurization system. It was determined that the solar pasteurization system could produce an average of 61.8 L/m^2 for each kWh of incident solar energy, for lower temperatures ranging from 56 - 58˚C and at higher temperatures (93 - 97˚C), the system could produce approximately 0.6 L/m^2 for each kWh of incident solar. Therefore throughout the year, including the winter months, large volumes of rainwater can be treated efficiently utilising solar pasteurization to produce water for drinking and domestic purposes.

CHAPTER 5: CONCLUSIONS & RECOMMENDATIONS

5.1 CONCLUSIONS

5.1.1 A survey of the quality of water collected in DRWH in the Kleinmond Housing Scheme

A survey of the quality of harvested rainwater was conducted in the Kleinmond Housing Scheme, situated in the Western Cape in 2012 (March to August). The chemical quality of the rainwater, in the domestic rainwater harvesting tanks sampled in the Kleinmond Housing Scheme, were within the guidelines as stipulated by the Drinking Water Specification 241 of the SANS (SABS, 2005), the South African Water Quality Guidelines for Domestic Water Use of the Department of Water Affairs and Forestry (DWAF, 1996), the Guidelines for Drinking-water Quality of the World Health Organisation (WHO, 2011) and the Australian Drinking Water Guidelines (NHMRC and NRMMC, 2011). Based on the microbial counts obtained on average for all the indicator organisms (including total coliforms, faecal coliforms and *Escherichia coli*), harvested rainwater, that has been stored in polyethylene tanks for a short period of time (> 1 year), is not suitable for drinking purposes as per standards stipulated by the DWAF (1996), the WHO (WHO, 2011) and the ADWG (NHMRC and NRMMC, 2011). According to the Spearman Rank Order correlations no significant correlation could be determined between COD and the microbiological indices ($p > 0.5$). However data sets showed significant correlations between the microbiological indicators and various metals and ions in the harvested rainwater samples ($p < 0.5$). Molecular typing also indicated that opportunistic pathogens and human pathogenic bacteria, such as *Aeromonas* spp., *Klebsiella* spp., *Salmonella* spp., various *Escherichia coli* strains, amongst many others, were isolated from the rainwater tanks. This study thus highlights the diverse array of pathogenic bacteria and the presence of pathogenic protozoa that occur in harvested rainwater during high rainfall periods. Similar observations were made for the rainwater in Hammanskraal in South Africa, where Nevondo and Cloete (1999) deemed the general quality of rainwater to be unacceptable. Other studies, world-wide, have also concluded that harvested rainwater is not suitable for drinking purposes without prior treatment (Yaziz et al,. 1989; Zhu et al,. 2004; Sazakli et al,. 2007).

5.1.2 Filter assessment – the efficiency of filtration systems in removing chemical and microbial contaminants from rainwater

The aim of this study was to monitor the effectiveness of filtration treatment systems in reducing the microbiological load of harvested rainwater. For this, three rainwater harvesting tanks were installed at the Welgevallen Experimental farm, Stellenbosch, South Africa. Four different systems of filtration were intermittently connected to the rainwater harvesting tanks installed at the Welgevallen Experimental farm, including two biological filtration systems, namely slow sand filtration and slow activated carbon filtration and two microfiltration systems, namely a polyvinyl (alcohol) (PVA) nanofibre membrane column with activated carbon and then the same system was tested without activated carbon.

Overall the results obtained for the chemical analysis of the slow activated carbon filtration system and the slow sand filtration system after biofilm formation indicated that the rainwater quality of unfiltered and filtered rainwater samples was within potable chemical standards in the filtered rainwater samples collected on the last day from each system. The total coliforms, *E. coli* and heterotrophic bacteria counts for all unfiltered and filtered rainwater samples using the slow activated carbon filtration systems and the slow sand filtration systems exceeded the guidelines stipulated by SANS 241 (SABS, 2005), the ADWG

(NHMRC and NRMMC, 2011) and DWAF (1996). Moreover, genus specific PCR based assays confirmed the presence of predominantly *Yersinia* spp., *Klebsiella* spp., *Pseudomonas* spp. and *Legionella* spp. in all unfiltered and in the filtrate of the slow sand filtration systems.

All cations and anions monitored in the rainwater samples collected before and after filtration through the activated carbon and nanofibre column were within the drinking water guidelines according to SANS 241 (SABS, 2005), DWAF (1996), ADWG (NHMRC and NRMMC, 2011) and WHO (2011). While the chemical quality of the rainwater was generally lower than the stipulated drinking water guidelines, the microbial quality of rainwater filtered through the PVA nanofibre system (without activated carbon) was inadequate according to drinking water guidelines stipulated by SANS 241 (SABS, 2005), DWAF (1996) and the ADWG (NHMRC and NRMMC, 2011) and could not be used for potable purposes. The addition of activated carbon to the PVA system may have provided an additional filtration barrier, but according to the microbial analysis performed, the system including the PVA nanofibre membrane and activated carbon could only be used for the filtration of one litre of rainwater before the components in the system need to be replaced. This was confirmed with the use of PCR based assays, as *Aeromonas* spp., *Klebsiella* spp. *Legionella* spp., *Pseudomonas* spp. and *Yersinia* spp. were detected in up five litres of filtered rainwater. Moreover, the average time required to filter one litre of rainwater through the PVA nanofibre system (without activated carbon) was approximately 37.48 minutes. The addition of activated carbon to the PVA nanofibre membrane filtration system then increased the filtration time, as the average time required to filter one litre of rainwater was 44.53 minutes.

This is also the first study known to detect the enteric virus, adenovirus, in rainwater harvesting tanks. The detection of specifically human adenovirus in the rainwater tanks is of great significance due to its high persistence and pathogenic nature (Vecchia et al., 2012) which poses a great threat to communities using DRWH as a potential potable water source. The route of transmission is most likely airborne originating from the tanks' surrounding environment. Furthermore, the filtration systems compared for the removal of adenovirus from potential potable water sources was not effective in producing water that meets the virological standards for drinking water and thus further research is needed to optimise these systems.

5.1.3 Solar pasteurization system – the efficiency of solar water pasteurization system in disinfecting water from domestic rainwater harvesting tanks

The aim of this study was to investigate the efficiency of a solar pasteurization system in treating harvested rainwater. Before the pasteurization pilot study, preliminary pasteurization experiments were performed in the laboratory and results indicated that overall, R2A agar was the most suited growth media for determining the recovery of heterotrophic bacteria from heat treated rainwater samples and was therefore used to assess the efficiency of the solar pasteurization system in the pilot scale study.

The solar pasteurization system was connected to one of the rainwater harvesting tanks that had been installed at the Welgevallen Experimental farm. Chemical analysis indicated that all cations and anions present in both the unpasteurized and pasteurized water were within the respective drinking water guidelines with the exception of iron, aluminium, lead and nickel. These four cations were shown to increase significantly after pasteurization at various temperatures and it is hypothesised that these elements could have leached from the stainless steel storage tanks of the pasteurization system. Microbial analysis indicated that rainwater samples pasteurized at 72°C and above (78 – 81ºC and 90 – 91ºC) could be utilised for potable purposes as total coliforms, *E. coli* and HPC were reduced to zero. However, PCR analysis of the pasteurized rainwater samples for the presence of pathogenic bacteria at the various temperature ranges showed that *Legionella* spp. and *Pseudomonas* spp. persisted even at the temperature range of 90 to 91ºC. The solar pasteurization system was ineffective in the removal of

enteric viruses from the harvested rainwater treated at 85°C and 90°C, and thus further research is needed to optimise these systems.

5.2 RECOMMENDATIONS

5.2.1 A survey of the quality of water collected in DRWH in the Kleinmond Housing Scheme

- The water in the domestic rainwater harvesting tanks in the Kleinmond Housing Scheme should not be used for potable and certain domestic purposes, without prior treatment.
- First flush diverters should be installed between the roof and the rainwater tank inlet to divert large amounts of debris which accumulates on the roof surfaces. This simple intervention could potentially significantly improve the microbial quality of the harvested rainwater.
- This study was conducted on rainwater samples harvested in a coastal area that experiences a winter rainfall and with limited point sources of chemical pollutants. To gain a complete perspective on the quality of harvested rainwater, it is thus suggested that future research should focus on the microbial and chemical quality of rainwater collected from inland, urban areas where the quality of the harvested rainwater may differ.

5.2.2 Filter assessment – the efficiency of filtration systems in removing chemical and microbial contaminants from rainwater

- The biological filtration systems need to be further analysed for an extended time period in order to effectively monitor the efficiency of a mature biofilm in the removal of pathogenic bacteria and certain contaminating chemicals. This is corroborated by literature as many studies have indicated that the biofilm used in biological filtration systems requires an extended time period to mature before it can provide any form of filtration.
- Future studies could include the quantification of the enteric viruses in harvested rainwater samples with the use of real time PCR reactions in order to determine whether a correlation exists between the concentration of the viruses present in the water samples before treatment and the efficiency of that specific treatment system. Culturing methods could also be employed to evaluate the number of infectious particles present in the water samples.

5.2.3 Solar pasteurization system – the efficiency of solar water pasteurization system in disinfecting water from domestic rainwater harvesting tanks

- The pasteurization system storage tank could be manufactured from an alternative material such as Safrene^{TM} (a high density based polyethylene) that will not allow for the leaching of metals into the rainwater at high temperatures. Future studies should thus investigate the effect of pasteurization on the chemical quality of rainwater using a pasteurization system that will not corrode during high temperature exposure.
- Although molecular techniques confirmed the presence of DNA of certain pathogenic bacteria, further studies are required to confirm the viability of specific bacterial pathogens at higher pasteurization temperatures. For this reason further studies are required to quantify and confirm

the presence of viable pathogenic organisms (bacteria and enteric viruses) in rainwater samples treated at temperatures greater than 72ºC.

- Of particular concern is the presence of *Legionella* spp. at such high temperatures. Previously this pathogen has been isolated from shower heads, whirlpools spas, cooling towers, air conditioning systems and humidifiers. Further studies should include the detection of viable *Legionella* cells as it is possible that the PCR assays used in this study detected the DNA of non-viable bacterial cells, which is a major drawback when utilising PCR analysis. As previously noted, misleading PCR results such as false positives or negatives could wrongfully deem the rainwater inadequate and this could lead to the installation of unnecessary expensive treatment systems. For this reason future studies should be conducted that will incorporate whole sample quantitative analysis, such as quantitative PCR (qPCR) and flow cytometry analysis using DNA probes specific for certain bacteria, in order to confirm the presence of viable pathogenic organisms within rainwater samples pasteurized at temperatures of greater than 72ºC.
- The effectiveness of the solar water pasteurization system for treating harvested rainwater should thus be monitored in a community (formal or informal settlement) as an alternative water supply. The investigation should include the following:
    - The social perception of implementing a solar water pasteurization system connected to a DRWH tank should be investigated in the earmarked community before such a study commences.
    - The design of the solar water pasteurization system connected to the DRWH should be optimised for optimal usage by the community members.
    - The effect of environmental factors on locally produced and utilised dwelling building materials on the chemical and microbial quality of the treated harvested rainwater should be analysed. The building material used in the preliminary solar pasteurization study could differ from other dwellings and could therefore have an impact on the quality of the treated harvested rainwater.
    - The operational sustainability and the capacity of the solar pasteurization system connected to a DRWH tank should be monitored.
    - One or two individuals in the community should be trained to supervise the major and continuous maintenance and repair of the DRWH tanks and the solar pasteurization treatment system.

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APPENDIX A:

CHAPTER 2

Table 1. List of the media used with corresponding colour reaction for each microorganism.

Medium	**Organism**	**Expected Cultural Colour Response**
m-Endo Agar	*Escherichia coli*	Green, metallic sheen
	Enterobacter aerogenes	Green, metallic sheen
	Salmonella typhimurium	Pink to red
ChromoCult® Coliform Agar	*Escherichia coli*	Dark-blue to violet
	Citrobacter freundii	Salmon to red
	Salmonella enteritidis	Colourless
m- FC Agar	Faecal coliforms	Shades of dark blue
Slanetz and Bartley Agar	*Enterococcus faecalis*	Red
Cetrimide Agar	*Pseudomonas aeruginosa*	Yellow-green to Blue-green/green
Salmonella Shigella Agar	*E. coli*	Slight growth, pink or red
	Enterobacter/Klebsiella spp.	Slight growth, pink
	Proteus spp.	Colourless, usually with black center
	Salmonella spp.	Colourless, usually with black center
	Shigella spp.	Colourless
	Pseudomonas spp.	Irregular, slight growth
Camplylobacter Blood-free Selective Medium	*Campylobacter* spp.	Pale to grey

Table 2. The concentration of anions present in the rainwater (n = 29) and control samples.

Sample number	Concentration of anions (mg/L)					
	Cl	**NO_3**	**SO_4**	**F**	**NO_2**	**PO_4**
1	19.9	1.9	8.8	ND	ND	ND
2	21.1	2	7.8	ND	ND	ND
3	24.4	1.4	12.5	ND	ND	ND
4	17.8	1.2	4.2	ND	ND	ND
5	20	1	5	ND	ND	ND
6	20.3	1.7	8.7	ND	ND	ND
7	16.7	1.6	3.7	ND	ND	ND
8	16.9	1.3	4	ND	ND	ND
9	16.9	1.1	3.9	ND	ND	ND
10	22.6	1.4	6.1	ND	ND	ND
11	26.3	1.6	15.8	ND	ND	ND
12	18.9	1.6	4.6	ND	ND	ND
13	25.4	1.4	9.1	ND	ND	ND
14	29.9	1.5	19.5	ND	ND	ND
15	17.9	1.4	4.8	ND	ND	ND
16	17.2	1.1	4.5	ND	ND	ND
17	18.9	1.5	5.6	ND	ND	ND
18	24	1.8	10.5	ND	ND	ND
19	21	1.2	6.2	ND	ND	ND
20	24.4	1.1	18.6	ND	ND	ND
21	18.8	1.3	5.2	ND	ND	ND
22	21.5	1.8	6.8	ND	ND	ND
23	20.5	1.6	4.4	ND	ND	ND
24	27	2.3	9.7	ND	ND	ND
25	19	2	5.6	ND	ND	ND
26	25.8	1.6	14.2	ND	ND	ND
27	18.8	1.3	5.6	ND	ND	ND
28	23.8	1.7	8.3	ND	ND	ND
29	19.2	1.8	6.9	ND	ND	ND
Control	24.7	ND	31.4	ND	ND	ND

Table 3. The concentration of metal ions present in the rainwater (n = 29) and control samples.

Sample	Concentration of metal ions (µg/L)									
	Al	**V**	**Cr**	**Mn**	**Fe**	**Co**	**Ni**	**Cu**	**Zn**	**As**
1	43.24	0.86	0.58	0.21	8.22	0.01	0.13	2.44	5.56	0.59
2	139.36	1.09	0.75	0.93	61.34	0.02	0.21	3.58	9.72	0.49
3	85.63	1.54	1.70	0.22	27.20	0.01	0.13	1.17	1.61	0.60
4	43.08	0.86	0.84	0.20	10.19	0.01	0.11	2.01	6.12	0.41
5	62.73	1.17	0.98	0.40	17.19	0.01	0.10	1.20	1.96	0.59
6	139.37	0.58	0.47	1.82	32.74	0.06	0.27	1.82	6.28	0.35
7	69.89	0.84	0.79	0.21	20.93	0.01	0.09	0.75	0.62	0.35
8	94.58	1.15	0.99	0.28	26.32	0.02	0.11	0.99	2.39	0.43
9	56.43	1.11	1.01	0.17	11.71	0.01	0.10	1.24	1.00	0.46
10	37.87	0.86	0.73	0.19	7.36	0.01	0.14	2.49	5.64	0.59
11	48.33	0.84	0.56	0.18	11.23	0.02	0.32	3.66	7.40	0.56
12	43.42	0.95	0.73	0.19	10.27	0.01	0.10	1.91	6.11	0.43
13	44.56	0.96	0.83	0.11	12.85	0.02	0.11	1.14	0.62	0.56
14	86.30	1.08	0.90	0.35	33.86	0.02	0.10	1.28	0.83	0.49
15	115.49	0.71	0.63	0.27	22.60	0.02	0.16	2.65	4.13	0.32
16	62.22	0.83	0.65	0.19	13.24	0.01	0.11	1.25	1.00	0.36
17	48.70	0.85	0.81	0.20	11.29	0.01	0.07	1.13	0.71	0.38
18	50.85	0.74	0.52	0.18	10.94	0.01	0.12	1.79	6.13	0.52
19	91.26	0.89	0.91	0.22	25.42	0.01	0.08	1.09	0.57	0.48
20	116.11	0.81	0.67	0.32	21.92	0.02	0.11	2.95	3.68	0.37
21	81.38	1.00	0.99	0.27	22.01	0.02	0.13	1.56	2.11	0.42
22	82.74	0.91	0.81	0.33	21.97	0.03	0.15	1.86	1.99	0.65
23	76.73	0.68	0.73	0.68	13.09	0.03	0.19	2.46	5.34	0.52
24	54.59	0.78	0.69	0.14	10.42	0.01	0.08	1.28	0.76	0.44
25	88.53	0.76	0.51	0.37	17.56	0.01	0.14	2.22	4.38	0.32
26	71.79	0.77	0.64	0.25	15.06	0.01	0.09	1.66	1.61	0.48
27	93.29	1.00	0.85	0.57	13.55	0.02	0.19	2.23	5.74	0.80
28	180.20	0.94	1.06	1.53	35.45	0.04	0.22	3.73	15.77	0.43
29	61.09	0.90	0.84	0.14	13.41	0.01	0.11	1.47	2.10	0.43
Control	1220.60	0.59	0.38	13.61	232.22	0.28	1.96	0.82	1.20	0.12

Table 3 (continued). The concentration of metal ions present in the rainwater (n = 29) and control samples.

Sample	Concentration of metal ions (µg/L)								
	Se	**Mo**	**Cd**	**Ba**	**Hg**	**Pb**	**Be**	**Li**	**Sn**
1	0.29	0.08	0.13	4.06	0.19	0.24	ND	ND	ND
2	0.28	0.14	0.41	5.32	0.17	0.30	ND	ND	ND
3	0.19	0.11	0.10	2.76	0.15	0.15	ND	ND	ND
4	0.45	0.09	0.16	4.18	0.13	0.10	ND	ND	ND
5	0.15	0.08	0.10	3.35	0.12	0.12	ND	ND	ND
6	0.40	0.06	0.12	4.86	0.11	0.22	ND	ND	ND
7	0.44	0.08	0.08	2.09	0.10	0.11	ND	ND	ND
8	0.22	0.10	0.06	4.21	0.09	0.18	ND	ND	ND
9	0.47	0.09	0.11	2.47	0.09	0.10	ND	ND	ND
10	0.32	0.08	0.14	4.07	0.08	0.13	ND	ND	ND
11	0.52	0.08	0.58	5.05	0.07	0.34	ND	ND	ND
12	0.48	0.10	0.18	4.36	0.07	0.20	ND	ND	ND
13	0.78	0.11	0.08	2.34	0.14	0.08	ND	ND	ND
14	0.40	0.10	0.10	2.58	0.10	0.12	ND	ND	ND
15	0.38	0.09	0.13	5.36	0.08	0.18	ND	ND	ND
16	0.79	0.07	0.14	2.14	0.06	0.15	ND	ND	ND
17	0.42	0.07	0.14	2.16	0.05	0.08	ND	ND	ND
18	0.44	0.07	0.13	4.75	0.04	0.15	ND	ND	ND
19	0.57	0.09	0.09	2.21	0.04	0.14	ND	ND	ND
20	0.61	0.08	0.18	4.75	0.04	0.15	ND	ND	ND
21	0.40	0.09	0.10	2.26	0.04	0.19	ND	ND	ND
22	0.25	0.09	0.14	2.27	0.03	0.24	ND	ND	ND
23	0.11	0.09	0.16	4.53	0.10	0.22	ND	ND	ND
24	0.42	0.06	0.14	2.34	0.04	0.14	ND	ND	ND
25	0.63	0.06	0.14	4.09	0.03	0.15	ND	ND	ND
26	0.34	0.06	0.08	1.95	0.03	0.14	ND	ND	ND
27	0.49	0.06	0.27	5.99	0.03	0.19	ND	ND	ND
28	0.40	0.08	0.14	7.35	0.03	0.39	ND	ND	ND
29	0.31	0.07	0.10	2.54	0.03	0.31	ND	ND	ND
Control	0.22	0.02	0.01	3.71	0.07	0.05	7.31	ND	ND

Table 4. The concentration of cations present in the rainwater (n = 29) and control samples.

Sample	Concentration of cations (µg/L)							
	Ba	**Ca**	**K**	**Mg**	**Na**	**P**	**Si**	**Sr**
1	0.04	11.32	2.21	1.68	15.39	0.03	3.03	0.14
2	0.08	13.46	3.72	1.85	19.10	0.03	3.87	0.18
3	0.02	10.60	6.31	1.30	15.00	0.03	4.98	0.15
4	0.03	10.98	3.15	1.66	16.72	0.02	3.29	0.14
5	0.02	11.31	3.94	1.46	14.30	0.03	4.10	0.16
6	0.07	11.77	1.83	1.94	19.91	0.02	2.79	0.10
7	0.02	10.80	2.71	1.45	13.47	0.01	2.94	0.13
8	0.03	11.29	3.42	1.44	14.32	0.03	3.79	0.17
9	0.02	10.94	3.43	1.47	14.06	0.02	3.34	0.17
10	0.04	11.73	2.07	1.78	16.59	0.02	3.27	0.13
11	0.09	12.96	1.78	1.91	18.19	0.02	3.36	0.13
12	0.02	11.20	2.88	1.59	14.89	0.02	3.07	0.15
13	0.02	11.70	2.36	1.48	13.16	0.02	4.04	0.17
14	0.05	12.33	2.59	1.60	15.08	0.02	4.02	0.16
15	0.05	11.98	1.77	1.95	17.90	0.02	3.10	0.12
16	0.02	10.80	1.75	1.37	11.81	0.02	3.32	0.14
17	0.03	11.69	2.01	1.52	13.48	0.02	3.56	0.15
18	0.03	11.92	2.21	1.81	17.70	0.02	2.83	0.13
19	0.02	11.47	2.81	1.47	13.57	0.01	3.43	0.15
20	0.03	11.73	1.96	1.62	15.55	0.02	3.29	0.14
21	0.02	11.21	2.83	1.39	13.06	0.02	3.40	0.12
22	0.02	11.09	2.65	1.36	13.09	0.01	3.40	0.14
23	0.04	11.90	2.38	1.60	14.41	0.02	3.01	0.13
24	0.02	10.83	2.57	1.33	12.21	0.01	3.13	0.16
25	0.07	11.05	2.01	1.47	14.07	0.02	2.96	0.13
26	0.02	10.52	2.47	1.31	12.10	0.02	2.90	0.15
27	0.18	14.19	2.14	2.15	22.27	0.02	4.27	0.14
28	0.02	11.89	2.99	2.11	21.33	0.03	3.31	0.12
29	0.02	11.28	2.64	1.45	12.93	0.01	3.48	0.15
Control	0.01	12.14	0.69	2.27	22.01	0.01	1.52	0.05

Table 5. The pH level of the rainwater samples (n = 29).

Sample	Sampling						
	2	**3**	**4**	**5**	**6**	**7**	**8**
1	6	5.5	5.5	5.5	5	5	5
2	6	5.5	5.6	5.6	5	5	5
3	6	5.5	5	5	6	5	5
4	6	5.5	6	6	5	5	5
5	6.5	5.5	5.5	5.5	5	5	5
6	6	5.5	5	5	5	5	5
7	6	5.5	5.5	5.5	5	5	5
8	6	5.5	5	5	5	5	5
9	6	5.5	5.5	5.5	5	5	5
10	6	5.5	5	5	5	5	5
11	6	5.5	5	5	6	5	5
12	6	5.25	5.5	5.5	5	5	5
13	6	5.5	5.6	5.6	5	5	5
14	6	5.5	5.5	5.5	5	5	5
15	6	5.5	5.5	5.5	6	5	5
16	6	5.5	6	6	5.5	5	5.5
17	6	5.5	5.5	5.5	4.5	5	5
18	6	5.5	6	6	6	5	5.5
19	6	5.5	5	5	6	5	5.5
20	6	5.5	5.5	5.5	5	5	5
21	6	5.5	5.5	5.5	5	5	6
22	6	5.5	5	5	4.5	5	5
23	6	5.5	5	5	5	5	5
24	6	5.5	5.5	5.5	5	5	5
25	6	5.5	5.5	5.5	5	5	5
26	6	5.5	5.5	5.5	5	5	5
27	6	5.5	5	5	5	5	5.5
28	6	5.5	5	5	5	5	5
29	6	5.5	5.5	5.5	5	5	6

Table 6. The temperature of the rainwater samples (n = 29).

Sample	Sampling						
	2	**3**	**4**	**5**	**6**	**7**	**8**
1	24	20	13	13	14	13	11
2	24	21	15	15	14	13	14
3	25	20	13	13	14	12	11
4	23	20	15	15	13	12	14
5	25	18	17	17	15	11	12
6	24	20	14	14	15	13	14
7	24	19	15	15	15	14	14
8	25	20	16	16	14	13	14
9	25	20	15	15	14	12	14
10	25	20	13	13	15	13	15
11	23.5	20	14	14	14	13	15
12	24	22	13	13	13	13	13
13	23	20	16	16	16	13	14
14	24.5	20	17	17	14	13	15
15	24	19	15	15	14	12	14
16	24	20	14	14	15	13	14
17	24	18	14	14	15	12	13
18	24	20	15	15	13	13	13
19	23	21	16	16	15	13	15
20	24	19	13	13	13	13	14
21	25	19	13	13	14	13	14
22	24	20	15	15	14	13	14
23	22	18	16	16	14	12	13
24	24	20	17	17	15	12	15
25	23	19	13	13	13	11	12
26	24	19	13	15	15	13	14
27	23	18	18	18	14	12	13
28	24	20	17	17	16	13	13
29	24	20	14	14	14	12	13

Table 7. Various organisms isolated during sampling one from *Salmonella Shigella* Agar (SS Agar).

Accession Number	Organism	Query of Coverage Sequence	Similarity of BLAST search
NZ_AKJX01000236.1	*Acidovorax* sp.	95%	92%
NZ_CACP01000004.1	*Aeromonas caviae*	90%	99%
AJXP01000035.1	*Enterobacter cloacae* subsp. *cloacae* GS1	99%	98%
NZ_ACIS01000017.1	*Lutiella nitroferrum*	90%	91%
AKCM01000008.1	*Pseudomonas aeruginosa*	99%	95%
NZ_CAIG01000019.1	*Pseudomonas pseudoalcaligenes*	100%	97%
AGCS01000013.1	*Pseudomonas putida* B6-2	98%	96%
NZ_ABAK02000001.1	*Salmonella enterica* subsp. *enterica serovar*	100%	94%
NZ_ADBX01000007.1	*Serratia odorifera*	94%	98%
AKNF01000069.1	*Shigella flexneri*	99%	98%
AGQO01000102.1	*Yersinia enterocolitica subsp. palearctica*	99%	97%
NZ_ACCD01000071.1	*Yersinia rohdei* ATCC 43380	96%	95%

Table 8. Organisms isolated during sampling two from *Salmonella Shigella* Agar (SS Agar).

Accession Number	Organism	Query Coverage	Max identity
NZ_CACP01000004.1	*Aeromonas caviae*	99%	98%
AGDP01000012.1	*Klebsiella oxytoca*	99%	98%

Table 9. Various organisms isolated during sampling three from *Salmonella Shigella* Agar (SS Agar).

Accession Number	Organism	Query Coverage	Max identity
NZ_ADMS01000149.1	*Achromobacter piechaudii* ATCC 43553	99%	99%
NZ_AKJX01000236.1	*Acidovorax* sp. CF316	85%	93%
NZ_CACP01000004.1	*Aeromonas caviae* Ae398	86%	99%
AHIL01000001.1	*Comamonas testosteroni* ATCC 11996	99%	95%
AJXP01000037.1	*Enterobacter cloacae* subsp. *cloacae* GS1	99%	99%
AIGY01000044.1	*Escherichia coli* DEC11D	100%	95%
AKCF01000001.1	*Klebsiella oxytoca*	99%	99%
NZ_ABVP01000020.1	*Proteus penneri* ATCC 35198 P	94%	97%
AGQO01000102.1	*Yersinia enterocolitica* subsp. *palearctica*	99%	97%
NZ_ACCD01000071.1	*Yersinia rohdei* ATCC	100%	98%

Table 10. Various organisms isolated during sampling four from *Salmonella Shigella* Agar (SS Agar).

Accession Number	Organism	Query Coverage	Max identity
NZ_CACP01000004.1	*Aeromonas caviae*	85%	99%
AGVO01000139.1	*Aeromonas salmonicida* subsp. *salmonicida*	93%	98%
AJXP01000037.1	*Enterobacter cloacae* subsp. *cloacae*	99%	97%
AGDM01000001.1	*Klebsiella oxytoca*	98%	97%
NZ_ABJD02000103.1	*Providencia stuartii* ATCC 25827	98%	95%
AEAJ01000992.1	*Pseudomonas syringae*	90%	98%
Z_ABEH02000008.1	*Salmonella enterica* subsp. *enterica serovar* Javiana	98%	99%
AJUV01000032.1	*Serratia marcescens*	86%	97%

Table 11. Various organisms isolated during sampling five from *Salmonella Shigella* Agar (SS Agar).

Accession Number	Organism	Query Coverage	Max identity
NZ_CACP01000004.1	*Aeromonas caviae*	92%	98%
AGVO01000139.1	*Aeromonas salmonicida* subsp. *salmonicida*	98%	93%
NZ_ACKQ02000003.1	*Chryseobacterium gleum* ATCC 35910	97%	97%
NZ_ADLG01000006.1	*Citrobacter freundii*	99%	96%
AJXP01000037.1	*Enterobacter cloacae* subsp. *cloacae* GS1	98%	96%
NZ_AFHR01000079.1	*Enterobacter hormaechei* ATCC 49162	99%	96%
NZ_AEXB01000008.1	*Enterobacter mori* LMG	92%	94%
AIGW01000043.1	*Escherichia coli* DEC11B	98%	96%
AGDI01000022.1	*Klebsiella oxytoca*	99%	97%
AFQK01000047.1	*Klebsiella pneumoniae* JH1	94%	94%
ALJX01000053.1	*Morganella morganii* subsp. morganii	98%	97%
NZ_ACLE01000013.1	*Proteus mirabilis* ATCC 29906	96%	96%
NZ_ABVP01000020.1	*Proteus penneri* ATCC 35198	95%	97%
NZ_ABXW01000071.1	*Providencia alcalifaciens*	93%	96%
NZ_ABJD02000103.1	*Providencia stuartii* ATCC 25827	92%	97%
NZ_ABEH02000001.1	*Salmonella enterica* subsp. *enterica serovar* Javiana	93%	99%
NZ_ABFH02000001.1	*Salmonella enterica* subsp. *enterica serovar* Virchow	96%	99%
NZ_AAYR01000007.1	*Yersinia pestis biovar* Antiqua	86%	94%

Table 12. Various organisms isolated during sampling six from *Salmonella Shigella* Agar (SS Agar).

Accession Number	Organism	Query Coverage	Max identity
AB698740.1	*Aeromonas hydrophila*	95%	98%
NZ_ADLG01000006.1	*Citrobacter freundii*	97%	98%
AGDP01000012.1	*Klebsiella oxytoca*	99%	98%
NZ_ABXW01000071.1	*Providencia alcalifaciens* DSM 30120	98%	97%
NZ_AHIP01000073.1	*Pseudomonas extremaustralis* 14-3	97%	98%
GU198116.1	*Pseudomonas fluorescens*	98%	97%
AKCL01000048.1	*Pseudomonas putida* SJTE	100%	95%
AB681874.1	*Serratia fonticola* 16S	96%	98%
NZ_ABCD01000005.1	*Yersinia pestis* CA88-4125	94%	97%

Table 13. Various organisms isolated during sampling seven from *Salmonella Shigella* Agar (SS Agar).

Accession Number	Organism	Query Coverage	Max identity
NZ_CACP01000004.1	*Aeromonas caviae*	96%	99%
AJXP01000037.1	*Enterobacter cloacae* subsp. *cloacae GS1*	99%	98%
AGDP01000012.1	*Klebsiella oxytoca*	98%	97%
ALJC01000005.1	*Pseudomonas psychrophila*	99%	97%
AJXI01000282.1	*Pseudomonas syringae pv. tabaci str. 6605*	100%	97%

Table 14. Organisms isolated during sampling three from *Legionella* CYE Agar base.

Accession Number	Organism	Query Coverage	Max identity
NZ_CACP01000004.1	*Aeromonas caviae*	96%	96%
AGDM01000015.1	*Klebsiella oxytoca*	99%	97%
NZ_ACCI02000101.1	*Providencia rettgeri* DSM	94%	98%
AHTR01000039.1	*Salmonella enterica* subsp. *Entericaserovar*	99%	97%
AJUV01000032.1	*Serratia marcescens*	92%	99%

Table 15. Organisms isolated during sampling six from *Legionella* CYE Agar base.

Accession Number	Organism	Query Coverage	Max identity
AB698740.1	*Aeromonas hydrophila*	98%	96%
AB680670.1	*Pseudomonas fluorescens*	98%	97%
NZ_AHPP01000001.1	*Pseudomonas synxantha* BG33R	99%	97%
CAKR01000050.1	*Yersinia massiliensis* CCUG	98%	96%
NZ_ACCD01000071.1	*Yersinia rohdei* ATCC 43380	100%	97%

Table 16. Organisms isolated during sampling four from *Campylobacter* Blood-free Selective Medium.

Accession Number	Organism	Query Coverage	Max identity
NZ_CACP01000004.1	*Aeromonas caviae*	95%	95%
NZ_ACQA01000001.1	*Ochrobactrum intermedium*	98%	98%

Table 17. Organisms isolated during sampling five from *Campylobacter* Blood-free Selective Medium.

Accession Number	Organism	Query Coverage	Max identity
NZ_AFQY01000001.1	*Acinetobacter lwoffii*	88%	84%
FJ860877.1	*Acinetobacter* spp.	100%	98%
ALOP01000231.1	*Brucella abortus* BCB034	95%	96%
AB680315.1	*Comamonas terrigena*	98%	96%
NZ_AEBT01000061.1	*Enterococcus faecalis* DAPTO 512	96%	97%
NZ_ACQA01000001.1	*Ochrobactrum intermedium*	98%	100%
AFOX01000032.1	*Paenibacillus polymyxa* ATCC 842	94%	93%
NZ_AHIP01000073.1	*Pseudomonas extremaustralis*	99%	98%
AHPN01000001.1	*Pseudomonas fluorescens* SS101	99%	97%

Table 18. Organisms isolated during sampling six from *Campylobacter* Blood-free Selective Medium.

Accession Number	Organism	Query Coverage	Max identity
FJ860877.1	*Acinetobacter* spp.	98%	95%
ALOP01000231.1	*Brucella abortus* BCB034	82%	84%
AKNI01000039.1	*Escherichia coli* EPEC C342-62	87%	81%
AFVD01000035.1	*Escherichia coli* O104:H4	98%	100%

Table 19. *Escherichia coli* strains isolated during sampling one to three using ChromoCult® Coliform Agar and MLGA, m-ENDO agar.

Sampling 1		Sampling 2		Sampling 3		Sampling 4	
Organism	**Accession number**	**Organism**	**Accession number**	**Organism**	**Accession number**	**Organism**	**Accession number**
Escherichia coli SE11 DNA, complete genome	AP009240.1	*Escherichia coli* O157:H7 partial 16S rRNA gene, strain WAB1892	AM184233.1	*Escherichia coli* O111:H- str. 11128 DNA, complete genome	AP010960.1	*Proteus vulgaris* strain BD2_1A 16S ribosomal RNA gene	JN644538.1
Escherichia coli W, complete genome	CP002967.1	*Escherichia coli* O157:H7 partial 16S rRNA gene, strain WAB1892	AM184233.1	*Escherichia coli* ETEC H10407, complete genome	FN649414.1	*Escherichia coli* strain 6 16S ribosomal RNA gene, partial sequence	JQ907530.1
Escherichia coli W, complete genome	CP002967.1	*Enterobacter hormaechei* subsp. *Steigerwaltii* 16S rRNA gene, type strain EN-562T	AJ853890.1	*Escherichia coli* W, complete genome	CP002967.1	*Proteus vulgaris* strain YRR06 16S ribosomal RNA gene	EU373433.1
Escherichia sp. B4 16S ribosomal RNA gene, partial sequence	EU722735.1			*Enterobacter cloacae* subsp. Cloacae partial 16S rRNA gene, type strain DSM 30054T	HE978272.1	*Escherichia coli* Xuzhou21, complete genome	CP001925.1
Escherichia coli partial 16S rRNA gene, strain DSM 1103	HE978270.1			*Escherichia coli* strain 3 16S ribosomal RNA gene, partial sequence	JQ907529.1	*Escherichia coli* partial 16S rRNA gene, strain DSM 1103	HE978270.1
Escherichia coli strain c163 16S ribosomal RNA gene, partial sequence	JQ781645.1			*Escherichia coli* APEC O1, complete genome	CP000468.1	*Escherichia coli* partial 16S rRNA gene, strain DSM 1104	HE978270.2
Escherichia coli strain 7 16S ribosomal RNA gene, partial sequence	JQ907519.1			*Escherichia coli* strain 7 16S ribosomal RNA gene, partial sequence	JQ907519.1	*Escherichia coli* partial 16S rRNA gene, strain DSM 1103	HE978270.1
Escherichia coli NA114, complete genome	CP002797.2			*Escherichia coli* strain 214 16S ribosomal RNA gene, partial sequence	JN180970.1	*Escherichia coli* BW2952, complete genome	CP001396.1
Escherichia coli strain 7 16S ribosomal RNA gene, partial sequence	JQ907519.1			*Escherichia coli* strain B13 16S ribosomal RNA gene, partial sequence	JN129480.1	*Escherichia coli* Xuzhou21, complete genome	CP001925.1
Escherichia coli strain 174 16S ribosomal RNA gene, partial sequence	JN180968.1			*Escherichia coli* O111:H- str. 11128 DNA, complete genome	AP010960.1	*Escherichia coli* Xuzhou21, complete genome	CP001925.1
Escherichia coli W, complete genome	CP002967.1			*Escherichia coli* strain Y31 16S ribosomal RNA gene, partial sequence	JN578646.1	*Escherichia coli* W, complete genome	CP002967.1
Escherichia coli ETEC H10407, complete genome	FN649414.1			*Escherichia coli* strain Y31 16S ribosomal RNA gene, partial sequence	JN578646.1	*Escherichia coli* W, complete genome	CP002967.2
Escherichia coli ETEC H10407, complete genome	JQ907528.1			*Escherichia coli* O111:H- str. 11128 DNA, complete genome	AP010960.1		

Table 20. *Escherichia coli* strains isolated during sampling five to seven using ChromoCult® Coliform Agar and MLGA, m-ENDO agar.

Sampling 5		Sampling 6		Sampling 7	
Organism	**Accesion number**	**Organism**	**Accesion number**	**Organism**	**Accesion number**
Serratia sp. OX11a_S5 16S ribosomal RNA gene, partial sequence	JF274793.1	*Escherichia coli* partial 16S rRNA gene, strain DSM 1103	HE978270.1	*Escherichia coli* Xuzhou21, complete genome	CP001925.1
Escherichia coli BW2952, complete genome	CP001396.1	*Enterobacteriaceae* bacterium 12 16S ribosomal RNA gene, partial sequence	JN613161.1	*Escherichia coli* BW2952, complete genome	CP001396.1
Escherichia coli BW2952, complete genome	CP001396.1	*Escherichia coli* strain sch69 16S ribosomal RNA gene, partial sequence	JX294889.1	*Escherichia coli* P12b, complete genome	CP002291.1
Escherichia coli O7:K1 str. CE10, complete genome	CP003034.1	*Escherichia coli* partial 16S rRNA gene, strain DSM 1103	HE978270.1	*Escherichia coli* partial 16S rRNA gene, strain DSM 1103	HE978270.1
Escherichia coli strain BJLsh5 16S ribosomal RNA gene, partial sequence	HQ857759.1	*Escherichia coli* partial 16S rRNA gene, strain DSM 1103	HE978270.1	*Escherichia coli* BW2952, complete genome	CP001396.1
Shigella boydii CDC 3083-94, complete genome	CP001063.1	*Escherichia coli* strain sch70 16S ribosomal RNA gene, partial sequence	JX294890.1	*Escherichia coli* partial 16S rRNA gene, strain DSM 1103	HE978270.1
Escherichia coli partial 16S rRNA gene, strain DSM 1103	HE978270.1	*Escherichia coli* partial 16S rRNA gene, strain DSM 1103	HE978270.1	*Escherichia coli* BW2952, complete genome	CP001396.1
Escherichia coli O111:H- str. 11128 DNA, complete genome	AP010960.1	*Escherichia coli* partial 16S rRNA gene, strain DSM 1103	HE978270.1	*Shigella* sp. AB5221 16S ribosomal RNA-like gene, partial sequence	GU366033.1
Escherichia coli partial 16S rRNA gene, strain DSM 1103	HE978270.1	*Shigella flexneri* strain VITRAMJ 16S ribosomal RNA gene, partial sequence	JX307691.1	*Escherichia coli* Xuzhou21, complete genome	CP001925.1
Escherichia coli partial 16S rRNA gene, strain DSM 1103	HE978270.1	*Escherichia coli* partial 16S rRNA gene, strain DSM 1103	HE978270.1	*Escherichia coli* partial 16S rRNA gene, strain DSM 1103	HE978270.1
Escherichia coli O7:K1 str. CE10, complete genome	CP003034.1	*Escherichia coli* partial 16S rRNA gene, strain DSM 1103	FN649414.1	Shigella boydii CDC 3083-94, complete genome	CP001063.1
Escherichia coli Xuzhou21, complete genome	CP001925.1	*Escherichia coli* partial 16S rRNA gene, strain DSM 1103	HE978270.1	Escherichia coli partial 16S rRNA gene, strain DSM 1103	HE978270.1
Escherichia coli strain skg007 16S ribosomal RNA gene, partial sequence	HQ286917.1	*Escherichia coli* strain BJLsh5 16S ribosomal RNA gene, partial sequence	HQ857759.1	Escherichia coli Xuzhou21, complete genome	CP001925.1
Escherichia coli partial 16S rRNA gene, strain DSM 1103	HE978270.1	*Escherichia coli* partial 16S rRNA gene, strain DSM 1103	HE978270.1	Shigella sp. AB5221 16S ribosomal RNA-like gene, partial sequence	GU366033.1
		Escherichia coli Xuzhou21, complete genome	CP001925.1	Escherichia coli partial 16S rRNA gene, strain DSM 1103	HE978270.1
		Escherichia coli O111:H- 16S ribosomal RNA gene, partial sequence	GU237022.1		
		Escherichia coli partial 16S rRNA gene, strain DSM 1103	HE978270.1		
		Escherichia coli strain sch69 16S ribosomal RNA gene, partial sequence	JX294889.1		

APPENDIX B:

CHAPTER 4 AND 5

Table 1. Evaluation of Adenovirus in Rainwater.

Sample Source	Description	Identity	Accession number
JC Smuts tank	Human adenovirus 40 strain M-364 structural protein gene, partial cds	92%	HQ268775.1
Tank A.2	Bovine adenovirus 3 strain HLJ0955	95%	JN381195.1
Tank B.1	Bovine adenovirus 3, complete genome	98%	AF030154.1
Tank B.2	Human adenovirus 40 strain M-364 structural protein gene, partial cds	92%	HQ268775.1
Tank C	Simian adenovirus B isolate BaAdV-1, complete genome	97%	KC693021.1

Table 2. Treatment Methods for Rainwater.

Treatment Method	Sample	Description	Identity	Accession number
PVA Nanofiber membrane and Activated Carbon	Before	Bovine adenovirus 3, complete genome	98%	AF030154.1
PVA Nanofiber membrane and Activated Carbon	After	Bovine adenovirus 3, complete genome	98%	AF030154.1
Slow Sand Filtration	Before	Bovine adenovirus 3 strain HLJ0955, complete genome	100%	JN381195.1
Slow Sand Filtration	After	Bovine adenovirus 3 strain HLJ0955, complete genome	98%	JN381195.1
Solar Pasteurisation	Before	Bovine adenovirus 3 strain HLJ0955	96%	JN381195.1
Solar Pasteurisation	After	Bovine adenovirus 3 strain HLJ0955	96%	JN381195.1

Table 3. PCR assays for the detection of various commonly found bacteria genera detected in untreated and pasteurized rainwater samples.

Sample temperature (˚C)		Organism						
		***Aeromonas* spp.**	***Klebsiella* spp.**	***Legionella* spp.**	***Pseudomonas* spp.**	***Salmonella* spp.**	***Shigella* spp.**	***Yersinia* spp.**
55	B	-	+	+	+	-	-	-
	A1	-	-	+	+	-	-	-
	A2	-	-	+	+	-	+	+
56	B	+	+	+	-	-	+	-
	A1	-	-	+	-	-	-	-
	A2	-	-	+	-	-	-	-
57	B	+	+	+	+	-	-	+
	A1	-	+	+	+	-	-	-
	A2	-	-	+	-	-	-	-
64	B	-	+	+	+	-	-	-
	A1	-	+	+	+	-	-	-
	A2	-	+	+	+	-	-	-
65	B	+	+	+	+	-	-	+
	A1	-	+	+	-	-	-	+
	A2	-	-	+	-	-	-	-
66	B	-	-	-	+	-	-	-
	A1	-	-	+	-	-	-	-
	A2	-	-	+	-	-	-	-
72	B	-	-	+	-	-	-	-
	A1	-	-	+	-	-	-	-
	A2	-	-	+	-	-	-	-
73	B	-	-	+	+	-	-	+
	A1	-	+	+	+	-	-	-
	A2	-	-	+	+	-	-	-
74	B	-	-	+	-	-	-	-
	A1	-	-	+	+	-	-	-
	A2	-	-	+	-	-	-	-
78	B	-	-	-	-	-	-	-
	A1	-	-	+	-	-	-	+
	A2	-	-	+	-	-	-	+
81	B	-	+	+	+	-	-	+
	A1	-	-	+	-	-	-	-
	A2	-	-	+	-	-	-	-
81[2nd]	B	-	-	+	+	-	-	-
	A1	-	-	+	-	-	-	-
	A2	-	-	+	+	-	-	-
90	B	-	-	+	-	-	-	-
	A1	-	-	+	+	-	-	-
	A2	-	-	+	+	-	-	-

91	B	-	+	+	+	-	-	-
	A1	-	-	+	+	-	-	-
	A2	-	-	+	+	-	-	-
91^{2nd}	B	-	-	+	+	-	-	-
	A1	-	-	+	+	-	-	-
	A2	-	-	+	-	-	-	-