ASSESSMENT OF THE MICROBIAL QUALITY OF VARIOUS DOMESTIC RAINWATER HARVESTING SYSTEMS AND THE SUITABILITY OF A NANO BASED TREATMENT METHOD

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ABSTRACT

In most developing countries, people from rural and peri-urban settlements depend on harvested rainwater (HRW) as an alternative water source for drinking and other household purposes. Despite this reliance, there is little monitoring of the microbial quality of HRW in these areas. The most important issue in relation to using untreated harvested rainwater for drinking and other domestic purposes is the potential public health risks associated with microbial pathogens. Unlike chemical contamination, microbial contamination my lead to disease occurring rapidly, hence the need for frequent monitoring. Thus, the current study investigated the microbial quality of various domestic rainwater harvesting systems and the suitability of a nano based treatment method. The first experiments involved determining the microbial (*Escherichia coli*) and physicochemical quality (pH, turbidity, nitrate and chemical oxygen demand (COD)) of HRW in the Eastern Cape Province, South Africa. Samples were collected from 11 tanks situated at the Rhodes University, Kenton-on-sea (coastal) and in homes in the Grahamstown area on a weekly basis between June and September 2016. The Colilert-18®/Quanti-tray® 2000 system was used for enumeration of *E. coli* while physicochemical parameters were measured using commercial kits. Results showed that all samples were contaminated with varying concentrations of *E. coli* ranging from 7 to 1055 MPN/100 mL. Physicochemical analysis revealed that pH ranged from 5.6 to 7.6 and Turbidity values obtained for all tanks were below 5 NTU except for tank 4 (5.12 ± 4.96 NTU) and 7 (5.58 ± 8.19 NTU). Nitrate levels (range: 5.95 to 28.12 mg L^{-1}) and COD (range: 66.53 to 191.12 mg L^{-1}) were higher than the recommended South African drinking water quality guidelines in most of the tanks. In the second experiments, the objective was to determine whether a modified hydrogen sulphide (H_2S) test kit with an improved detection rate is an effective preliminary screening qualitative test that can be used for rainwater quality monitoring. The hydrogen sulphide method is a low-cost microbiological field-based test which can be used in areas where water testing facilities are limited. Harvested rainwater samples were collected from various tanks in the Eastern Cape and tested for contaminants of faecal origin using the modified hydrogen sulphide test kit, Colilert-18/Quanti-tray®/2000 and membrane filtration technique. Faecal coliforms were measured using membrane filtration, *E. coli* was measured using Colilert and correspondence rates were calculated with results of the improved hydrogen sulphide test kit. *E. coli* results ranged from <1 – >2419.6 MPN/100 mL while the faecal coliforms ranged from 0 – >300 CFU/mL. The agreement rate with hydrogen sulphide test and membrane filtration was 88% while the agreement rate for the Colilert and hydrogen sulphide test was 76%. The third experiments investigated the prevalence of pathogenic *E. coli* strains and their antimicrobial resistance patterns in HRW tanks in the Eastern Cape, South Africa. *E. coli* isolates obtained in the first experiments were further screened for their virulence potentials using polymerase chain reaction (PCR) and subsequently tested for

antibiotic resistance using the disc-diffusion method against 11 antibiotics. The pathotype most detected was the neonatal meningitis *E. coli* (NMEC) (*ibeA* 28%) while pathotype enteroaggregative *E. coli* (EAEC) was not detected. The highest resistance of the *E. coli* isolates was observed against Cephalothin (76%). All tested pathotypes were susceptible to Gentamicin, and 52% demonstrated multiple-antibiotic resistance (MAR). The fourth experiments shed light on the occurrence of *Legionella*, zoonotic and fungal pathogens in the rainwater harvesting systems (RWHS) situated in different regions of South Africa. Rainwater samples were collected in urban and semi-urban areas from tanks situated in various areas in South Africa (Johannesburg, Pretoria and Grahamstown). Pathogenic organisms investigated were *Salmonella, Shigella, Vibrio cholerae, Legionella* and fungal isolates. Pure isolates were obtained and screened using PCR. Results revealed the presence of pathogenic bacteria and fungi in all the tested RWHS. In Grahamstown the most detected pathogen was *Salmonella* (73%) while *Vibrio Cholerae* was not detected. All the tested pathogens were present from the RWHS situated in Pretoria. *Shigella* was not detected from the RWHS in Johannesburg while others were detected. Identification of fungal isolates from HRW showed the presence of pathogenic fungi such as *Aspergillus fumigatus, Cryptococcus laurentii, Aureobasidium pullulans* and *Mucor circinelloides.* The last experiments, focussed on exploring a suitable treatment method for HRW where a nano compound quaternary imidazolium modified montmorillonite (MMT) was used as a potential household rainwater treatment option. Harvested rainwater samples were collected from the RWHS situated at the Council for Scientific and Industrial Research (CSIR), Pretoria South Africa. River and borehole water samples were included in the study to check the efficiency of the treatment method on various water sources. River water samples were collected from Olifants River, Witbank, South Africa while borehole water was collected from a privately-owned borehole in Pretoria. For inoculation studies, all the water sources were sterilised in batches of 1 and 2 L and inoculated with approximately 10^7 CFU/mL of overnight *E. coli*. Approximately 200 mg of the quaternary imidazolium modified MMT was added to the inoculated water and samples collected immediately after inoculation (time 0) and thereafter every hour for 5 hrs. The analyses were further conducted using unsterilised water samples (total bacterial count) and 500 mg of the treatment material. Complete inactivation of *E. coli* in sterilised HRW was achieved in 2 hrs for the 2 L water samples and 3 hrs for the 1 L water samples. Sterilised river water achieved complete *E. coli* inactivation in 4 hrs for the 1 L and 5 hrs for the 2 L samples while borehole water samples achieved complete *E. coli* inactivation in 5 hrs (2 L) and 6 hrs for the 1 L samples. In the unsterilised water sources (total bacteria), complete bacterial inactivation was observed in 5 hrs for both the 1 and 2 L harvested rainwater samples, 6 hrs in river water samples (both 1 and 2 L) and 8 hrs for borehole water samples (1 and 2 L). The results suggest that the treatment option was more efficient in harvested rainwater (required less time for bacterial inactivation compared to river and borehole water). The results of the current study are of public health concern since the use of untreated HRW for potable purposes may pose a risk of transmission of pathogenic and antimicrobial-resistant *E. coli* and other pathogenic organisms such as *Salmonella, Shigella* and *Vibrio*

cholerae. It is therefore recommended that in cases where the tested harvested rainwater is used for potable purposes, simple treatment methods such as boiling and SODIS be applied so the harvested rainwater is fit for human consumption.

Keywords: *Escherichia coli*, domestic rainwater harvesting, microbial contamination, physicochemical quality, household water treatment

DECLARATION

"I declare that the dissertation hereby submitted to Rhodes University, for the degree Doctor of Philosophy has not previously been submitted by me for a degree at this or any other University; that it is my own work in design and in execution, and that all material contained herein has been duly acknowledged."

Signature of the candidate_____

_____Day of_____2019

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ABBREVIATIONS

AGNPs	Silver nanoparticles
APEC	Avian pathogenic *E. coli*
ANOVA	Analysis of Variance
BSF	Biosand filter
COD	Chemical Oxygen Demand
CFU	Colony Forming Units
CSIR	Council for Scientific and Industrial Research
DEC	Diarragenic *E. coli*
DRWH	Domestic rainwater harvesting
DWAF	Department of water affairs
EAEC	Enteroaggregative *E. coli*
EIEC	Enteroinvasive *E. coli*
EHEC	Enterohaemorragic *E. coli*
EPEC	Enteropathogenic *E. coli*
EXPEC	Extraintestinal pathogenic *E. coli*
GBCSA	Green Building Council of South Africa
H_2S	Hydrogen sulphide
HRW	Harvested rainwater
INPEC	Intestinal pathogenic *E. coli*
MF	Microfiltration
MFT	Membrane filtration
MMT	Montmorillonite
MAR	Multiple antibiotic resistance
MDH	Malate dehydrogenase
MPN	Most probable number
NF	Nano filtration
NOM	Natural organic matter
NPV	Negative predictive value

NMEC	Neonatal meningitis *E. coli*
NTU	Nephelometric turbidity units
PVA	Polyvinyl alcohol
PCR	Polymerase chain reaction
PPV	Positive predictive value
RWH	Rainwater harvesting
RWHS	Rainwater harvesting system
RU	Rhodes University
SANS	South African National Standards
SODIS	Solar disinfection
SOCO-DIS	Solar collector disinfection
SPSS	Statistical Package for the Social Science
UF	Ultrafiltration
UV	Ultraviolet
UPEC	Uropathogenic *E. coli*
UNICEF	United Nations Children's Fund
VGs	Virulence genes
WHO	World Health Organisation
WRC	Water Research Commission

CHAPTER ONE: INTRODUCTION

1.1 PROBLEM STATEMENT

Rainwater harvesting (RWH) is gaining popularity worldwide and South Africa is among the countries which adopted the concept of RWH to overcome the shortage of clean water. Communities situated in rural and peri-urban areas often experience shortage of water. In rural areas where there is no piped water or municipal water supply, people rely on harvested rainwater for their daily water needs such as drinking, bathing and cooking. However, in developed regions harvested rainwater is mostly used for non-potable purposes such as vehicle washing, laundry and gardening. Rainwater harvesting is therefore regarded as a potential method in order to alleviate the problems of water shortage. However, the quality of harvested rainwater is reported to be poor with the potential of causing diseases if consumed without any prior treatment. Ahmed *et al*. (2011) reported that despite the general perception that harvested rainwater is safe to drink, the presence of potential pathogens such as *E. coli*, *Aeromonas* spp., *Campylobacter* spp., *Salmonella* spp., *Legionella pneumophila, Giardia* spp., *Cryptosporidium* spp., *Vibrio* spp. and enteric viruses have been isolated from harvested rainwater. In South Africa, few studies have reported on the quality of harvested rainwater. Mostly, in South Africa, more focus is placed on the quantity of harvested rainwater with a lesser focus on the quality of such water. Rainwater quality can differ depending on the atmospheric pollution, harvesting method and storage. It is clear that in most cases the quality of harvested rainwater is poor and therefore more research is needed regarding the quality of such water. This will allow proper understanding of the type of contaminants found in harvested rainwater under local conditions. If the quality of harvested rainwater is well understood it will be easier to advice users on the type of treatment they should undertake. The end results of efforts made in documenting contaminants found in harvested rainwater and treatment of such water will be improved health for communities consuming harvested rainwater. Furthermore, people who depend on the use of harvested rainwater should be taught on the dangers of consuming untreated rainwater and also encouraged to continuously treat harvested rainwater in the homes. By providing communities with means to treat water at household level, many potential water-borne infections could be avoided.

1.2 AIMS AND OBJECTIVES OF THE STUDY

1.2.1 AIM

This study aimed to monitor the quality of harvested rainwater in selected regions of South Africa and to improve the quality of harvested rainwater by exploring a suitable treatment method.

1.2.2 OBJECTIVES

The above stated aim will be achieved by addressing the following objectives:

- To monitor the quality of harvested rainwater in different areas of South Africa.
- To determine the efficiency of an early warning water quality monitoring tool for harvested rainwater at household level: A low cost hydrogen sulphide (H_2S) kit.
- To investigate the presence of *E. coli* virulence genes and antimicrobial resistance in harvested rainwater.
- To investigate the presence of pathogenic organisms (*Salmonella, Legionella, Vibrio cholerae, Shigella* and fungal isolates) in harvested rainwater.
- To evaluate the efficiency of a nano compound clay-based material in the treatment of harvested rainwater.

1.3 GENERAL INTRODUCTION

One of the most significant environmental issues in developing countries is water scarcity, which is the lack of fresh water resources to meet water demand (Lee *et al.,* 2017). Furthermore, the demand for water exceed supply due to rapid population and industrial growth (Lee *et al*., 2017). The World Health Organization (WHO) and the United Nations Children's Fund (UNICEF) (2015) reported that more than 90% of the world's population has access to improved sources of drinking water and about 663 million people do not have access to improved drinking water globally. As the demand for water grows in South Africa, alternative sources must be explored to augment conventional water supply. Rainwater harvesting (RWH) captures and stores rain runoff from roofs and other surfaces which is later used for agricultural and domestic purposes (Jebamalar and Ravikumar, 2011). This method is further categorized into three groups based on type of catchment surface used, namely in-field, ex-field and domestic rainwater harvesting (Mwenge Kahinda and Taigbenu, 2011). Domestic rainwater harvesting (DRWH) involves the collection of rainwater from rooftops, courtyards and similar compacted or

treated surfaces and stored in underground tanks or aboveground tanks which is used for domestic purposes, garden watering and small-scale productive activities (Mwenge Kahinda *et al*., 2007). Various benefits of harvested rainwater include that it requires simple and inexpensive technologies that are easy to install and maintain, because of its simplicity, RWHS can be expanded, reconfigured or relocated to meet each household's needs, furthermore, it relieves pressure on available supplies (Liuzzo *et al*., 2016). Rainwater may also help to mitigate flood in low-lying areas and the integration of RWHS into buildings is an effective way to minimize the use of municipal supply for non-potable purposes and supply drinking water in places where water is scarce (Liuzzo *et al*., 2016). However, there are certain disadvantages associated with RWH such as supply limitation and the reliability of rainfall (both in terms of spatial and temporal distribution). In certain instances, RWHS depending on tank size cannot supply water for all domestic uses and are unlikely to make the households independent of the conventional water supply system (Liuzzo *et al*., 2016). Countries with limited water resources globally in terms of quantity and quality turn to rely on harvested rainwater as an alternative for drinking and various domestic purposes (Igbinosa *et al*., 2017). This is especially visible in developing countries where harvested rainwater is increasingly used on a daily basis (Igbinosa *et al*., 2017). Currently, many countries are supporting updated implementation of RWH practices in order to address the increase in water demand pressures associated with climatic, environmental and societal changes (Amos *et al*., 2016).

Although RWH may be a potential water resource, studies have reported that it can pose a public health risk because of its potential to carry microbial pathogens (Ahmed *et al*., 2008; Simmons *et al*., 2008; Lim and Jiang, 2013; Alves *et al*., 2014; Jesmi *et al*., 2014; Lye, 2014). The quality of harvested rainwater is affected by several factors including materials used in the construction of the RWHS and the environment in which it is located (Lee *et al*., 2010). Furthermore, limitations that need to be overcome for DRWH to be widely used include an investigation into possible health risks associated with the usage of DRWH systems. Other limitations of DRWH include the need to train households on how to construct, operate and maintain the system and also the costs involved when implementing DRWH systems (Mwenge Kahinda *et al*., 2010). Studies performed in South Africa show that the quality of harvested rainwater is generally poor. Even with the reports showing poor rainwater quality there is minimal research either previously or ongoing regarding the quality of harvested rainwater across South Africa. This study aims to shed light on the quality of various domestic rainwater harvesting systems and to explore a suitable treatment method.

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CHAPTER TWO: LITERATURE REVIEW[1]

2.1 DOMESTIC RAINWATER HARVESTING

Domestic RWH is gradually becoming an important potable water source to improve household water security, especially for most developing countries (Musayev *et al*., 2018). The practice of household RWH has been conducted for over 4000 years for both potable use and irrigation of food crops (Londra *et al*., 2015). Depending on the local needs and resource availability, different countries have different purposes for rainwater. Developed countries mostly use rainwater for non-potable needs such as irrigation, laundry and toilet flushing (Muthukumaran *et al*., 2011), while in developing countries harvested rainwater is normally used for potable purposes like drinking, cooking and personal hygiene (Kisakye *et al*., 2018). The main advantage of DRWH is to provide water as close as possible to the household, reducing the need for long distance walks in order to collect water (Helmreich and Horn, 2010). Other advantages of DRWH include that it can provide water for small-scale productive activities and lead to improved food security. RWH is not new in South Africa, catching roof runoff into various storage containers such as drums and containers is common especially in remote villages (DWAF, 2010). Furthermore, over the years different organisations have also promoted the harvesting of rainwater not only from rooftops but also from ground surfaces, particularly for agricultural production (DWAF, 2010). Non-governmental organisations (NGO's), private organisations and the government have implemented rooftop rainwater harvesting projects over the years as a response to the expressed need by poor households in rural areas for water (DWAF, 2010). In 1996, a Water Harvesting Programme (WHP) was initiated which was funded by the Eastern Cape Provincial Government and the Inter church Organisation for Development Co-operation. The focus of the WHP was on three main activities namely the ferrocement tank project, Small-scale irrigation project and the catchment dam project (DWAF, 2010). The projects focussed on building RWH tanks for households, schools and clinics. These projects were all aimed at providing rainwater for uses such as garden irrigation (to increase food security), daily household purposes such as drinking and cooking as well as watering

[1] This chapter is based on: Shirley Malema, Akebe Luther King Abia, Jean-Marc Mwenge Kahinda and Eunice Ubomba-Jaswa (2017). Gaining a Better Understanding of the Factors that Influence the Quality of Harvested Rainwater in South Africa: A Review. Novel approaches to rainwater harvesting and sanitation in developing countries. Nova Science Publishers, Hauppauge, Ney York, USA, ISBN: 9781634858267/9781634858540, 2017.

livestock (DWA, 2009). The various projects were implemented in areas such as Alice, Butterworth, Cala, Cofimvaba, Idutywa, Lady Frere, Middledrift, Mqannduli, Mount Fletcher, Mount Frere Ngqamakwe, Ntabankulu, Tsomo, Umtata and Willowvale (DWA, 2009). The Department of Science and Technology (DST) commissioned the CSIR to investigate technologies for improving the lives of low-income households with the aim of developing houses that are more comfortable, durable, faster to build, easily extendable and less dependent on municipal services. A water tank was installed next to the house for harvesting rainwater off the roof, which acts as a gutter due to its specific construction (CSIR, 2010). Ethekwini Municipality (2010) also promoted the use of RWH for individual household purposes such as toilet flushing, laundry and garden irrigation. Furthermore, Rand water in partnership with the department of water and environmental affairs started the Schools Rainwater Harvesting initiative in 2013, which focuses on schools' water supply, water demand management and household poverty eradication. Implementing RWH practices can also bring considerable economic and environmental benefits. Liang and Dijk (2011) stated that a financial comparison between harvested rainwater and groundwater for agricultural irrigation in the rural areas of Beijing showed that RWH was economically feasible and had positive effects for society. Furthermore, Tam *et al*. (2010) evaluated the cost effectiveness of RWH in 7 cities of Australia and indicated that the use of harvested rainwater is economically feasible in Gold Coast, Brisbane and Sydney due to more rainfall and higher reliability.

2.2 FACTORS AFFECTING THE QUALITY OF HARVESTED RAINWATER

Microbial quality of harvested rainwater is influenced by several factors such as contamination of the catchment area by air, dust, birds and animal droppings, insects, organic matter, pollutants from human activities and other modes of contamination such as atmospheric deposition of environmental organisms (Ahmed *et al*., 2011; Farreny *et al*., 2011). Figure 2.1 shows several pathways in which contamination of the rooftop RWH can occur.

2.2.1 Catchment area (Roof)

The rooftop as the most commonly used method of RWH can harbour enteric pathogens of which the source of contamination is likely to be faecal waste from birds, lizards, mice, rats, possums, dead animals and insects (Ahmed *et al*., 2010; Lee *et al*., 2012). Sources of pollutants commonly encountered in rooftop runoff include precipitation, atmospheric deposition and materials used in the construction of the roof (Abbasi and Abbasi, 2011).

2.2.2 Gutters

Gutters which connect the roof and the storage tank have also been reported to contribute to rainwater contamination. Gutters are made from different materials such as PVC plastic and galvanized metal. PVC gutters are mostly recommended as they do not rust and water quality will be maintained over a long period of time (Mosley, 2005). Correctly installed gutters must not have flat areas where debris and water may pool as these may provide sites for mosquitoes to breed (Mosley, 2005). Protective coatings are often applied to the outside of metal downspouts to protect the material from corrosion; however, runoff water comes into contact with the unprotected inside. Applying protective coatings to the inside of downspouts may be one of the means of preventing metal contamination of harvested rainwater from gutters and downspouts (Ward et al., 2010).

2.2.3 Storage tank

Storage tanks used in harvesting rainwater can also pose a serious health risk as small animals and birds can enter the tanks (Ahmed *et al*., 2010). Rainwater tanks in some instances can provide habitats for mosquito breeding (Schets *et al*., 2010). Thomas and Martinson (2007) reported that poorly designed storage tanks can result in increases of microbial contamination. If the top of the tank is not properly covered, light and oxygenation can cause rapid bacterial growth in the tank, resulting in poor quality water.

2.2.4 Air quality

Kaushik *et al*. (2012) studied the influence of air quality on the composition of microbial pathogens in fresh rainwater. Rainwater was collected using an automated rainwater collector with negligible interference from dustfall or bird dropping. They reported that the only mode of microbiological contamination of rainwater was through atmospheric pathways namely; scavenging of airborne microorganisms or bioaerosols by cloud or rain droplets. Their results indicated that of the 50 samples tested 25 (50%) were found to be positive for at least one of the four pathogens tested. Furthermore, of the 25 positive samples, 42% were found to be positive for *E. coli*, 32% for *Pseudomonas aeruginosa*, 12% for *Klebsiella pneumoniae*, and 2% for *Aeromonas hydrophila.* Ekström (2010) also reported that the presence of *E. coli* and *P. aeruginosa* in fresh rainwater is most likely associated with their presence in bioaerosols or air.

2.2.5 Seasonal changes

Studies by Lighthart (2000) and Jones and Harrison (2004) reported that seasonal and meteorological changes influenced atmospheric concentrations of bacterial and fungal spores and found that the microbial concentrations correlated with the incidence of allergic and infectious disease outbreaks.

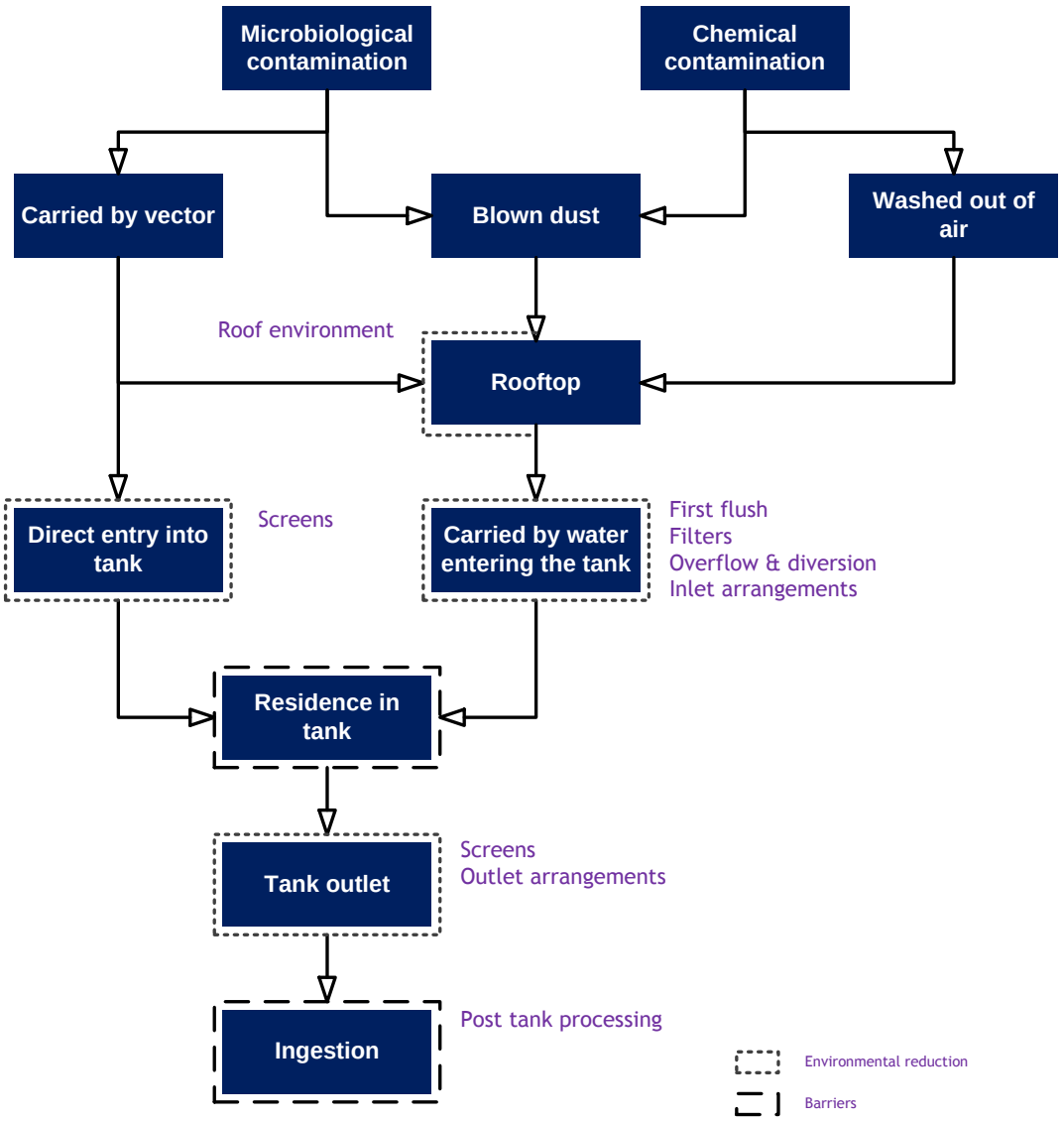

Figure 2.1: *Contamination pathways of rooftop RWH systems (adapted from Martinson and Thomas, 2003)*

During dry periods, dust, faecal deposits, rodents and birds are the major sources of heavy pollution (Ahmed *et al*., 2008). After long dry periods caused by less rainfall the quality of harvested rainwater may be of serious health risks due to accumulation of these pollutants especially if the water is harvested from rooftops. Microbial contaminants such as *E. coli*, faecal coliforms, *Salmonella* spp. and *Giardia lamblia* may be detected during this time of harvesting (Ahmed *et al*., 2011). Dobrowsky *et al*. (2014a) reported that rain allows pathogens from animal droppings and other organic debris to be flushed into the tanks via the gutters and *E. coli* counts and toxin genes increases during the higher-rainfall period. Furthermore, faeces of birds, insects, and mammals could be filtered from the roof tops into the rainwater tank, which results in faecal contamination of the water source. Despins *et al*. (2009) collected

samples from rainwater cisterns and reported the significant correlation between total coliforms, faecal coliforms and temperature. Furthermore, higher concentrations of total coliforms and faecal coliforms were observed during summer (17°C) and lower concentrations during winter (-5°C). They concluded that this could be attributed to decreased microbial activity or decreased animal presence during colder months.

2.2.6 Location of the rainwater harvesting system

The location of the RWH system is also an important aspect to consider when assessing the level of contamination in harvested rainwater. Campisano *et al*. (2017) reported that microbial populations in collected and stored rainwater may exhibit substantial variations between different locations depending on climatic conditions (e.g. wind speed and direction, regime of rainfall events). In urban areas, rainwater might be already contaminated before it reaches the catchment area (Sánchez *et al*., 2015). This is regarded as the first stage of contamination which occurs when rainfall washes out and scavenges aerosols, gases and thin volatile particles from the urban atmosphere (Sánchez *et al*., 2015). Air quality can also be affected by organic pollutants derived from fuel leakage of vehicles, petrochemical and plastic-chemical industries which may in turn contaminate the harvested rainwater (Huston *et al*., 2012). Factors attributed to the quality of harvested rainwater range from environmental pollution from industries, automobiles and anthropogenic activities and the presence of particles (Igbinosa and Osemwengie, 2016). Mostly in urban areas, harvested rainwater may be important to augment municipal supply and also to offer flexibility in water use during water restriction periods. However, in rural and peri-urban areas where water supply is often limited harvested rainwater is used for potable water purposes. In some rural areas in developing countries where defecation in open lands is practiced, it is possible that faecally contaminated dust may get blown on to the roofs or the falling rain may capture airborne pathogens (Abbasi and Abbasi, 2011). Nair and Ho (2009) listed microbial contamination as a primary health risk because it varies depending on location, season, environment and maintenance practices, which leads to unpredictable water quality. The importance of location when installing rainwater harvesting systems were also evident in a study by Simmons *et al*. (2001) which stated *Legionella sp*. were not detected in samples from 23 tanks in rural New Zealand, but were present in 5 of 7 tanks sampled in urban Denmark (Albrechtsen, 2002). Jongman and Korsten (2016) studied the quality of harvested rainwater in rural villages and results indicated the presence of *Salmonella*, *E. coli* and fungal isolates such as Penicillium and Cryptococcus species. The study further reported extensive disparities in the level of water pollution and bacterial composition between sites.

2.3 IMPLICATIONS OF QUALITY ON THE USES OF HARVESTED RAINWATER

Rainwater harvesting is usually used for both potable and non-potable applications in countries such as Australia, South Africa, USA, Germany and Japan (Silva *et al*., 2015). Indoor non-potable applications include toilet flushing and cooling towers while outdoor non-potable applications are irrigation systems and vehicle washing (Georgia RWH guidelines, 2009). A study in Queensland involving rainwater tank owners has reported on various potable and non-potable uses of roof harvested rainwater including drinking, cooking, laundry, showering, pool top-up, gardening, car washing, ornamental water features, toilet flushing, filling fish tanks, and pet washing (Hamilton *et al*., 2016). In most urban areas harvested rainwater is used to augment water supply and reduce consumption from centrally supplied sources. A study by GhaffarianHoseini *et al*. (2016) suggested that the overall global household water uses account for 80-90 % of water consumption and highlight the importance of water conservation benefits associated with RWH implementation. Certain uses of roof harvested rainwater used in many aspects of daily life, such as washing family cars, sprinkling gardens with water and watering flowers and plants may result in the formation of potentially infectious aerosols (Kobayashi *et al*., 2014). Schets *et al*. (2010) also reported that harvested rainwater used for toilet flushing may pose a health risk when aerosols containing *Legionella* are formed during toilet flushing and then inhaled. They concluded that the pathogens present in untreated rainwater, used for toilet flushing, can have negative consequences for public health. In a follow up study, Schets *et al*. (2010) conducted a three-year study using roof-harvested rainwater used for toilet flushing, cleaning floors and watering gardens. The study demonstrated that harvested rainwater was faecally contaminated and incidentally contained potential human pathogenic microorganisms such as *Campylobacter*, *Legionella*, *Cryptosporidium* and *Giardia*. A recent study by Dobrowsky *et al*. (2017) indicated that individuals that utilize harvested rainwater may be at risk of *Legionella* and *Acanthamoeba* infection as these opportunistic pathogens were detected at high concentrations in all the tank water samples collected.

2.4 CURRENT KNOWLEDGE IN SOUTH AFRICA ON RWH QUALITY

There are several studies on the quality of harvested rainwater in South Africa (Table 2.1). The available studies conducted in South Africa on the quality of harvested rainwater suggest that the water is not fit for potable purposes. Therefore, more studies should be carried out in various provinces of South Africa in order to fully understand the type of contaminants from different locations. Without a full understanding of the different pathogens contaminating harvested rainwater and their associated concentrations, achieving safe harvested rainwater will result in unsuccessful outcomes (Shanks *et al*., 2010). South African research into RWH has been biased towards understanding the dynamics associated with improving the quantity of harvested rainwater. Nevertheless, several studies conducted on the quality of harvested rainwater in South Africa do provide baseline information from which intensive studies on quality can be performed.

Table 2.1: *Studies performed in South Africa on the quality of harvested rainwater*

Location/ study area	Contaminants detected	Rainwater application	Reference
South Africa (Cape Town)	*Pseudomonas Klebsiella, Enterobacter, Legionella* spp, *Salmonella*, *Shigella* spp., *Yersinia* spp.	Drinking, laundry, dish washing	Dobrowsky *et al*. (2014 a)
South Africa (Cape Town)	*E. coli*, total coliforms, faecal coliforms	Drinking, laundry, dish washing	Dobrowsky *et al*. (2014 b)
South Africa (Eastern cape)	*E. coli*, total coliforms	Drinking, irrigation, laundry	Tandlich *et al*. (2012, 2014)
South Africa (Eastern Cape)	*Legionella, Acinetobacter, Pseudomonas, Clostridia*	Drinking, laundry, dishwashing	Chidamba and Korsten (2015a)
South Africa (Gauteng)	*E. coli*, total coliforms, *enterococci*	Not available	Chidamba and Korsten (2015b)
South Africa (Cape Town)	Acanthamoeba spp., *Naegleria fowleri* Vermamoeba, vermiformis *Legionella spp.*	Not available	Dobrowsky *et al*. (2016)
South Africa (Cape Town)	*Legionella spp, E. coli*, total coliforms, *Acanthamoeba spp*, nitrites, nitrates, barium, sodium, arsenic, silicon, phosphates, magnesium	Not available	Dobrowsky *et al*. (2017)
South Africa (Cape Town)	Bacteroides,Adenovirus, Lachnospiraceae and *E. coli*	Drinking, laundry, dishwashing	Waso *et al*. (2018a)
South Africa (Cape Town)	Heterotrophic bacteria, *E. coli*, total coliforms and faecal coliforms	Not available	Waso *et al*. (2018b)

2.5 DOMESTIC RAINWATER HARVESTING PRACTICES IN SOUTH AFRICA

Domestic RWH is gaining popularity among South African households both in the rural and urban areas. As the demand for alternative water sources increases a number of provinces explore the potential of DRWH as a low-cost solution to supplement existing water supplies and mitigating the effects of drought as well as storm runoff. Furthermore, the South African government has started to recognise and support the national RWH programmes, which have a narrow but important focus on the construction of above and below-ground rainwater storage tanks to be used by rural households for food gardens and other household purposes such as drinking and bathing (after treatment) (DWAF, 2010). Figure 2.2 shows the number of households using RWH tanks as their main source of water in South Africa while Figure 2.3 depicts a RWH system situated at Rhodes University, South Africa. Rooftop

RWH is a source of drinking water in rural areas especially in the Eastern Cape and KwaZulu-Natal (Mwenge Kahinda *et al*., 2010, Tandlich *et al*., 2014). In most cases the installation of the RWHS in urban areas and rural areas is similar and made of three key aspects such as the catchment area (roof), the conveyance system and the storage tank (Thomas and Martison, 2007).

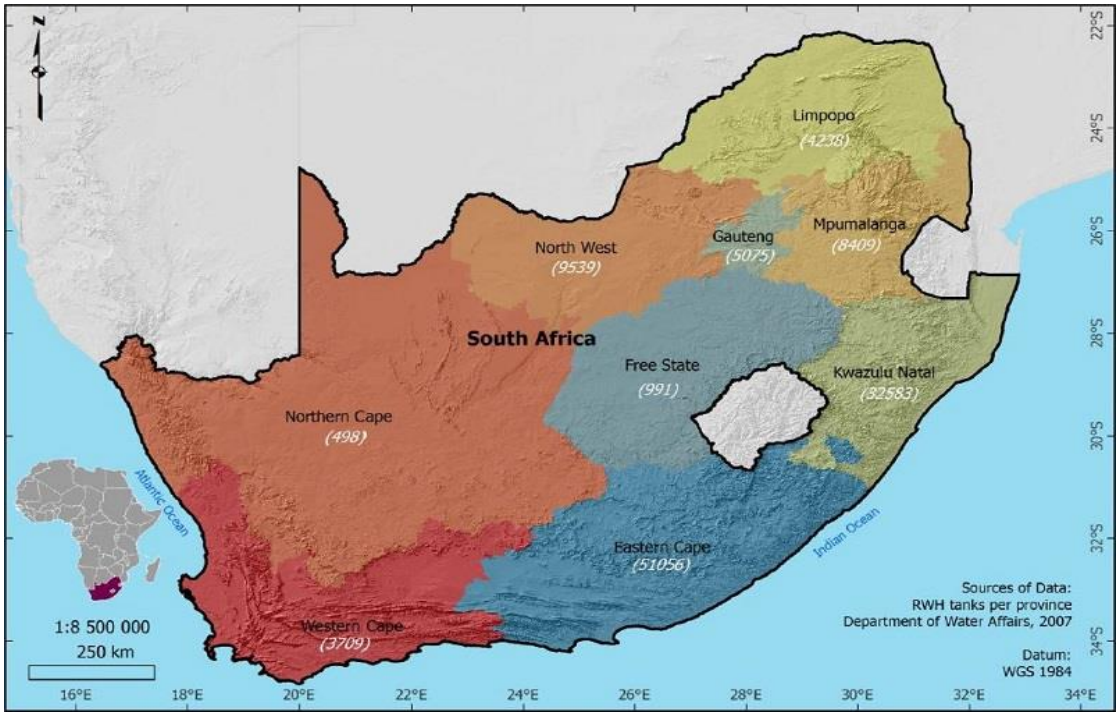

Figure 2.2: *Number of households using RWH tanks as main water source in each of the nine provinces of South Africa (DWS, 2014)*

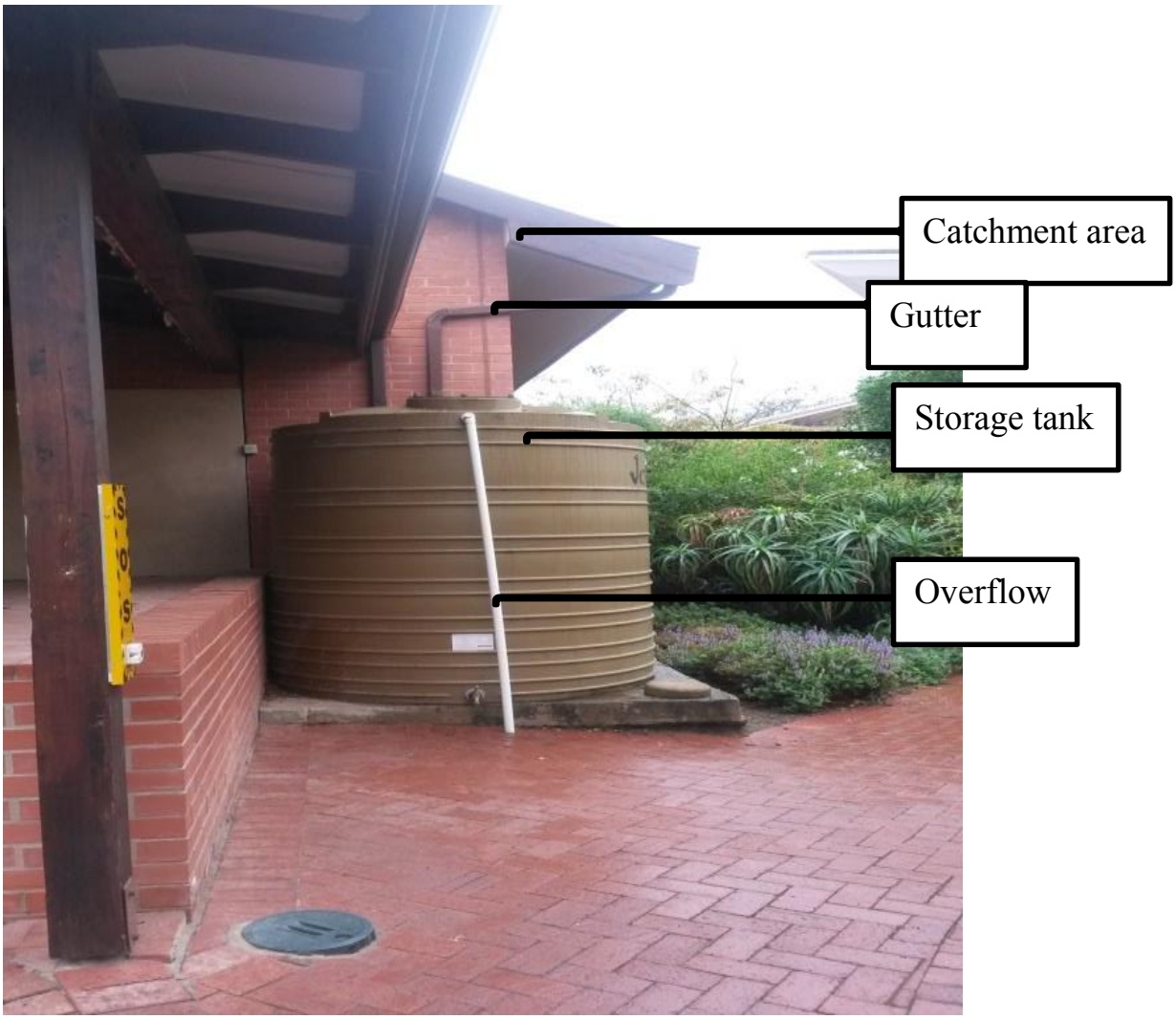

Figure 2.3: *Picture showing a rainwater harvesting system at Rhodes University, South Africa*

2.6 HEALTH IMPLICATIONS OF THE QUALITY OF HARVESTED RAINWATER

The most serious and immediate health risk associated with roof-collected drinking-water is microbial contamination (Stewart *et al*., 2016). While many of the micro-organisms found in roof-collected supplies are harmless, the safety of roof-collected rainwater for human consumption will depend on excluding or minimising enteric pathogens (Stewart *et al*., 2016). Rainwater harvesting systems are used to provide water for both potable and non-potable applications (Achadu *et al*., 2013; Dobrowsky *et al*., 2014c). Roof-collected tank rainwater is a major source of drinking and domestic water supply in Australia, New Zealand and other countries (Simmons *et al*., 2001). Using untreated harvested rainwater for household purposes enables the transmission of pathogens to humans, which may translate

to disease outbreak in more severe cases (Burns *et al*., 2012). Furthermore, consumption of untreated rainwater if contaminated with pathogens such as *Salmonella* and *Giardia lamblia* can cause gastrointestinal illnesses which may cause fatalities in immunocompromised people (Yu, 2000). Pathogenic organisms are usually excreted from faeces of animals and transported to rainwater storage system via roof runoffs and ground surface harvesting methods. Some of the pathogenic organisms in harvested rainwater include *Aeromonas*, *Shigella*, *Vibrio cholera*, *Cryptosporidium, Legionella* and *Campylobacter (*Ahmed *et al*., 2008). Ashbolt and Kirk (2006) reported that *Salmonella mississippi* was implicated in the case of infections in Australia after consumption of contaminated harvested rainwater. Dobrowsky *et al*. (2014d) investigated prevalence of virulence genes associated with pathogenic *E. coli* strains isolated from domestically harvested rainwater in South Africa during low- and high-rainfall periods. Their results revealed that pathogenic *E. coli* genes were detected in 3% (EPEC and EHEC) and 16% (EAEC) of the 80 rainwater samples collected during the sampling period from the 10 domestic harvested rainwater tanks. Although, there is limited research regarding contamination of harvested rainwater by viruses, adenoviruses isolated from harvested rainwater can cause diseases ranging from acute respiratory diseases, pneumonia, conjunctivitis, and gastroenteritis (Jiang, 2006).

Consumption of contaminated rainwater can result in diseases such as cholera, gastroenteritis and typhoid fever (WHO, 2003). Infants, elderly and individuals with compromised immune systems are mostly affected by water-borne illness acquired from contaminated rainwater which can pose a serious and sometimes deadly health risk (Ahmed *et al*., 2012). Unlike microbial contamination, chemical contamination of water leads to health problems primarily through long term exposure to the presence of harmful chemical in water (Okonko *et al*., 2008). When RWH systems are situated close to industrial areas, contamination by heavy metals can occur in storage tanks (Mosley, 2005). These metals are associated with chronic diseases such as renal failure, liver cirrhosis, hair loss and anaemia (Baez *et al*., 2007). Magyar *et al*. (2008) studied lead and other heavy metals which are common contaminants of rainwater tanks in Melbourne where results showed that concentrations of cadmium, iron, and zinc were found at levels exceeding acceptable standard limits.

2.7 GUIDELINES FOR INSTALLATION AND MONITORING THE QUALITY OF HARVESTED RAINWATER

Monitoring the quality of harvested rainwater is necessary due to the potential health risks as a result of chemical and microbiological contaminants. In South Africa, drinking water quality guidelines stipulated by the Department of Water Affairs (DWAF) (1996) and the South African Bureau of Standards (SANS 241:2015) are used to assess the quality of harvested rainwater. The stipulated

guidelines states that indicator organisms such as *E. coli*, total coliforms and enterococci should be used on regular basis to test for potential possible contaminants in drinking water. DWAF (2007) published rainwater harvesting guidelines for intensive family food production which detail the step by step household tank construction. However, families needed to establish an intensive food garden to qualify for the tank. Substantial progress has been made to develop guidelines for harvested rainwater in South Africa and a current study by Ndiritu *et al*. (2018) reported on the development of hydrologic guidelines for RWHS design and assessment of the city of Johannesburg, South Africa and used the data from multiple daily simulations of potential RWHS in the Johannesburg city. The developed guidelines focused on the potable and non-potable uses of RWHS such as toilet flushing, air conditioning and irrigation. They concluded that the developed guidelines are considered adequate for the preliminary design and assessment of the feasibility of RWHS in the city of Johannesburg.

Furthermore, a report on the development of resource guidelines for RWH was produced by Mwenge Kahinda *et al.* (2016). The report focused on producing a technical manual that provides guidance for the design, installation and management of domestic rainwater harvesting systems in South Africa. The World Health Organisation recommend that monitoring the quality of harvested rainwater should focus on sanitary inspections such as checking the cleanliness of the catchment area and storage, the structural integrity of the system and the physical quality of rainwater (turbidity, colour and smell). The level of pH should be monitored frequently in case of new concrete, ferrocement or masonry storage tanks (WHO, 2003). Microbial quality of rainwater needs to be monitored for *E. coli* or thermotolerant coliforms. The levels of lead, zinc or other heavy metals in rainwater should also be measured occasionally when it is in contact with metallic surfaces during collection or storage (WHO, 2003). In states like Georgia (USA), the guidelines for rainwater harvesting were documented to assist regulators, rainwater systems designers and end users in implementing rainwater harvesting best management practices (Georgia RWH guidelines, 2009). However, these guidelines mainly focus on installation of the rainwater harvesting system (Gutter sizing, installation of leaf filters and flush diverters). Ontario in Canada also published their guidelines for residential rainwater harvesting systems with the help of the government and the industry stakeholders (Ontario residential RWH system guidelines, 2010). The design and installation guidelines in Ontario are for catchment and conveyance, storage and sizing and rainwater quality and treatment. For rainwater quality and treatment, the Ontario guidelines specify that pre-storage treatment devices must be incorporated as part of the conveyance network and rely on gravity flow to facilitate the treatment process. Australia is one of the countries which have clear guidelines regarding rainwater harvesting, all designs and constructions of roof water harvesting and drainage systems need to adhere to minimum standard specified in the Australian Standards (Australian standards, 2008). Australian cities have implemented communal RWHS which serve groupings of households. Communal systems collect, store, treat and re-distribute rainwater to households

(Australian standards, 2008). The water treatment plant uses filtration, UV treatment and chlorination for redistribution of potable water to each household and a community centre (Cook *et al*., 2013).

2.8 ANTIMICROBIAL RESISTANCE PREVALENCE OF PATHOGENIC *ESCHERICHIA* COLI STRAINS FROM HARVESTED RAINWATER

Microbial resistance to antibiotics is a major concern globally and over the years the subject of antibiotic resistance has attracted scientific research (van den Honert *et al*., 2018). Furthermore, the high rate of antibiotic resistance and the simultaneous decline of new antimicrobial developments pose major threats to global health, leading to a higher rate of treatment failure, increased infection and severity of infection (Capita *et al.*, 2016). *Escherichia coli* is a member of faecal coliforms that contaminate drinking water from human and animal faecal waste and has been the foremost indicator of faecal contamination in water quality monitoring for many decades (Odonkor *et al*., 2018). Moreover, *E. coli* has also been shown to be a significant reservoir of genes coding for antimicrobial drug resistance and therefore is a useful indicator for resistance in bacterial communities (Arsène-Ploetze *et al*., 2018; Katakweba *et al*., 2018). Some of the *E. coli* strains are commensal while others are pathogenic. The pathogenic strains are responsible a variety of human diseases (Kaper *et al*., 2004). There exist six types of pathogenic *E. coli* including Shiga toxin-producing *E. coli* (STEC), enteropathogenic *E. coli* (EPEC), enterotoxigenic *E. coli* (ETEC), enteroaggregative *E. coli* (EAEC), diffusely adherent *E. coli* (DAEC) and enteroinvasive *E. coli* (EIEC). These strains are classified by virulence properties and pathogenicity mechanisms causing gastrointestinal diseases such as diarrhoea, urinary tract infections and meningitis (Bien *et al.*, 2011). Enterohaemorrhagic *E. coli* (EHEC) is one type of STEC that can cause severe enteric diseases, such as haemolytic uraemic syndrome and haemorrhagic colitis leading to acute renal failure and often death (Puno-Sarmiento *et al.*, 2013). *Escherichia coli* O157:H7, the most well-known serotype of EHEC, has caused many outbreaks of water and food-borne diseases in many countries. The incidence of non-O157 STEC has been increasing in recent years, including those caused by serotypes O26, O45, O103, O111, O121 and O145 (Farrokh *et al*., 2013). Some *E. coli* strains can also cause extraintestinal diseases and are called extraintestinal pathogenic *E. coli* (ExPEC). The ExPEC, which were defined by disease association, include uropathogenic *E. coli*, neonatal meningitis-associated *E. coli* and sepsis-causing *E. coli* (Dale and Woodford, 2015). Pathogenic *E. coli* strains are implicated in many water-borne disease outbreaks, STEC and EPEC have been frequently reported to be responsible for water-borne disease outbreaks worldwide (Chandran and Mazumder, 2015). Several studies have reported on pathogenic *E. coli* strains and antibiotic-resistant bacteria in roof-harvested rainwater (Meays *et al*., 2004; Donovan *et al*., 2008; Kinge *et al*., 2010; Chidamba *et al*., 2015b; Malema *et al*., 2018). In rainwater harvesting, urban birds are a major source of faecal contamination

as they have direct access to the roof catchment surface and have been implicated as reservoirs and vectors for the spread of antibiotic-resistant strains of *E. coli* (Radimersky *et al*., 2010).

2.9 LOW-COST HYDROGEN SULPHIDE TEST KITS FOR HARVESTED RAINWATER QUALITY MONITORING

Frequent water quality monitoring plays an integral part in ensuring the correct operation of water supplies, verifying the safety of drinking water, investigating disease outbreaks and validating processes and implementing preventative measures (Bain *et al*., 2012). The most serious public health risk associated with drinking water is microbiological contamination as pathogens originating from bacteria, viruses, protozoa and helminths can cause a wide range of health problems, however, the primary concern is infectious diarrheal diseases transmitted by people drinking water contaminated with faeces (UNICEF, 2008). In under resourced or remote areas with shortage of infrastructure, capital and availability of trained personnel, water quality monitoring may be a difficult task to implement (McMahan *et al*., 2012). The need for simple effective household water quality monitoring techniques is especially great for rural and small community and household water supplies that are located in remote areas and cannot afford commercial laboratory testing. On-site testing methods with portable simplified test kits have provided opportunities in developing countries. One of the potential solutions to address the issue of water quality monitoring in such areas is the use of low-cost field tests for drinking water quality. Due to high costs when using standard methods such as membrane filtration and Colilert for water quality monitoring, low cost tests such as the hydrogen sulphide (H_2S) test offer promising solutions (Khush *et al*., 2013).

The H_2S is a presence/absence test which is cost effective, easy-to-use, and a portable alternative field-based water quality test which has been used globally for more than two decades and gained popularity as a low-cost assay for assessing faecal contamination (Manja *et al*., 1982; Gupta *et al*., 2008; Gandhi, 2006). In order to test the efficiency of the H_2S method, an affordable and convenient on-site method, rainwater samples were tested from a total of 221 tanks from the households in Perth, Western Australia (Nair *et al*., 2001). The efficiency of the H_2S method was tested against the presence of total coliform and faecal coliform bacteria, determined by the standard membrane filtration method. The agreement of the results from the H_2S method and the standard methods was greater at a coliform count of >10 CFU/100 mL. Gupta *et al*. (2008) investigated water samples from a variety of sources including rainwater using locally produced H_2S test kits and laboratory-based membrane filtration for the detection of *E. coli*. Results indicated that for water sources with *E. coli* contamination of 1000 to 9999 CFU/100 mL, sensitivity of the H_2S kit was 94%. Gupta and colleagues further concluded that The H_2S test, when incubated at 24 hrs, is a promising alternative for assessing water quality where *E. coli* levels may be high. In rural Tanzania, the H_2S test kit was produced using easily sourced imported and local

materials at a cost of 0.41 USD per 100 mL test and the H_2S test successfully detected faecal bacteria in a range of improved and unimproved water samples (Matwewe *et al*., 2018). Furthermore, Tambekar *et al*. (2013) evaluated the efficiency of the H_2S test in drinking water from various water sources and reported that the H_2S test showed a 100, 84 and 89 % correlation with the Eijkman test, membrane filtration technique and the most probable number (MPN) respectively. Vasudevan and Tandon (2008) determined the microbial quality of rainwater from roof surfaces and tested 54 rainwater samples using the H_2S test and the most probable number methods. Results reported a 65-75% agreement between the H_2S test and the MPN method. Furthermore, the authors noted that the disagreements between the H_2S test and the MPN method was more pronounced at low bacterial count. In South Africa, the H_2S test kit was tested on harvested rainwater by Tandlich *et al*. (2014) and reported a correspondence rate of 71% between the m-TEC *E. coli* test and the H_2S test method. The need for cost-effective, simple and efficient water quality monitoring tools in under resourced communities is an important factor considering that water related diseases affect many people across the world. With effective low-cost water monitoring tools, people in remote areas will endure great benefits which include testing the quality of the water from the comfort of their own homes as well as reduction of water-borne diseases.

2.10 APPROPRIATE HOUSEHOLD WATER TREATMENT METHODS FOR HARVESTED RAINWATER

Microbial and chemical contaminants have been detected in DRWH tanks. Therefore, if the water is used for potable purposes, it could produce adverse health effects. Treatment of harvested rainwater in rural communities, especially in the developing world, should be inexpensive, simple, and easy to use (De Kwaadsteniet *et al*., 2013). Household water treatment has been extensively promoted as the solution to the problem of poor-quality drinking water in developing countries (Sobsey *et al*., 2008). There are various household water treatment methods intended for domestic use such as solar disinfection (SODIS), chlorination, biosand filters (BSF) and boiling for deactivation of microbes (Sobsey, 2002). A summary of treatment methods used in harvested rainwater is presented in Table 2.2.

2.10.1 DISINFECTION METHODS

Disinfection is a simple treatment method which can be effectively applied at household level (Jordan *et al*., 2008). However, the common disadvantage associated with most disinfection methods is reduced treatment efficiency on turbid water (Sobsey *et al*., 2002). Filtration is often required when using disinfection methods to reduce turbidity which can shield certain microorganisms thereby resulting in treatment inefficiency (Qualls *et al*., 1983). The volume of water that can be treated with disinfection methods such as solar radiation is also of concern as it can only treat small volumes of water (Jordan *et*

al., 2008). Treatment of harvested rainwater with disinfection has been proved to be successful by other studies (Despins *et al*., 2009; Mendez *et al*., 2011, Ahmed *et al*., 2012).

A. **Solar disinfection**

Solar disinfection (SODIS) has shown to be an effective treatment method at household level. In SODIS treatment, water is exposed to sunlight for about 6–8 hrs and pathogens are inactivated by the synergistic effect of both temperature and sunlight radiations (Sichel *et al*., 2007; Ubomba-Jaswa *et al*., 2009; Dayem *et al*., 2011). Ahammed and Meera (2008) studied the effectiveness of SODIS in the treatment of roof harvested rainwater and reported that complete inactivation of total coliforms was observed after 6 hours when solar radiation exceeded 500 W/m^2. Limitations of SODIS include inefficiency in treatment of large volumes of water, its ineffectiveness during cloudy or rainy days and it cannot be used on turbid water (EAWAG, 2012). Amin and Han (2009) investigated the benefits of solar collector disinfection (SOCO-DIS) as a potential treatment system for harvested rainwater for small scale water supply. SOCO-DIS was compared to SODIS with the aim of overcoming the limitations of SODIS reported that in the SOCO-DIS system, disinfection improved by 20–30% compared with the SODIS system and that rainwater was fully disinfected even under average weather conditions due to the effects of concentrated sunlight radiation and the synergistic effects of thermal and optical inactivation. An advantage of using SODIS on low pH waters include increased inactivation rates due to the depletion of Adenosine triphosphate, the main energy transfer molecule in the cells (Amin and Han, 2009). Dowbrowsky *et al*. (2015b) investigated the efficiency of a closed-coupled solar pasteurization system in reducing the microbiological load in harvested rainwater and to determine the change in chemical components after pasteurization. Cations analysed were within drinking water guidelines, with the exception of iron, aluminium, lead and nickel which were detected at levels above the respective guidelines in the pasteurized tank water samples. Indicator bacteria including, heterotrophic bacteria, *E. coli* and total coliforms were reduced to below the detection limit at pasteurization temperatures of 72 °C and above. However, with the use of molecular techniques *Yersinia spp*., *Legionella* spp. and *Pseudomonas* spp. were detected in tank water samples pasteurized at temperatures greater than 72 °C (Dowbrowsky *et al*., 2015b).

B. **Ultraviolet light**

Ultraviolet (UV) radiation is defined as a physical method where water is exposed to a lamp producing light at a wavelength of nearly 250 nm. The wavelength is located in the middle of the germicidal band and is the one responsible for damaging the DNA of microorganisms (Bolton and Colton, 2008).

Ultraviolet light treatment method often requires filtration as a pre-treatment step since it is not effective on turbid water (Qualls *et al*., 1983; Macomber, 2010). Several studies have reported on the effectiveness of UV as a disinfection method for harvested rainwater (Jordan *et al*., 2008; Ahmed *et al*., 2012). Kim *et al*. (2005) reported that the number of total coliform present in rainwater is reduced by 50% even at low exposure to UV. Advantages of using Ultraviolet in treating harvested rainwater include: its high efficiency in the removal of microbes from water and the fact that it does not introduce chemicals or produce harmful disinfection by-products (Vilhunen *et al*., 2009). Despite its positive attributes in the treatment of harvested rainwater, UV treatment has disadvantages which include: (i) lack of disinfectant residual to protect the water from recontamination or microbial regrowth after treatment, (ii) turbidity and certain dissolved constituents can interfere with or reduce its disinfection efficiency and (iii) high electricity usage is required to power the UV lamps (Kowalski *et al.*, 2000).

C. **Chlorination**

This method of disinfection has been practiced since ancient times. Chlorination requires that the appropriate dosage be administered. Chlorination is known to be effective against bacteria, viruses and protozoa. Several studies reported on chlorination as an effective intervention strategy to prevent diarrhoeal diseases (Semenza *et al*., 1998; Quick *et al*., 1999 and Quick *et al*., 2002). Free chlorine inactivates more than 99.99% of enteric pathogens except *Cryptosporidium* and *Mycobacterium* species (WHO, 2002). One of the disadvantages of water chlorination process is the formation of disinfection by-products which may pose a health risk to consumers (Nsikak *et al*., 2017). Nath *et al*. (2006) reported that chlorination is less effective in turbid water of >30 NTU and that microbial contaminants may be protected by particulates in the water.

2.10.2 FILTRATION METHODS

A. Sand filters

Several studies have reported that biosand filters (BSF) are capable of removing 81–100% bacteria and 99.98–100% protozoa from harvested rainwater. Sobsey *et al*. (2008) reported that treatment of harvested rainwater with BSF can reduce bacteria, viruses and protozoa by up to 4-log reduction. In another study by Bauer (2011) slow sand filtration removed *E. coli* by 2.25 and 3.92 logarithmic units. Furthermore, a study done by Rahmat *et al*. (2008) reported on the efficiency of sand filters for reduction of physico-chemical properties of harvested rainwater. They found that turbidity was reduced by 76%, suspended solids by 54% and pH by 36%. Ahammed and Meera (2010) used a sand filter medium coated with iron hydroxide and manganese oxide to remove bacteria and heavy metals from harvested

rainwater. Results showed that the coated filter medium was able to remove 99% of coliforms and 96% lead.

B. Membrane filters

Treatment of water using membrane separation has gradually gained popularity because it effectively removes various contaminants. Nanofiltration (NF) membranes are an effective technology to remove dissolved organic contaminants (Petersen, 1993). This type of treatment offers an attractive approach to meeting multiple objectives of advanced water treatment, such as the removal of disinfection by product precursors, natural organic matter (NOM), endocrine disrupting chemicals and pesticides (Escobar *et al*., 2000; Nghiem *et al*., 2004). Disadvantages of using nanofilters include the decrease of permeate flux (membrane fouling) which is a major obstacle to the application of NF membranes to water treatment. Fouling worsens membrane performance and ultimately shortens membrane life, resulting in the increase of operational cost (Hörsch *et al*., 2005; Li and Elimelech, 2006). Membrane filters applied as post-treatment helps to remove pathogens and suspended solids. Advances in low pressure driven membrane technologies such as microfiltration (MF) and ultrafiltration (UF) have been used in water and wastewater treatment due to their high efficiency, ease of operation and small footprint (Quin *et al*., 2006). Dobrowsky *et al*. (2015a) evaluated the efficiency of a polyvinyl (alcohol) (PVA) nanofiber membrane/activated carbon column, for the treatment of harvested rainwater. Results indicate that 3 L of potable water may be produced by the PVA nanofiber membrane/activated column as *E. coli*, total coliform, and heterotrophic bacterial numbers were reduced to within drinking water guidelines according to the DWAF (1996). Furthermore, in separate experiments, molecular techniques were utilised to investigate the bacterial and viral removal efficiencies from DRWH tanks. Genus-specific PCR assays revealed the presence of potentially pathogenic bacteria, commonly associated with tank water. Results indicated that *Klebsiella* spp., *Legionella* spp., *Pseudomonas* spp., and *Yersinia* spp. were detected in all the unfiltered tank water samples and were then sporadically detected in the filtered tank water. *Legionella* spp. and *Yersinia* spp. were the most persistent genera, as these bacteria were detected in all the unfiltered tank water samples and in 85 and 80% of the 20 filtered tank water. The PCR assays and BLAST analysis also confirmed the presence of bovine adenovirus 3 in all of the tank water samples collected before microfiltration for both tanks sampled. Other adenovirus strains detected in the rainwater tanks included simian adenovirus B isolate BaAdV-1 and human adenovirus 40 strain M-364. Moreover, once the tank water had undergone filtration through the PVA nanofiber membrane/activated carbon column, the presence of adenovirus was indicated in 75 % of the filtered tank water samples.

2.10.3 BOILING

Boiling is one of the oldest methods used in household water treatment (Conant, 2005). The World Health Organisation recommends bringing water to a rolling boil to indicate that a disinfection temperature is reached (WHO, 2008). Feachem *et al*. (1983) reported that heating water to 55°C has be shown to inactivate most pathogens such as bacteria, viruses, helminths and protozoa. Other studies also reported on boiling as a water treatment option (Sobsey and Leland, 2001; Conant, 2005). A major disadvantage of boiling is its consumption of energy, cost and sustainability of fuel. In areas of the world where wood and other biomass fuels or fossil fuels are in limited supply and must be purchased, the costs of boiling water are excessive (Sobsey, 2002). The use of wood and wood-derived fuels is also a concern because it contributes to the loss of woodlands and the accompanying ecological damage caused by deforestation (Sobsey and Leland, 2001).

2.10.4 NANOMATERIALS

Nanomaterials have unique size-dependent properties related to their high specific surface area such as fast dissolution, high reactivity and strong sorption (Gehrke *et al*., 2015). Several commercial and non-commercial technological developments are employed on daily basis but nanotechnology has proved to be one of the advanced ways for water treatment (Amin *et al*., 2014). Different nanocomposites such as silver, zinc oxide, copper oxide and magnesium oxide are at various stages of research and development for the remediation of drinking water (Chong *et al*., 2011; Rana and Kalaichelvan, 2011; Loo *et al*., 2013; Quang and Chau, 2013). Silver is the most widely used material due to its low toxicity and microbial inactivation in water (Kumar *et al*., 2004). Silver nanoparticles (Ag NPs) are highly toxic to microorganisms and thus have strong antibacterial effects against a wide range of microorganisms, including viruses, bacteria and fungi (Krishnaraj *et al*., 2012; Kalhapure *et al*., 2015; Borrego *et al*., 2016). Silver nanoparticles are derived from its salts like silver nitrate and silver chloride, and their effectiveness as biocides is documented in the literature (Shrivastava *et al*., 2007). Nawaz *et al*. (2012) used silver disinfection in the treatment of harvested rainwater and reported a success rate of 95-99% for removal of *Pseudomonas* and *E. coli*. The combination or modified forms of any of the clay minerals have also been shown to be effective in adsorption of certain contaminants from drinking water and wastewaters (Motshekga *et al*., 2013). Montmorillonite, bentonite, kaolinite and zeolite are common clay minerals that have been used as fillers for preparing nano clay materials (Motshekga *et al*., 2013). Due to the abundant nature of the montmorillonite, nano based clay materials could provide cost effective, simple and easy to use household water treatment technologies.

Table 2.2: *Summary of methods used to treat harvested rainwater*

Location	Treatment method	Organisms detected in harvested rainwater	Percentage removal after treatment	Reference
Australia	Boiling	*E. coli*	100%	Spinks *et al*. (2003)
Arizona	Filtration + UV	Total coliforms, *E. coli*, and Enterococci	100%	Jordan *et al*. (2008)
Brazil	Slow filtration	*Cryptosporidium*	99%	Heller *et al*. (2006)
Ethiopia	Moringa *stenopetala* seed, sand filter and boiling	Turbidity, Total coliforms	53.84-94.63%	Taffere *et al*. (2016)
India	Sand filter	Total coliforms	99%	Ahammed and Meera (2010)
Saudi Arabia	SODIS	*P. aeruginosa*	92-100%	Amin *et al*. (2014)
South Korea	UV	Total coliforms	50%	Kim *et al*. (2005)
South Korea	Membrane filters	Total coliforms	78-98%	Kim *et al*. (2005)
South Africa	PVA nanofiber membrane	*E. coli, HPC, total coliforms*	>99%	Dobrowsky *et al.* (2015a)
South Africa	Closed couple solar pasteurizer	*E. coli, HPC, total coliforms*	>99%	Dobrowsky *et al*. (2015b)
South Africa	Closed couple pasteurization	*Legionella*	99%	Reyneke *et al*. (2016)
South Africa	Solar pasteurization and Solar disinfection	*E. coli*, *Pseudomonas, Legionella*	47.27-99.61%	Strauss *et al.* (2016)
Thailand	Granular activated carbon filtration	Dissolved organic solids, nitrate, phosphate	40-80%	Areerachakul *et al*. (2009).

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CHAPTER THREE: THE QUALITY OF HARVESTED RAINWATER IN THE EASTERN CAPE, SOUTH AFRICA: IMPLICATIONS ON TREATMENT AND USE[2]

3.1 INTRODUCTION

Most developing countries around the world experience frequent water shortages. The lack of access to water of sufficient quantity is further worsened by the amount of pollution occurring in available water resources and the effects of climate change (Karl *et al*., 2009). South Africa experienced an El Niño-related drought between late 2014 and June 2016 which is the warm phase of an irregularly periodical variation in winds and sea surface temperatures over the tropical eastern Pacific Ocean (Pearce, 2016). The drought and heat conditions have impacted on the already dry and drought-stricken country, affecting sectors such as water and agriculture (Manderson *et al*., 2016). The National Water Act of 1998 established the basic human needs reserve, which has been defined as 25 litres per capita per day (L/c/d) or 6000 litres per household per month. However, studies carried out in 2015 show that the average South African suburban family of 4 uses 300 litres per person per day (DWS, 2017/2018). In order to reduce the burden of water demand, alternative water sources (groundwater, greywater reuse, storm water and rainwater harvesting), which have the potential to alleviate water scarcity are being explored (Mwenge Kahinda *et al*., 2009). Nevertheless, the quality of the water provided by these potential water sources may not be adequate for some uses, without appropriate treatment. Many households in all nine provinces of South Africa use rooftop RWH for domestic purposes (Mwenge Kahinda *et al*., 2010; Malema *et al*., 2016). In 2007, over 116,000 domestic rainwater harvesting (DRWH) tanks were in use in all the nine provinces with the Eastern Cape alone having almost half the total number of the tanks (51,056) (DWS, 2014). In the Eastern Cape Province, for example, the unreliability of water supply has resulted in a regular water shortage for household and personal use (Tandlich *et al*., 2012). As a result, a substantial number of households in the province have resorted to domestic rainwater harvesting (DRWH) systems as their primary water source (Mwenge Kahinda *et*

[2] This chapter is based on: Shirley Malema, Akebe Luther King Abia, Jean-Marc Mwenge Kahinda and Eunice Ubomba-Jaswa, 2018. The quality of harvested rainwater in the Eastern Cape, South Africa. Paper submitted to Water SA.

al., 2010; Malema *et al*., 2016). The conjunctive use of various water sources also known as multiple-use water services augments water supply and therefore holds the potential to realise a more comprehensive and interconnected set of water-related outcomes. Although rainwater harvested from rooftops is used by many communities as an alternative or a primary water source, several studies have reported that DRWH can pose a public health risk because of its potential to carry microbial pathogens and chemicals detrimental to human health (Lee *et al*., 2010; Akoto *et al*., 2011 and Dobrowksy *et al*., 2014). The quality of the harvested rainwater differs depending on the levels of atmospheric pollution, the harvesting method and the storage medium. There are numerous ways in which contaminants can enter the DRWH system and compromise the water quality. Chemical contaminants may dissolve from the atmosphere during precipitation and or leach due to the characteristics of the rainwater system such as the collection surface (Adeniyi, 2004).

Microbial pollutants are introduced into the RWH system through contaminated catchment areas (e.g. bird droppings on roof surfaces), poor collection methods and storage design (Pathak and Heijnen, 2005). The existence of various sources and pathways of contaminants demonstrates the complexity of maintaining and safeguarding public health when relying on roof water harvesting systems for domestic supply (Ahmed *et al*., 2014). Depending on the intended use, desirable properties of water include low organic content, a neutral pH value, adequate amount of dissolved oxygen, moderate temperature, the absence of infectious agents as well as toxic substances (Adeniyi, 2004). The South African water quality guidelines (DWAF, 1996a-h) do cater for water meant for various uses. However, as RWH guidelines for South Africa do not currently exist, the directives as set by the South African National Drinking Water Standards (SANS 241-1, 2015) determine the suitability of harvested rainwater for potable use. Although several households in the Eastern Cape depend on harvested rainwater (usually without prior treatment) for their daily water requirements, there are very few monitoring programmes or studies that have been conducted to determine the quality of the water obtained in this area (Tandlich *et al*., 2012; Tandlich *et al*., 2014). Despite the reports on the poor quality of harvested rainwater in many regions of the world, especially in rural communities in developing countries, there exist household water treatment options that could help protect the health of the users. Some of these methods, which include solar disinfection (SODIS), chlorination, biosand filter (BSF) and boiling (Malema *et al*., 2016) should be inexpensive, simple, and easy to use (De Kwaadsteniet *et al*., 2013). Household water treatment has been extensively promoted as a solution to the problem of poor drinking water quality in developing countries (Sobsey *et al*., 2008). However, to choose an appropriate method, knowledge of the quality of the water is needed. Therefore, the present study aimed at determining the microbial and physicochemical quality of harvested rainwater so as to recommend appropriate treatment methods before use.

3.2 MATERIALS AND METHODS

3.2.1 Study area

Grahamstown/Makhanda (33°18′36″S; 26°31′36″E) under Makana Local Municipality is situated in the Eastern Cape Province, South Africa. It is located 60 km inland, 130 km northeast of Port Elizabeth and 170 km southwest of East London (Figure 3.1). The town, which was founded in 1820 as a military outpost, has a population of approximately 80,390 inhabitants (Statistics SA, 2016). Depending on the season, mean temperatures in Grahamstown fluctuate between 5 °C in winter (Jun-Sept) and 35 °C in summer (Nov-Apr). Mean annual rainfall in the years 1970-2010 was 650 mm (Zengeni *et al*., 2016), with bimodal peaks in October -November and again in March-April. Kenton-on-sea of Ndlambe Local Municipality (33° 42' 0" South, 26° 41' 0" East), commonly referred to as Kenton, is a small coastal town on the Sunshine Coast, in the Eastern Cape of South Africa.

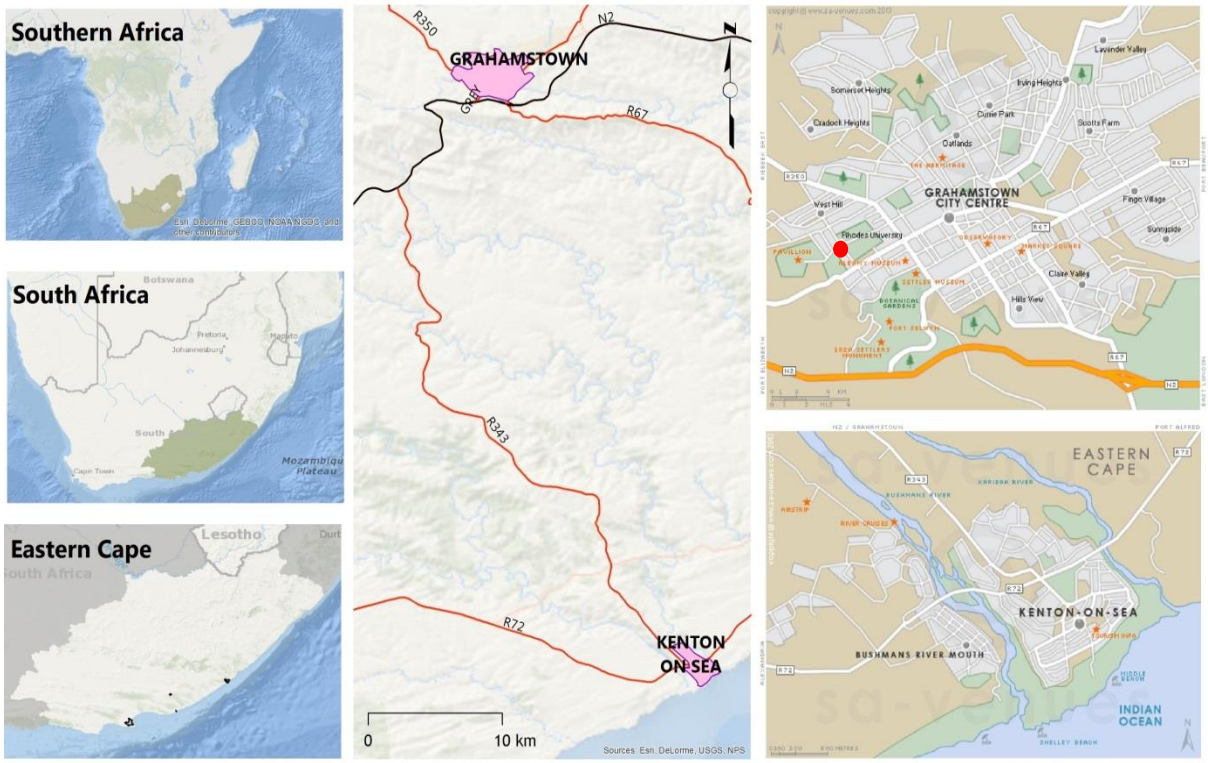

Figure 3.1: *Map showing the three study sites: Rhodes University (●), Kenton-on-sea and Grahamstown west*

Kenton-on-sea is situated between the Bushmans and the Kariega Rivers and lies between the industrial centres of East London (180 km) and Port Elizabeth (130 km). The distance between Rhodes University (RU) and Grahamstown west is approximately 4 km while the distance between RU and Kenton-on-sea is 59.2 km. These three sites were selected for sample collection based on diversity in environmental conditions (e.g. presence of foliage and birds), the age of tanks, as well as the various uses of the water

stored in the tanks (Table 3.1). The rainwater harvesting systems were made of the catchment area (roof), gutters and finally a storage tank. The rainwater harvesting systems were situated in both semi-urban and urban areas. Five of the selected systems had tile roofs (three on campus and two in Kenton-on-sea), while the other five had galvanised roofing (three in Grahamstown west and two on campus). One system located at Rhodes University had an asbestos roof (Table 3.1). Tanks in all three areas, however, were made of the same polyethylene material. None of the systems was equipped with pre-tank treatment devices such as first flush diverters or gutter guards. Out of the eleven DRWH systems selected, only one system located on the RU campus (T5, Table 3.1) had a chlorinator to treat the water post-storage. No cleaning maintenance was carried out neither on the tanks nor the roof.

Table 3.1: *Site characteristics of rainwater harvesting tanks*

Site	Location	Overhanging Tree branches and birds	Treatment	Rainwater usage	Tank age (years)	Roof type
T1	Rhodes University	Yes	None	Emergency Flushing of toilets	3	Tile
T2	Rhodes University	Yes	None	Emergency Drinking water source	3	Galvanized
T3	Rhodes University	Yes	None	Drinking	3	Galvanized
T4	Rhodes University	No	None	Emergency Flushing of toilets	6	Asbestos
T5	Rhodes University	Yes	Chlorinator	Drinking	-	Tile
T6	Rhodes University	No	None	Emergency Flushing of toilets	3	Tile
T7	Kenton (coastal)	No	None	Dishwashing	8	Tile
T8	Kenton (coastal)	No	None	Dishwashing	8	Tile
T9	Grahamstown west	No	None	Drinking	4	Galvanized
T10	Grahamstown west	No	None	Watering the garden	6	Galvanized
T11	Grahamstown west	No	None	-	6	Galvanized

3.2.2 Sample collection

Harvested rainwater samples were collected from 11 tanks where 6 tanks were situated at Rhodes University, 2 tanks at Kenton-on-sea and finally 3 tanks situated at Grahamstown West. Rainwater

samples were collected from the outlet of the tanks over a three-month period (July-September) in 2016. Furthermore, each rainwater harvesting tank was sampled 12 times in the 3-month period of the study bringing the total number of samples tested in 3 months to approximately 132. Five-litre plastic bottles were used to collect the water samples. The bottles were sterilised by first washing with antibacterial soap, rinsed with tap water followed by soaking in 30% HCl for 5 minutes and finally rinsed with sterile distilled water. Once a week, sterile 5 L bottles were used to collect rainwater samples by first rinsing the tap connected to the tanks with 70% Ethanol and letting the tap run for 30 seconds before collection. Samples were then transported to Rhodes University's Microbiology laboratory on ice for microbial analysis within 6 hours. An aliquot of 1 L was preserved from each sample and was used to determine the physicochemical parameters.

3.2.3 Microbial analysis

Escherichia coli was enumerated in triplicates using the Colilert-18® / Quanti-tray®/2000 (IDEXX Laboratories, Inc., Johannesburg South Africa). Briefly, 100 mL of water sample was mixed with the Colilert reagent, allowed to dissolve and transferred to a Quanti-tray®/2000. The trays were then sealed with a Quanti-tray® sealer and incubated at 37˚C for 18-24 hrs. A positive (*E. coli* ATCC 29522 inoculated into sterile water) and negative (sterile water) controls were included. Following incubation, the Quanti-tray®2000 were examined under UV light for fluorescent wells. The most probable number (MPN)/100 mL values were recorded according to a tabulation of 95% confidence intervals provided by the manufacturer.

3.2.4 Physicochemical analysis

Turbidity was measured in duplicate using a turbidity meter (Lutron TU-2016, Lutron Electronic Enterprise CO.LTD, Taiwan) and pH was tested in triplicate using a pH meter (Oakton, multi-parameter PC TestR-35, Eutech Instruments). Nitrate was determined using the US EPA 353.2 method (US EPA, 1993) and COD was determined using the closed reflux colourimetric method (APHA, 1998). Samples were measured in triplicate using the Shimadzu 1240 UV/VIS spectrophotometer according to the manufacturer's instructions.

3.2.5 Statistical analysis

Data analysis was done using the Statistical Package for the Social Sciences (SPSS) version 16.0 (Norusis, 2008). The non-parametric Spearman's rank correlation was used to assess the relationship between *E. coli* counts, pH, turbidity, nitrate, COD and tank age. Further non-parametric spearman's

rank correlations were performed to determine the relationship between *Escherichia coli*, roof type, tank location, tank age and presence/absence of tree branches using indicative variables. One-way Analysis of Variance (ANOVA) was used to determine if there was any significant difference between the concentrations of *E. coli* observed in tanks located in the Grahamstown west and those located in RU, given that the sites were 4 km apart. ANOVA results were considered significant when $p < 0.05$.

3.3 RESULTS

3.3.1 Microbial quality of harvested rainwater

Escherichia coli was detected in all the tested rainwater tanks (Figure 3.2). The mean concentrations of *E. coli* obtained were above the minimum requirements as set out by the World Health Organisation (0 CFU/100 mL) for drinking water. The mean *E. coli* counts in T1 to T6 situated at RU ranged between 220 and 1055 MPN/100 mL. The mean concentration of *E. coli* in the two tanks (T7 and T8) in Kenton-on-sea ranged between 129 and 244 MPN/100 mL, while in tanks T9 and T11 located in the Grahamstown west the mean *E. coli* counts ranged between 7 and 82 MPN/100 mL. The three highest mean *E. coli* concentrations were recorded in tanks that were three years old, while the lowest mean *E. coli* concentrations were observed in tanks that were four years and eight years old.

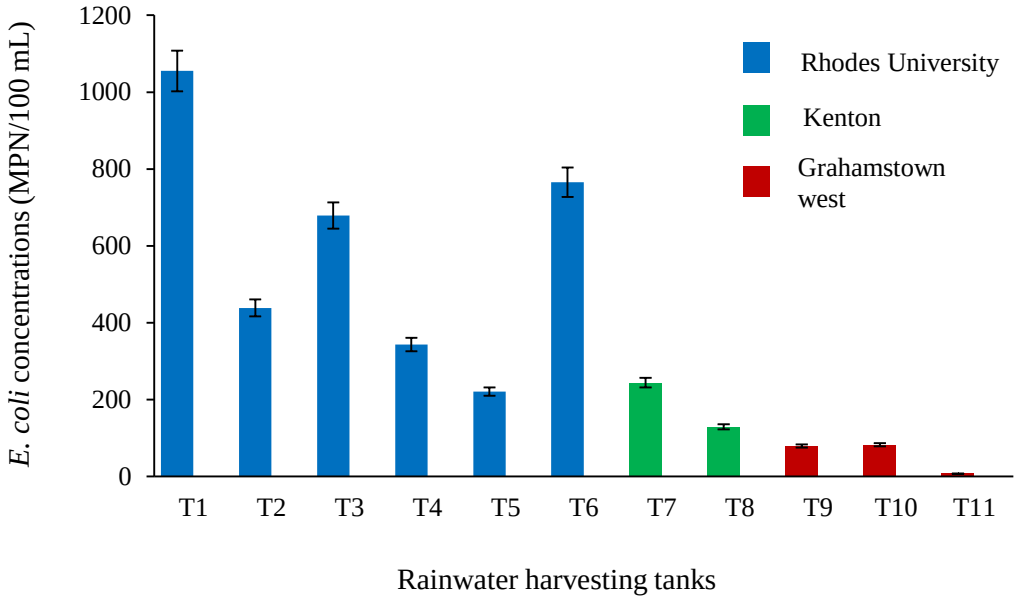

Figure 3.2: *The mean concentration of E. coli sampled from 11 tanks situated at Rhodes University, Kenton-on-sea and Grahamstown west*

Though tanks in RU and Grahamstown west were situated only 4 km away from each other, there was a statistically significant difference in *E. coli* counts ($p = 0.000$, $p < 0.05$) between the two locations.

3.3.2 Physicochemical parameters of harvested rainwater

The results of the physicochemical analysis of the water samples are presented in Table 3.2 and are compared to the South African drinking water quality guidelines. The pH values of all water samples collected fell within the drinking water guidelines (pH 5.0-9.7) with approximately 80% of samples having a neutral pH of about 7. Turbidity values obtained for all tanks were below 5 NTU except for tank 4 (5.12 ± 4.96 NTU) and 7 (5.58 ± 8.19 NTU). Elevated (> 10 mg L^{-1}) nitrate levels were observed in all tanks except in T1, which had a nitrate level of 5.9 ± 2.12 mg L^{-1}. Also, apart from T1 (66.53 ± 68.00 mg L^{-1}) and T6 (69.34 ± 83.21 mg L^{-1}), the COD levels in all tanks exceeded the recommended limit (< 75 mg L^{-1}) as set by the South African drinking water quality guidelines (DWAF, 1996a).

Table 3.2: *Physicochemical parameters for harvested rainwater collected from tanks situated at Rhodes University, Kenton-on-sea and Grahamstown west (values: mean ± standard deviation)*

Sample ID	pH SA limit 5.0-9.7	Turbidity (NTU) SA limit <5 NTU	Nitrate (mg l^{-1}) SA limit <10 mg l^{-1}	COD (mg l^{-1}) SA limit <75 mg l^{-1}
T1	7.6±0.7	1.21±0.78	5.95±2.12	66.53±68
T2	6.4±0.4	2.24±1.73	28.12±14.39	**148.07±118.57**
T3	5.6±0.6	3.01±2.16	13.2±12.35	**79.68±82.00**
T4	7.5±0.6	**5.12±4.96**	8.71±5.25	**191.12±216.37**
T5	7.4±0.3	2.43±3.12	**15.36±8.60**	**102.94±106.19**
T6	7.6±0.5	1.24±1.43	**11.03±4.78**	69.34±83.21
T7	7.3±0.3	**5.58±8.19**	**12.82±4.45**	**124.92±54.46**
T8	7.3±0.2	2.83±1.67	**11.63±6.76**	**158.88±154.31**
T9	7.6±0.5	3.18±2.69	**12.12±8.74**	**90.54±65.17**
T10	6.9±0.3	3.39±3.50	**16.63±5.49**	**105.5±96.69**
T11	7.0±0.3	0.49±0.67	**17.03±11.31**	**82±90.3**

Note: values in bold show parameters exceeding the recommended South African drinking water quality standard

3.3.3 Correlation between *Escherichia coli* and physicochemical parameters

The correlations between *E. coli* counts in the various tanks and measured physicochemical parameters are shown in Table 3.3. There was no statistically significant correlation between the *E. coli* counts and the physicochemical parameters in most of the tanks. There was, however, a statistically significant positive correlation between *E. coli* and pH ($p = 0.014$ and $p = 0.036$) observed in T2 and T3 respectively (Table 3.3). For the COD, a statistically significant negative correlation was observed in

T1 and T2 (p = 0.010 and p = 0.050, respectively). There was a statistically significant positive correlation (p = 0.011), between the *E. coli* counts and turbidity in T8 and a statistically significant negative correlation observed between the *E. coli* count and turbidity in T5 (p = 0.043).

Table 3.3: *Spearman's rank correlation coefficients for E. coli and physicochemical parameters of rainwater samples*

Sampling sites	Correlations	pH	Turbidity	Nitrate	COD
T1	r_s*	0.347	-0.354	-0.378	-0.873
	*P***	0.399	0.389	0.403	**0.010**
	N***	8	8	7	7
T2	r_s	0.857	0.408	-0.257	-0.812
	P	**0.014**	0.364	0.623	**0.050**
	N	7	7	6	6
T3	r_s	786	0.055	-0.543	-0.754
	P	**0.036**	0.908	0.266	0.084
	N	7	7	6	6
T4	r_s	0.048	0.304	-0.286	0.252
	P	0.911	0.464	0.535	0.585
	N	8	8	7	7
T5	r_s	-0.235	-0.722	-0.559	0.073
	P	0.576	**0.043**	0.192	0.877
	N	8	8	7	7
T6	r_s	-0.132	0.019	0.143	0.180
	P	0.756	0.964	0.760	0.699
	N	8	8	7	7
T7	r_s	-0.314	0.706	-0.300	0.000
	P	0.544	0.117	0.624	0.1000
	N	6	6	5	5
T8	r_s	-0.174	0.192	0.359	0-.821
	P	0.742	**0.011**	0.553	0.089
	N	6	6	5	5
T9	r_s	-0.200	-0.464	0.100	0.400
	P	0.704	0.354	0.873	0.505
	N	6	6	5	5
T10	r_s	-0.029	0.116	-0.300	0.000

Sampling sites	Correlations	pH	Turbidity	Nitrate	COD
	P	0.957	0.827	0.624	1.000
	N	6	6	5	5

*Spearman's rank correlation coefficient, ***p*-value, ***Number of samples. #Correlation is significant at the 0.05 level.

3.3.4 Correlation between *Escherichia coli*, roof type, tank location, tank age and presence/absence of tree branches

There was no statistically significant correlation between the *E. coli* counts and the type of roofing materials used ($p = 0.266$; $p > 0.05$). On the other hand, a statistically significant negative correlation ($p = 0.000$; $p < 0.05$) was observed between the mean *E. coli* counts and tank age, *E. coli* and tank location and *E. coli* and the presence/absence of tree branches.

3.4 DISCUSSION

3.4.1 Microbial quality of harvested rainwater

Water-borne pathogens may be present in urban roofs due to biological activity associated with depositions of wind-blown dirt (Sanchez *et al*., 2015). Ahmed *et al*. (2010) and Lee *et al*. (2012) reported that the rooftop as the most commonly used method of RWH can harbour enteric pathogens of which the source of contamination is likely to be faecal waste from birds, lizards, mice, dead animals and insects. In addition, during dry periods, dust, faecal deposits, rodents and birds are the major sources of heavy pollution (Ahmed *et al*., 2008). The finding by Ahmed *et al*. (2008) may further explain the results obtained in the current study as the sampling occurred during the dry season (June-September). After long dry periods caused by less rainfall the quality of harvested rainwater may be of serious health risks due to the accumulation of these pollutants especially if the water is harvested from rooftops (Ahmed *et al*., 2011).

Evans *et al*. (2006) investigated the effect of weather on roof-harvested rainwater and reported that microbial contamination of the rooftop was influenced by wind velocities. Thus, the findings of Evans *et al*. (2006) may provide some explanation for the mean *E. coli* counts observed in the current study. The *E. coli* counts from the current study exceeded the WHO and South African drinking water quality guidelines of 0 CFU/100 mL. Several studies have also reported on the high *E. coli* counts in harvested rainwater (Ahmed *et al*., 2012; Dobrowksy *et al*., 2014; Chidamba and Korsten, 2015), indicating the need to treat water from this source before use. In the current study, only one RWH system had a post-storage treatment device (chlorinator) which significantly improved the quality of the harvested rainwater during the early weeks of sampling. However, the treatment was interrupted when the

chlorinator clogged, and continued monitoring indicated an increase in *E. coli* counts. These findings stress the importance of the timely inspection and maintenance of treatment devices as these bacteria could be transferred to storage containers.

Previous studies have reported high concentrations of faecal and total coliforms in stored rainwater (Tandlich *et al*., 2012). Both pre-tank (gutter guard, first flush diverters) and post-tank (boiling, chlorination and filtration) treatment methods could significantly improve the quality of harvested rainwater. In the absence of post-tank treatment, it is advisable that the water be used only for non-potable outdoor purposes such as irrigation and vehicle washing (Tandlich *et al.,* 2012) as the South African water quality guidelines for irrigation purposes are less stringent on the microbial quality of the harvested rainwater (1-10 CFU/100 mL faecal coliforms) (DWAF, 1996d) thus, only one tank (T10) in Grahamstown west which was used for watering a garden met these criteria.

Three RWH systems situated on campus (T2, T3, T5) and one (T9) situated in the Grahamstown west were used for drinking purposes. Also, two of the RWH systems located in Kenton-on-sea (T7 and T8) were used for dishwashing. Given all the tanks in the current study did not meet the WHO and South African drinking water quality guidelines, they could be a potential health risk associated with the consumption of the water from these tanks without prior treatment. A recent study has shown that rainwater harvesting tanks in the Eastern Cape harboured antibiotic-resistant pathogenic *E. coli* strains (Malema *et al*., 2018). Although three of the RWH systems (T1, T4 and T6) situated on campus were used for emergency flushing, which is an indoor non-potable application, these tanks could also represent a potential health risk. It has been reported that substantial quantities of potentially infectious aerosols may be produced during flushing and that aerosolization can continue through multiple flushes to expose subsequent toilet users (Johnson *et al*., 2013). For example, the presence of *Legionella* spp. in rainwater used for toilet flushing may pose a health risk due to the inhalation of the organism through aerosols formed during toilet flushing (Schets *et al*., 2010).

3.4.2 Physicochemical parameters of harvested rainwater

Water used mainly for potable purposes such as drinking should be colourless, tasteless, odourless, free of turbidity and must not contain any chemicals or microorganisms capable of causing diseases (Ubuoh, 2014). However, physicochemical parameters tested in this study such as COD, nitrate and turbidity exceeded the South African drinking water quality standards. Elevated levels of COD such as those recorded in the current study (which ranged from 82±90.3 - 191.12±216.37) may indicate the risk of oxygen depletion due to degradation of organic matter in the storage system (Eriksson *et al*., 2002). Although it is suggested that the greatest threat associated with the consumption of polluted water is the presence of microorganisms in such water, excessive amounts of COD recorded may also indicate

the presence of oxygen-requiring microorganisms in the tanks. Similarly, nitrate levels in most of the tanks were above the country's drinking water quality guidelines. Similar results had been reported by Rim-Rukey *et al*. (2007) who suggested that using simple methods as bone char could considerably remove contaminants such as nitrate and COD from harvested rainwater.

Results of the physicochemical parameters obtained in the current study were not in agreement with a study earlier conducted in the country. The authors reported that the nitrate and COD in the rainwater harvesting tanks in their study were within the recommended limits set by South African drinking water quality guidelines (Dobrowksy *et al*., 2014). These discrepancies could be attributed to the difference in the sampling season and the study sites. For example, Ge *et al*. (2017) reported that high particulate matter resulted in high nitrate levels and that such observations occurred more in winter compared to summer. This could therefore explain the findings of the current study given that sampling was conducted in the winter season. Although no studies in South Africa has previously reported on elevated levels of COD and nitrate in harvested rainwater, such high values have been reported in other countries (Ikhifa *et al*., 2005 and Rim-Rukey *et al*., 2007).

3.4.3 Correlation between *Escherichia coli* and physicochemical parameters

The relationship between microorganisms and physicochemical parameters in harvested rainwater have previously been reported. For example, Kobayashi *et al*. (2014) reported that there was a correlation between the presence of *Legionella* species in rainwater tanks and COD values. Results from the current study corroborate those of Kobayashi and colleagues as positive correlations between *E. coli* counts and physicochemical parameters such as COD, pH and turbidity were observed. The observed positive correlations between pH and *E. coli* in the current study could be attributed to the fact that the pH levels in the tanks favoured *E. coli* growth as the organism grows best at a near neutral pH of 6-8 (Desmarchelier and Fegan, 2003). There was no observed correlation between nitrate levels and *E. coli* in all tanks. A similar study by Le Nhung *et al*. (2011) also reported the lack of a significant correlation between nitrate content and bacterial parameters. On the other hand, unlike the current study and that of Le Nhung *et al*. (2011), a recent study conducted in Philadelphia, USA did not find a significant correlation between all studied physicochemical parameters and the presence of pathogens in roof-harvested rainwater barrels Hamilton *et al*. (2018). Elevated levels of turbidity associated with organic matter can promote microbial growth in the storage tank (Momba and Notshe, 2003). Although high turbidity levels as those recorded in T8 could have contributed to the high *E. coli* counts in the tank, this was not the case with other tanks in the current study. No statistically significant correlation was observed between turbidity and the mean *E. coli* counts in the other tanks except T5 where a statistically significant negative correlation was observed between the two parameters. These differences could be associated to several other factors such as the tank material, roof materials and the prevailing air quality

around a particular location of the RWH storage tank, hence the relationship with the microbial life inside the tanks.

3.4.4 Correlation between *Escherichia coli*, roof type, location, tank age and presence/absence of tree branches

The Spearman's rank correlation was used to determine if there was any relationship between site characteristics of the RWHS such as overhanging tree branches, tank age, roof type and tank location. Correlation analysis yielded no significant positive results. The statistical analyses yielded negative correlations instead. The lack of a positive correlation between *E. coli* counts and factors such as overhanging tree branches, tank age, roof type and tank location in the current study suggest that the high *E. coli* counts found in the rainwater tanks may have originated from other sources. A study by Chubaka *et al*. (2018) took to assess whether building materials used in the catchment areas; building rooftops, gutters and tanks played a role in rainwater microbial contamination. It was found that there was no relationship between building structure materials and rainwater microbial content. This finding was similar to the one observed in the current study where factors such as roof type did not yield any relationship with the amount of *E. coli* found in rainwater.

Another study by Meera and Ahammed (2018) on the factors affecting the quality of roof-harvested rainwater noted that a high positive correlation ($r > 0.8$) was found between total coliforms and dry periods in all runoffs except asbestos. Furthermore, the authors reported that the positive correlation between the water quality parameters and dry periods was presumably due to increase in dry deposition and dissolution of roofing material. Though the current study did not determine the relationship between dry periods and *E. coli* counts, results suggest that dry deposition which is the accumulation of pollutants on the catchment area may have played a role in the high *E. coli* counts observed in this study hence the lack of a positive relationship between *E. coli* counts and factors such as roof type, location, tank age and presence/absence of tree branches. Furthermore, Chapman *et al*. (2008) studied water quality and health risks from urban rainwater tanks and revealed that there were similar levels of indicator bacteria in tank water irrespective of whether or not trees overhung the roof catchment supplying the rainwater tank i.e. prevalence of *E. coli* counts from tanks with overhanging trees were 23% while *E. coli* counts from tanks with trees within 5 m proximity were at 21%. Their study concluded that seasonality in bacterial counts is associated primarily with rainfall (i.e. run-off and associated top-up of tanks) and not with proximity of trees to the rainwater tank roof catchment. Results from Chapman *et al*. (2008) were similar to those from the current study where there was no relationship between the presence of overhanging tree branches and *E. coli* counts.

3.5 CONCLUSION

The physicochemical and microbial quality of rainwater samples examined during this three-month study indicates that in Grahamstown, Eastern Cape, the rainwater harvested is polluted. Microbial and physicochemical indicators tested exceeded allowable limits for potable uses. It is critical to have a comprehensive understanding of the contamination pathways and to determine the concentration of microbial and chemical contaminants in the harvested rainwater. This will not only guide the choice of the appropriate (pre- and post-tank) treatment methods but also take steps to mitigate rainwater contamination through maintenance best practices of the risk factors identified. Future research must include monitoring of DRWHS from various geographical regions in South Africa in order to fully document the type of contaminants found in these regions.

3.6 REFERENCES

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CHAPTER FOUR: THE EFFICIENCY OF A LOW-COST HYDROGEN SULPHIDE (H_2S) KIT: AN EARLY WARNING WATER QUALITY MONITORING TOOL FOR HARVESTED RAINWATER AT HOUSEHOLD LEVEL[3]

4.1 Introduction

Several methods are used to detect and quantify *E. coli* and other faecal coliforms in drinking water. However, these methods are complex, time-consuming and require laboratory facilities (WHO, 2011). World Health Organization recommends that the microbial quality of drinking water be measured using faecal indicator bacteria, preferably *E. coli*, to test for the presence of faecal contamination rather than identifying pathogens directly (WHO, 2008). Although *E. coli* is recommended as a standard indicator organism for this purpose, hydrogen sulphide (H_2S)-producing bacteria are alternative faecal indicator bacteria that have been shown to correlate with *E. coli* levels and their detection has been developed into field test products (Murcott *et al.*, 2015). The H_2S test kit (Manja *et al*., 1982) was developed to equip public health workers with a simple but reliable field test kit that can detect faecal contamination in drinking water. Most developing countries lack water testing facilities due to financial constraints and a shortage of trained water technician staff which further hampers their ability to ensure accurate assessments (Wright *et al.*, 2012). A study on the overall cost of monitoring microbial drinking water quality in Sub-Saharan Africa indicated that financial constraints are assumed to be the main barrier to conducting water quality tests (Delaire *et al*., 2017). Conducting a microbial water quality test involves four types of expenses, namely; consumables, laboratory equipment, labour (for collecting and analysing samples) and logistics such as transport and communication (Hunter *et al*., 2009; Crocker and Bartram, 2014; Delaire *et al*., 2017). Delaire and colleagues also revealed that the estimated cost of monitoring piped water supplies in sub-Saharan African countries at the time of their study, based on the WHO recommendations, varied between 1403 USD (Liberia) and 1655 672 USD (South Africa)

[3] Malema, M.S., Abia, A.L.K., Tandlich, R., Zuma, B., Mwenge Kahinda, J., Ubomba-Jaswa, E., 2018. The suitability of a low cost modified hydrogen sulfide (H2S) kit to determine the microbial water quality of harvested rainwater for potable use. Manuscript submitted to the Journal of Water and Health.

and amounted to 10931 000 USD per year for all the sub-Saharan African countries. Furthermore, the cost per-test of the H_2S as a water quality testing method in the monitored sub-Saharan countries was lower (8.3 USD) compared to membrane filtration (12.5±8.1 USD) and most probable number (MPN) techniques (14.0±12.4 USD). The lower costs of the H_2S test method is mainly because it is a simple field-based method without the need for specialised equipment and trained personnel (Delaire *et al.*, 2017). The modified H_2S test method was tested on harvested rainwater in South Africa as part of a pilot study of a community-based rainwater monitoring and treatment programme in the Grahamstown area in the Makana Local Municipality between March and June 2013 (Tandlich *et al.*, 2014). A total of 55 samples were collected from eight rainwater tanks during the period of the study. Results indicate that the modified H_2S test kit, which is a low-cost microbiological field-based test, was successfully used by non-governmental organisation volunteers to detect faecal contamination in harvested rainwater. Results further revealed a 71% rate of correspondence between the membrane-thermotolerant *Escherichia coli* (m-TEC) enumeration and the H_2S test kit. In rural and certain peri-urban areas, the use of Colilert® and the membrane filtration technique (MFT) for monitoring the microbial quality of water may not be a feasible choice due to the lack of investment capacity in building laboratories, thereby reducing logistical costs (US EPA, 2016). Therefore, to strengthen testing programs, capacity building must not only include laboratory development and staff training (US EPA, 2016) but also simple, robust and accurate early warning water quality tools which can give an indication of water quality at the point of use.

The H_2S test is based on detecting the presence of bacteria that produce H_2S (Sobsey and Pfaender, 2002). The test uses a medium with thiosulphate as a sulphur source and ferric ammonium citrate as an indicator. In this test, H_2S is produced by the reduction of thiosulphate which then reacts with the ferric salt (ferric ammonium citrate) to form an insoluble black ferrous sulphide precipitate (Mosley and Sharp, 2005). Members of the *Enterobacteriaceae* group such as *Salmonella, Citrobacter, Clostridia, Klebsiella* and *Proteus* can produce H_2S in such a medium (Sobsey and Pfaender, 2002). Other non-enteric bacteria, typically absent in drinking water, can reduce thiosulphate into H_2S under anaerobic conditions (Sobsey and Pfaender, 2002). The inhibition of non-faecal bacteria takes place by the addition of 0.5% deoxycholate (Sobsey and Pfaender, 2002). In the year 2017, it was reported that diarrheal disease was the second leading cause of death among children aged below 5 years. Globally nearly 1.7 billion cases of childhood diarrheal disease are reported and responsible for killing around 525,000 children every year (WHO, 2017). Although the incidences of diarrheal diseases mostly affect the low-income populations with poor access to safe water, sanitation, and urgent medical care, acute infectious diarrhea is also a common cause of outpatient visits and hospital admissions in high-income regions (Wazny *et al.*, 2013). Thus, there is a need for a wide range of rapid and affordable water quality tests at household level to ensure that users treat their water before use to prevent getting diarrheal diseases. This study aims to report on the efficiency of the improved modified H_2S test kit by comparing

it to two known standard tests, namely Colilert® and MFT. The sensitivity of the modified H_2S test was improved by the addition of 0.5% deoxycholate. The inclusion of bile salts such as sodium deoxycholate in the H_2S test facilitates the maximum recovery of intestinal faecal pathogens (WHO, 2002). Although Tandlich *et al*. (2014) compared the efficiency of the modified H_2S test kit to that of the m-TEC enumeration method for the evaluation of the microbial quality of harvested rainwater, the comparison of the modified H_2S test kit to the MFT and the defined substrate method (Colilert®) has not been reported.

4.2 MATERIALS AND METHODS

4.2.1 Sample collection

Sampling was conducted over a three-month period (June – September 2016) in the Eastern Cape from tanks situated at the Rhodes University, Kenton-on-sea (coastal) and in homes in the Grahamstown area on a weekly basis (Figure 3.1). Sample collection was done according to the procedure outlined in section 3.2.2.

4.2.2 Microbiological analysis

4.2.2.1 Preparation of the hydrogen sulphide (H_2S) test kits

The H_2S test kits were prepared as previously described by Luyt *et al*. (2011). The modified H_2S medium consisted of 40 g peptone, 3 g dipotassium hydrogen phosphate, 1.5 g ferric ammonium citrate, 2 g sodium thiosulfate, 2 ml Teepol, 0.5 g sodium deoxycholate, 2.5 mg L-Cysteine and 100 mL distilled water. The medium was mixed in a glass beaker and dissolved using a stirrer without heating for 30 minutes. One (1) mL of the medium was pipetted onto a strip of Whatman no.1 paper (5 x 10 cm), and this strip was then placed into a single 40 mL urine jar. The H_2S test kits were dried overnight at 54°C in an incubator (Panasonic Biomedical Sales, UK) with lids off. Following incubation, the H_2S kits were stored away from direct sunlight at room temperature (25°C) until use. The expected shelf life of the prepared H_2S test kits is 6 weeks.

4.2.2.2 Hydrogen sulphide method

The H_2S test method was performed following home-based instructions to test for faecal bacteria as previously described by Tandlich *et al*. (2014) with slight modifications on the incubation temperature. Briefly, five sterile H_2S test kits per sampling site were filled with 20 mL of the collected rainwater samples (total volume of urine jars was 40 ml). The contents of the H_2S test kits were hand shaken for

about 10 seconds and incubated at 37°C for 72 hrs instead of keeping the H_2S test kits in a warm place (room temperature) as described by Tandlich *et al*. (2014). The incubated H_2S test kits were checked every 12 hrs for any colour change. A black colour indicated the presence of H_2S-producing organisms (positive) while no colour change indicated the absence of H_2S-producing organisms. Both positive (*E. coli* ATCC® 25922) and negative (sterile distilled water) controls were included in each test.

4.2.2.3 Colilert

Escherichia coli was enumerated in triplicates using the Colilert-18® / Quanti-tray®/2000 (IDEXX Laboratories, Inc., Johannesburg South Africa). Briefly, 100 mL of water sample was mixed with the Colilert reagent, allowed to dissolve and transferred to a Quanti-tray®/2000. The trays were then sealed with a Quanti-tray® sealer and incubated at 37˚C for 18 -24 hrs. Positive *E. coli* culture (inoculated in sterile water) and negative (sterile water) controls were included. Following incubation, the Quanti-tray®2000 were examined under UV for fluorescence wells. The MPN/100ml values were recorded according to a tabulation of 95% confidence intervals provided by the manufacturer.

4.2.2.4 Membrane filtration technique (MFT)

The MFT was performed in triplicates to test for faecal coliforms where 100 mL of the harvested rainwater sample was filtered through a 0.45 µm sterile membrane filter (Millipore, Tokyo, Japan). The membrane filters were placed on m-FC agar (Merck, South Africa) plates and incubated at 44.5°C for 24 hours. After incubation, presumptive faecal coliforms (blue colonies) were counted.

4.2.3 Statistical analysis

Data analysis was done using the Statistical Package for the Social Sciences (SPSS) version 16.0. The non-parametric Spearman's rank correlation was used to determine if there was any correlation in terms of faecal coliform detection between the three methods (Colilert, MFT and H_2S test). Furthermore, a 2x2 contingency table (95% confidence level) method (Mack and Hewison, 1988) was used to determine sensitivity, specificity, positive predictive value, negative predictive value, false positive, false negative and accuracy. The correspondence rates were calculated as a+d/grand total x100 where a, is the true positive result, d is the true negative result and grand total represents the total number of samples tested.

4.3 RESULTS

4.3.1 Efficiency of the H_2S test to detect faecal bacteria in harvested rainwater

A total of 88 samples from 11 harvested rainwater tanks were analysed to determine the performance of the H_2S test kit in the detection of faecal contaminants compared to two standard methods (Colilert and MFT). The correspondence rates (Table 4.1) were calculated to monitor the performance of the new method (H_2S test) in comparison to the two standard methods. Figure 4.1 shows the difference between a negative and a positive H_2S test kit.

Table 4.1: *Correspondence rate of the H2S test when compared to MFT and Colilert*

		Colilert		**MFT**	
		Presence	Absence	Presence	Absence
H_2S test	Presence	52	0	46	6
	Absence	21	15	5	31
MFT	Presence	51	0	-	-
	Absence	22	15	-	-

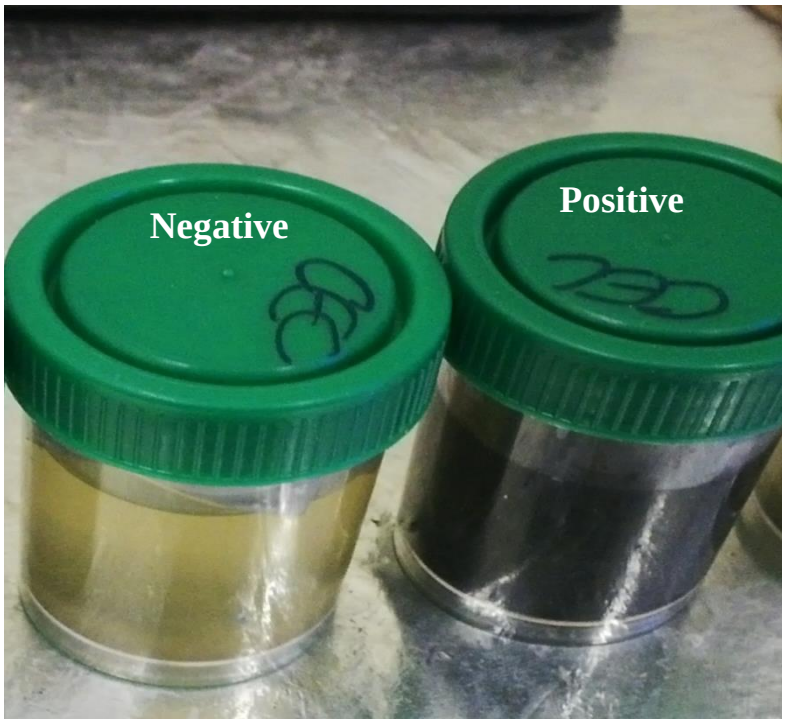

Figure 4.1: *Water samples analysed by modified H_2S kit indicating negative (left) and positive (right) results*

Comparison of the H_2S test as the new method and MFT as the standard method showed a 93% sensitivity and 88% accuracy. The H_2S test showed a false positive of 16% and a false negative of 9.8%

compared to the MFT. Using Colilert as the standard method, the H_2S test showed a sensitivity of 71% and a specificity of 100% to detect the presence or absence of faecal bacteria in harvested rainwater (Table 4.2) when compared to Colilert, the H_2S test had a false positive result of 0% and a false negative of 29%.

Table 4.2: *Comparison of H2S test with Colilert and MFT for performance efficiency*

Parameter	H_2S vs MFT %	H_2S vs Colilert %	MFT vs Colilert %
Sensitivity[1]	93	71	70
Specificity[2]	88	100	100
Positive predictive value[3]	92	100	100
Negative predictive value[4]	91	42	41
Accuracy[5]	88	76	75

Senssitivity[1]= true positive results/ (true positive results + false negative results)

Specificity[2]= true negative results/ (true negative results + false positive results)

Positive Predictive value[3]= true positive results/ (true positive results +false positive)

Negative predictive value[4]= true negative results/ (true negative results +false negative)

Accuracy[5]= (true positive results + true negative results)/total number of samples

4.3.2 Performance of H_2S test on the detection of faecal bacteria from individual rainwater tanks

The H_2S test detected faecal bacteria in all the tanks except at sampling site 11 (Figure 4.2). Sampling sites referred to as S1-S11 in this chapter are the same as T1-T11 from chapter 3. Sampling site 11 presented low bacterial counts compared to the other rainwater harvesting tanks throughout the sampling period. At sites S1 S2, S4 and S5, there was a correlation between the two methods (H_2S and MFT) in the detection of faecal bacteria. Furthermore, the H_2S test performed better in S6, S8, S9 and S10 when compared to MFT. Table 4.3 shows the correspondence of the H_2S test when compared to Colilert and MFT.

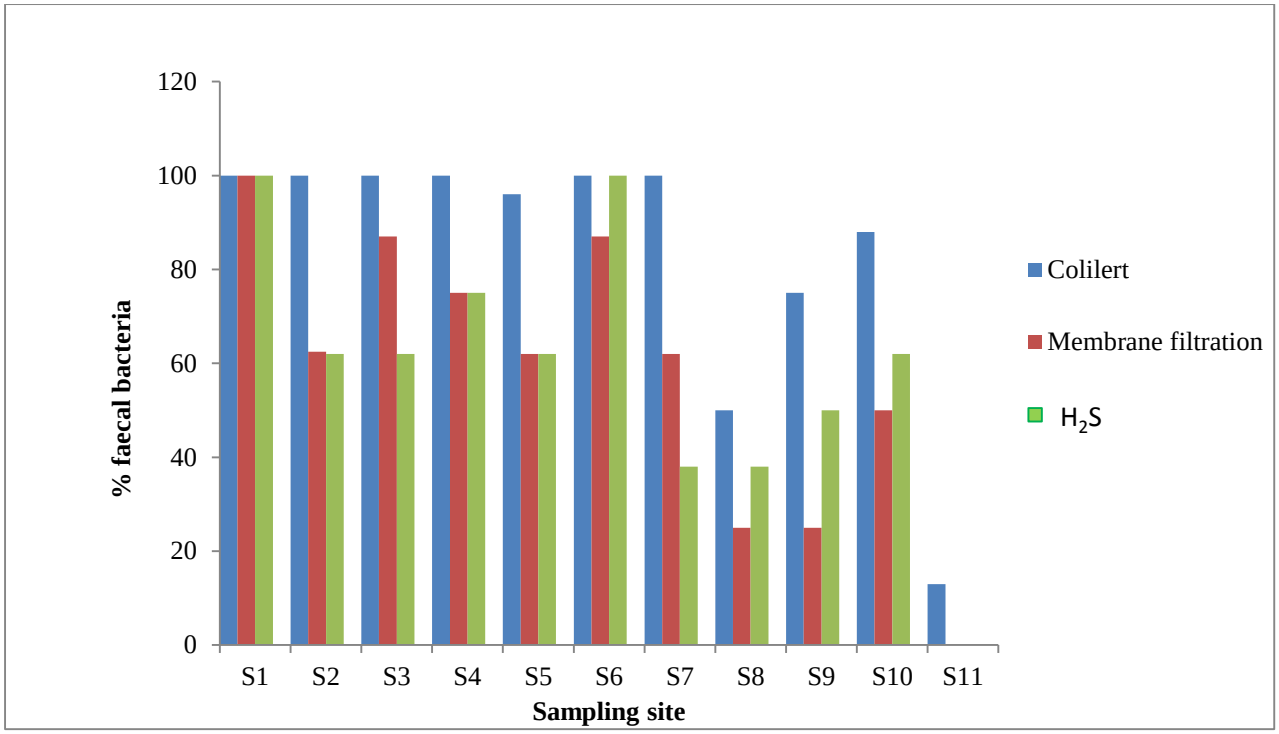

***Figure 4.2**: Percentage detection of faecal bacteria using the H_2S test, Colilert and membrane filtration*

Table 4.3: *Correspondence of the H_2S test in comparison to Colilert and MFT*

Sampling frequency	**S1**			**S2**			**S3**			**S4**			**S5**			**S6**			**S7**			**S8**			**S9**			**S10**			**S11**		
	Colilert	MFT	H_2S	Colilert	MFT	H_2S	Colilert	MFT	H_2S	Colilert	MFT	H_2S	Colilert	MFT	H_2S	Colilert	MFT	H_2S	Colilert	MFT	H_2S	Colilert	MFT	H_2S	Colilert	MFT	H_2S	Colilert	MFT	H_2S	Colilert	MFT	H_2S
1	>2419.6	>300	+	1161	>300	+	652	>300	+	>2419.6	>300	+	10	0	-	>2419.6	>300	+	181	9	+	202	5	+	8	0	+	<1	0	-	9	0	-
2	1073	>300	+	99	0	-	437	>300	+	5	0	-	19	0	-	346	0	+	506	6	+	187	18	+	<1	0	-	448	>300	+	<1	0	-
3	1769	>300	+	576	38	+	533	>300	+	153	0	+	<1	0	-	1081	>300	+	237	7	+	86	0	+	76	0	-	338	50	+	<1	0	-
4	408	>300	+	206	0	-	435	76	-	666	>300	+	325	54	+	986	>300	+	185	4	-	<1	0	-	651	>300	+	8	0	-	<1	0	-
5	962	>300	+	511	0	-	819	0	-	293	>300	-	634	>300	+	613	>300	+	66	0	-	76	0	-	1120	56	+	267	44	+	<1	0	-
6	1859	81	+	673	53	+	1841	114	-	724	48	+	734	140	+	994	>300	+	847	0	-	<1	0	-	36	0	-	198	0	-	<1	0	-
7	1859	>300	+	678	28	+	>2419.6	>300	+	627	42	+	977	>300	+	979	>300	+	583	5	-	<1	0	-	59	0	+	218	6	+	<1	0	-
8	994	>300	+	569	42	+	>2419.6	>300	+	750	>300	+	977	>300	+	>2419.6	>300	+	445	0	-	<1	0	-	<1	0	-	312	0	+	<1	0	-

S1-S11 represents sampling sites, Colilert measured *E. coli* counts, MFT measured faecal coliform counts, + represent presence of H_2S producing bacteria, - represent absence of H_2S producing bacteria

4.3.3 Correlation between results of Colilert test, membrane filtration and H_2S test in the detection of faecal bacteria

Statistical analysis was performed to determine if there was any correlation in terms of faecal bacterial detection between the three methods (Table 4.4). Results showed a positive significant correlation between Colilert and H_2S (r = 0.02, p<0.05). A similar significant positive correlation was observed between MFT and H_2S (r= 0.001, p<0. 05).

Table 4.4: *Correlation between results of the H_2S, Colilert and MFT*

		Correlations	**Colilert**	**MFT**	**H_2S**
	Colilert	r_s	1.000	.869**	.686*
		P	-	**0.001**	**0.020**
Spearman's rho		N	11	11	11
	MFT	r_s	.869**	1.000	.865**
		P	**0.001**	-	**0.001**
		N	11	11	11
	H_2S	r_s	.686*	.865**	1.000
		P	**0.020**	**0.001**	-
		N	11	11	11

**. Correlation is significant at the 0.05 level (2-tailed).

*. Correlation is significant at the 0.01 level (2-tailed).

4.4 DISCUSSION

4.4.1 Efficiency of the H_2S test to detect faecal bacteria in harvested rainwater

The H_2S test method was assessed in this study for its efficiency in the detection of faecal bacteria in harvested rainwater. When compared to the MFT, the H_2S test showed greater correspondence rate in the detection of faecal bacteria rendering it effective at 88% detection rate. However, in comparison to Colilert, the H_2S test showed a correspondence rate of 76%. It was also observed that the H_2S test kit performed better at higher contamination level (≥50 MPN/100mL) in the rainwater samples. This observation could explain the difference in correspondence rate between Colilert and the H_2S test because Colilert was able to detect contaminants at a count of 5 MPN/100 mL. In a previous study by

Tandlich *et al*. (2014) where the efficiency of the H_2S test was test in comparison to the membrane filtration method, a 71% correspondence rate was obtained for the modified H_2S test. These findings agree with the results of the current study where the H_2S test method was effective at 88% for faecal bacterial detection when compared to the standard MFT method. The accuracy of the H_2S test method was thus confirmed by the current study. The H_2S test kit may be used as a home-based test to monitor the quality of harvested rainwater. The test offers users of harvested rainwater the ability to test the water at household level. It should also be emphasised that should the H_2S test show a positive result, the water should not be used for any potable purposes such as drinking without treatment. The contaminated harvested rainwater could be treated at household level using treatment options such as boiling and chlorination prior to use. The modified H_2S test kit used in this study was first introduced and piloted by Luyt *et al*. (2011) and locally produced at a cost of five Rands (R5) or 0.34 USD using basic laboratory chemicals and everyday household materials such as urine jars. The modified H_2S test is a good early warning system that does not require laboratories or sophisticated equipment to establish whether or not water is contaminated by faecal bacteria (Luyt *et al*., 2011). A study by Wallis (1991) in Thailand reported that rainwater tanks had a 20 % false positive and 41 % false negative using the H_2S test compared to the MFT.

These results differ from our findings were false positives were 16% and false negatives were at 9.8% when H_2S was compared with MFT. False negatives occur when standard methods indicate the presence of contamination, a positive result but the new test indicates that the water is safe, a negative result. Alternatively, false positives occur when a new test indicates that a water source is contaminated, a positive result when in fact it is not. The improvement in detection ability of the H_2S kit in this current study can be attributed to the modification that the kit had undergone. Furthermore, a false positive result is less likely to lead to a risk of disease because it would result in the contaminated water either not being used, subjected to additional testing or treated. However, a false negative is of great concern as it means the contaminants are not detected by the new test (WHO, 2002) and hence users might be exposed to microbial contaminants that could make them sick. In the current study, the H_2S test gave a false negative of 9.8% with MFT and 29% when compared to the Colilert method.

4.4.2 Performance of H_2S test on the detection of faecal bacteria from individual rainwater tanks

Analysis from the current study showed that in most cases where rainwater samples had high faecal bacterial counts, the H_2S test showed a positive test as well (Table 4.3). The observation between the Colilert and the H_2S test suggests that the H_2S test is more efficient when the *E. coli* count in the rainwater samples is ≥50 MPN/100 mL. The results indicate the importance of using standard methods together with the H_2S test and moreover, the results further suggest that the correspondence rates must be measured on an ongoing basis throughout the programme where the H_2S test kit is used. In almost all the tested samples (S1-S10) the performance of H_2S test was satisfactory and proved to be competent when compared to MFT and Colilert. Water samples with low faecal bacterial counts resulted in negative H_2S test. This finding suggests that in low contaminated water, the H_2S test may not be a suitable method as pathogenic organisms may be present while the H_2S test shows the water is safe.

Furthermore, it is advisable to validate the H_2S test against standard methods, prior to distributing the test in a new setting. This will ensure that issues regarding both the quality of the water (to be tested regularly with the H_2S test) and the reliability of the H_2S test are properly addressed. Therefore, knowledge on the quality of the water to be tested is very important in order to minimize false negatives, especially in cases where contamination is seldom encountered. With sufficient public education on the H_2S test, households will be in charge of their own water safety. If results indicate high risk, households would be instructed to treat their water to make it fit for human consumption (Matwewe *et al*., 2018).

4.4.3 Correlation between results of Colilert test, membrane filtration and H_2S test in the detection of faecal bacteria

Results showed a higher positive correlation coefficient of ($r = 0.001$, $p<0.05$) in terms of faecal coliform detection between the H_2S test method and MFT. However, a weak positive correlation (r =

0.02, $p<0.05$) was observed in terms of faecal bacterial detection between Colilert and H_2S test. This observation could be attributed to the fact that the H_2S test failed to detect faecal bacteria in low contaminated water while Colilert consistently detected faecal bacteria even in low contaminated water. However, sensitivity levels between the H_2S method and standards methods may differ according to the type of standard method used. Vasudevan and Tandon (2008) conducted research on the microbial quality of rainwater from roof surfaces and performed similar tests using MPN, MFT and H_2S and their results were in agreement with the results obtained in this study in terms of the positive correlations in terms of faecal bacterial detection observed between the 3 methods during high bacterial counts.

Vasudevan and Tandon (2008) further stated that in some cases, H_2S showed that the water was potable while the MPN method disagreed. This finding differs from the current study in that no false positives were identified between Colilert and H_2S. Omar and Bhutada (2016) studied the bacteriological assessment of drinking water and reported that the H_2S test recorded, 20 positive samples out of the 38 samples tested, while 24 samples were positive for faecal contamination with MFT. The study from Omar and Bhutada (2016) further reported that 10.52% of the total number of samples tested as false negatives when the H_2S test was compared to MFT. Based on the low difference between the H_2S and the MFT observed in their study, the authors concluded that the H_2S test was a reliable and alternative method for the detection of faecal contamination during drinking water quality surveillance and screening of large numbers of water samples in a short duration in the field where laboratory facilities are limited.

4.5 CONCLUSION

The findings of this study suggest where harvested rainwater has ≥ 50 MPN/100 mL of *E. coli*, the H_2S kit is an effective and accurate preliminary test and has a positive correlation with the Colilert and MFT tests and can indicate if the water is safe for consumption. The findings of this study suggest that the H_2S test is efficient in water contaminated with >50 MPN/100 mL of *E. coli*, it can be concluded that with the rainwater tanks tested in the current study (with an average of 362 MPN/100

mL, the H_2S test will be an effective water quality monitoring tool. However, in some instances where *E. coli* contamination was < 50 MPN/100 mL and still could contain pathogenic microorganisms, the H_2S kit is not as sensitive and accurate. Therefore, determine the quality of water with standard methods in conjunction with the H_2S test is very important prior to distributing the H_2S test to every community to eliminated chances of encountering false negatives.

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CHAPTER FIVE: ANTIBIOTIC-RESISTANT PATHOGENIC *ESCHERICHIA* COLI ISOLATED FROM ROOFTOP RAINWATER-HARVESTING TANKS IN THE EASTERN CAPE, SOUTH AFRICA[4]

5.1 INTRODUCTION

Several countries around the world, including South Africa, make use of harvested rainwater (HRW) to meet their daily water needs. However, the most significant issue relating to the use of harvested rainwater is the potential health risk associated with the presence of various pathogenic organisms in such water (Ahmed *et al*., 2011). Indicator organisms like *E. coli* have been used to determine the microbiological safety of water meant for drinking and other human needs. Although most *E. coli* strains are non-pathogenic, certain strains may be pathogenic and carry virulence genes (VGs) (Masters *et al*., 2011). Pathogenic *E. coli* strains which can cause diseases in both humans and animals are categorised as intestinal pathogenic *E. coli* (InPEC) and extraintestinal pathogenic *E. coli* (ExPEC) (Russo and Johnson, 2000). Intestinal strains are mostly referred to as diarrheagenic *E. coli* (DEC) due to their ability to cause diarrhea using diverse mechanisms (Canizalez-Roman *et al*., 2013). The ExPEC strains have been reported to cause diseases such as urinary tract infections, neonatal meningitis, sepsis and wound infections and some examples include neonatal meningitis *E. coli* (NMEC) and uropathogenic *E. coli* (UPEC) (Russo and Johnson, 2000). Six groups of DEC strains known to

[4] Malema, M.S., Abia, A.L.K., Tandlich, R., Zuma, B., Kahinda, J.M.M., Ubomba-Jaswa, E. (2018). Antibiotic-resistant pathogenic Escherichia coli isolated from rooftop rainwater-harvesting tanks in the Eastern Cape, South Africa. Int. J. Environ. Res. Public Health 15 (5), 892. doi:10.3390/ijerph15050892

cause intestinal infections include enterotoxigenic *E. coli* (ETEC), enteropathogenic *E. coli* (EPEC), enterohemorrhagic *E. coli* (EHEC), enteroaggregative *E. coli* (EAEC), diffusely adherent *E. coli* (DAEC) and enteroinvasive *E. coli* (EIEC). Among all *E. coli* pathotypes, ETEC strains cause a cholera-like diarrheal disease and are the most common cause of childhood and travellers' diarrhea in developing countries (Jafari *et al*., 2012). Diffusely adherent *E. coli* pathotypes were previously implicated in intestinal infections (diarrhea in children between the ages of 18 months and 5 years) and extraintestinal infections (urinary tract infections and pregnancy complications) (Servin, 2014). Enteroinvasive *E. coli* strains shows pathogenic phenotypic and genetic similarities with *Shigella* spp. and can be identified by their epithelial cell invasiveness mediated in part by the *ipaH* and *virF* genes and association with dysentery (Karmali *et al*., 2010). EHEC is associated with bloody diarrhea and haemolytic uremic syndrome and expresses one or two Shiga-like toxin-encoding genes *stx1* and *stx2* (Viazis and Diez-Gonzalez, 2011). Several virulence genes in these *E. coli* pathotypes are responsible for a wide array of infections such as diarrhoea or haemolytic colitis, neonatal meningitis, nosocomial septicaemia, haemolytic-uraemic syndrome and urinary tract infections (Anastasi *et al*., 2010). Current molecular-based techniques such as polymerase chain reaction (PCR) allow for the identification of these VGs by amplifying specific target regions (Costa *et al*., 2014). Virulence genes associated with these pathogenic strains have been isolated in diverse environments in South Africa. For example, the presence of DEC virulence genes in 60% of samples collected from the Apies River (water and sediments) was reported by Abia *et al*. (2016).

In another study, a high prevalence of virulence genes associated with four pathogenic *E. coli* types (EAEC, EHEC, EPEC, and EIEC) in domestic rainwater harvesting tanks in Kleinmond, Cape Town was documented by Dobrowsky *et al*. (2014). Apart from being pathogenic, some of these microorganisms have developed resistance to many of the drugs designed to treat the infections they cause. For example, the antimicrobial resistance patterns of *E. coli* isolates in outpatient urinary tract infections in South Africa was studied and the results revealed that the isolated *E. coli* were resistant to trimethoprim-sulfamethoxazole (TMP-SMX; 68%), amoxicillin (65%) and ciprofloxacin (41%) (Bosch *et al*., 2011). Another study focused on the hospital, and community isolates of uropathogens at a tertiary hospital in South Africa and results revealed that the most isolated bacterial pathogen was *E. coli* (39%) (Habte *et al*.,

2009). Furthermore, levels of *E. coli* resistance to amoxicillin and co-trimoxazole ranged from 43–100% and 29–90%, respectively.

The presence of such drug-resistant bacteria in human settings has placed constraints on the choice of safe, effective and inexpensive antibiotics, especially for low- and middle-income countries (Detels *et al*., 2015). As such, the progression of resistant bacteria and the increasing incidence of antibiotic resistance genes (ARGs) are thus of significant public health concern (Xu *et al*., 2016). Although studies have been carried out on the presence of virulence genes and antibiotic-resistant bacteria in various water sources such as wastewater effluents, taps, wells and boreholes in South Africa, very few studies have investigated their presence in harvested rainwater (Dobrowsky *et al*., 2014; Kinge *et al*., 2010; Adefisoye and Okoh, 2016; Abia *et al*., 2017). This study aimed at reporting on the prevalence of pathogenic *E. coli* strains and their antibiotic resistance patterns in harvested rainwater collected from tanks in the Eastern Cape Province of South Africa. Such results would highlight the need for appropriate development and implementation of effective household water treatment methods, thereby protecting the lives of populations using such water for their daily needs. Moreover, results of the current study will also add to existing research databases which report on the circulating strains of antimicrobial-resistant organisms.

5.2 MATERIALS AND METHODS

5.2.1 Study Site and Sample Collection

Rooftop-harvested rainwater samples were collected from 11 rainwater-harvesting systems situated at various sites around Grahamstown west, Rhodes University campus and Kenton-on-sea in the Eastern Cape Province, South Africa. The distance between Rhodes University (33°31′36″ S, 26°51′63″ E) and Grahamstown west (33°18′36″ S; 26°31′36″ E) is approximately 4 km while the distance between Rhodes University and Kenton-on-sea (33°42′0″ S, 26°41′0″ E) is 59.2 km. Mean annual rainfall precipitation in Grahamstown is 650 mm, with bimodal peaks in October–November and again in March–April. All the sites were selected based on the diversity in environmental conditions (e.g., presence of foliage and birds) as well as the various uses of the water stored in the tanks. A total of 110 water samples were collected

from the 11 selected tanks from June 2016 to September 2016 and tested for *E. coli*. Sample collection was done as outlined in section 3.2.2.

5.2.2 Enumeration and isolation of *Escherichia coli*

Enumeration of E. coli was carried out using the Colilert-18® Quanti-tray®/2000 (IDEXX Laboratories, Inc., Johannesburg, South Africa). The test was performed following the manufacturer's instructions. After incubation at 37 °C for 18–24 h, presumptive *E. coli* isolates were obtained from fluorescent quanti-tray wells as described by Abia et al. (2015). The Colilert method has a detection limit ranging from <1 MPN/100 mL to >2419.6 MPN/100 mL. *E. coli* ATCC® 25922 was used as a positive control and Pseudomonas aeruginosa ATCC 49189 as a negative control. One hundred (100) *E. coli* isolates were then selected from the various tanks. Of the 100 isolates selected, 66 isolates were chosen from T1-T6 (11 isolates from each tank), 20 isolates were from T7 and T8 (10 isolates from each tank) and 14 isolates from T9 and T11. T10 was excluded from further analysis due to poor growth of the selected isolates from the culture media.

5.2.3 Identification of pathogenic *Escherichia coli* strains using polymerase chain reaction (PCR)

5.2.3.1 DNA Extraction and detection of virulence genes in *E. coli* isolates

One hundred (100) presumptive *E. coli* isolates were randomly selected and inoculated separately into 5 mL Erlenmeyer flasks containing 2 mL nutrient broth (Merck, Johannesburg, South Africa). The flasks were incubated overnight at 37 °C on a rotary shaker at 100 rpm. DNA was extracted from 1 mL of the overnight culture using the InstaGene™ Matrix (Bio-Rad Laboratories, Johannesburg, South Africa) following the manufacturer's instruction. The template DNA was stored at −20 °C for PCR assays. All selected samples were first confirmed as *E. coli* by testing for the presence of the malate dehydrogenase (*mdh*) gene which is found in most *E. coli* strains (Hsu and Tsen, 2001). After that, the presence of a total of eight VGs (*eaeA (*EPEC/EHEC), *eagg* (EAEC), *ipaH* (EIEC), *ST* (ETEC), *ibeA* **(**NMEC), *stx1* (EHEC), *stx2* **(**EHEC) and *flicH7* (EHEC)) were investigated. The primer sequences and the PCR-cycling conditions for the identification of the various VGs were as previously described by Abia *et al*. (2017). Both multiplex and singleplex PCR assays were performed for the target genes. Multiplex PCR assays were divided into 3 sets where set 1 contained *eaeA, eagg* and *ipaH,* set 2 contained *flicH7* and *Stx1* and finally set 3 contained *ST* and *ibeA* genes (Abia *et al*., 2017; Caine *et al*., 2014; Titilawo *et al*., 2015). Singleplex real-time PCR assays were performed for the *mdh* and *stx2* target genes (Omar and Barnard, 2014; Omar *et al*., 2010).

5.2.4 Screening for Antibiotic-Resistant *E. coli*

The remaining 1 mL from the overnight culture was used for antibiotic resistance analysis using the disk-diffusion method (Stange *et al*., 2016). Briefly, 100 µL of overnight *E. coli* culture was spread on Mueller–Hinton agar (Lasec, Cape Town, South Africa) and antibiotic mastrings (Davies diagnostics, Johannesburg, South Africa) were carefully placed onto inoculated plates, incubated at 37 °C for 18–20 h. Following incubation, the diameters (in millimetres) of clear zones of growth inhibition around the antibiotic disks were measured using a ruler and compared with the Clinical Laboratory Standard Institute (CLSI) 2013 reference values. The different phenotypic profiles (resistant, intermediate or susceptible) of the isolates were then determined following the interpretation of the zones of inhibition. A total of 11 antibiotics were selected for this study (Table 5.1). The antibiotics were chosen for their frequent use in the treatment of bacterial infections in South Africa Both positive (*E. coli* strain ATTC 25922) and negative controls (*E. coli* strain ATTC 35218) were included in the experiments.

Table 5.1: *Antibiotics used to determine antibiotic resistance of E. coli isolates*

Class	Antibiotic	Abbreviation	Concentration (µg)
β-Lactams	Ampicillin	AP	10
	Cephalothin	KF	5
Polypeptides	Colistin sulphate	CO	25
Aminoglycosides	Gentamicin	GM	10
Aminoglycosides	Streptomicin	S	10
Tetracyclines	Tetracycline	T	25
Folate pathway inhibitors	Cotrimoxazole	TS	25
Fluoroquinolones	Ciprofloxacin	CIP	5
Penicillin combination	Augmentin (amoxillin-clavulanate)	AUG	30
Sulfonamides	Trimethoprim	TM	5
Nitrofurans	Nitrofurantoin	NI	300

5.2.5 Data Analysis

Data were analysed using the Statistical Package for the Social Sciences (SPSS) (Version 16.0, Prentice Hall Press Company, New Jersey, USA, State if USA) (Norusis, 2008). The *E. coli* counts were $\log_{10}$ transformed before computation of the means and standard deviations. A multiple antibiotic resistance (MAR) index was performed following the procedure described by Krumperman (1983). A MAR index for an isolate was calculated using the formula: MAR = a/b where 'a' is the number of antibiotics from each group to which a particular isolate was resistant and 'b' is the total number of antibiotics against which the isolate was tested. A resistance index greater than 0.2 shows that *E. coli* isolates are likely to be from a high-risk source.

5.3 Results

5.3.1 Concentration of *E. coli* in Harvested Rainwater (HRW)

The log transformed ($\log_{10}$) *E. coli* counts and the mean *E. coli* counts in most probable number per 100 mL (MPN/100 mL) from individual tanks are shown in Table 5.2. The abundance of *E. coli* in the rainwater-harvesting tanks differed according to the location of the HRW system. The highest concentrations of *E. coli* were detected in tanks situated at Rhodes University (T1–T6).

5.3.2 Identification of virulence genes among *E. coli* Isolates

Samples which generated fluorescence from the Quanti-tray®/2000 cells were selected for the identification of the *E. coli* VGs. The most detected pathotypes were the NMEC and EHEC while the least detected pathotype was EAEC (Table 5.3). Of the 100 isolates tested for the VGs, 28% were identified as *ibeA* positive (Figure 5.1). The EAEC pathotype (*eagg* gene*)* was not detected among the tested isolates. Similarly, the *Stx1* gene of EHEC was not detected in any of the isolates.

Table 5.2: *Log transformed E. coli (MPN/100 mL) concentrations from various rainwater tanks*

Tank ID	*n*	Minimum	Maximum	Mean ± Standard Deviation
T1	11	2.55	3.29	3.02 ± 0.21
T2	11	1.95	3.11	2.62 ± 0.35
T3	11	2.58	3.29	2.84 ± 0.25
T4	11	1.64	2.89	2.52 ± 0.42
T5	11	0.79	3.00	2.18 ± 0.82
T6	11	2.53	3.04	2.88 ± 0.19
T7	10	1.73	2.96	2.36 ± 0.37
T8	10	1.78	2.41	2.09 ± 0.22
T9	7	0.61	3.04	1.71 ± 0.82
T10	10	0.3	3.19	1.57 ± 1.04
T11	7	0.61	1.12	0.85 ± 0.26

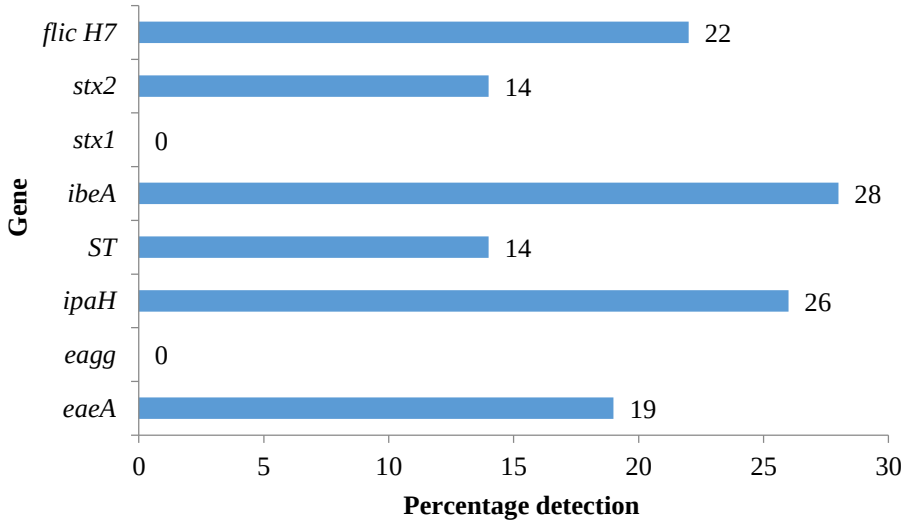

Figure 5.1: *Overall prevalence of virulence genes in isolated E. coli from harvested rainwater (HRW) tanks*

Table 5.3: *Number of virulence genes detected from rainwater-harvesting tanks*

Tank Location	**Tank ID**	**Number of *E. coli* Isolates Tested**	***eaeA* (EPEC/EHEC)**	***Eagg* (EAEC)**	***ipaH* (EIEC)**	***ST* (ETEC)**	***ibeA* (NMEC)**	***Stx1* (EHEC)**	***Stx2* (EHEC)**	***flichH7* (EHEC)**
Rhodes University	T1	11	6 (55%)	0	4 (36%)	0	4 (36%)	0	2 (18%)	4 (36%)
Rhodes University	T2	11	0	0	0	1 (9%)	1 (9%)	0	0	2 (18%)
Rhodes University	T3	11	1 (9%)	0	0	0	3 (27%)	0	0	1 (9%)
Rhodes University	T4	11	2 (18%)	0	1 (9%)	0	4 (36%)	0	0	3 (27%)
Rhodes University	T5	11	1 (9%)	0	0	0	4 (36%)	0	0	2 (18%)
Rhodes University	T6	11	1 (9%)	0	2 (18%)	2 (18%)	8 (72%)	0	0	3 (27%)
Kenton-on-sea	T7	10	2 (20%)	0	0	0	2 (20%)	0	0	2 (20%)
Kenton-on-sea	T8	10	0	0	4 (40%)	0	2 (20%)	0	1(10%)	2 (20%)
Grahamstown west	T9	7	1 (14%)	0	0	0	1 (13%)	0	0	0
Grahamstown west	T11	7	0	0	0	0	1 (13%)	0	0	0

Note: EPEC=Enteropathogenic *E. coli*, *EHEC*= Enterohemorrhagic *E. coli*, EAEC= Enteroaggregative *E. coli*, EIEC= Enteroinvasive *E. coli*, NMEC=Neonatal meningitis *E. coli*.

5.3.3 Antibiotic-Resistance Profiles of *E. coli* Isolated from the Harvested-Rainwater Samples

5.3.3.1 Overall antibiotic resistance profiles of the *E. coli*

All the 100 *E. coli* isolates tested for the presence of VGs were further tested for antibiotic resistance. Of the 11 antibiotics tested, the highest resistance displayed by *E. coli* isolates was against Cephalothin (76%) while complete susceptibility (100%) was observed to Gentamycin. The overall percentage of antibiotic resistance found in the tested isolates is shown in Figure 5.2. *E. coli* isolates were resistant to 10 of the 11 antibiotics used in this study with the resistant rate ranging from 9% to 76%. Furthermore, a low percentage of the isolates showed resistance to Ciprofloxacin (15%) and Nitrofurantoin (9%).

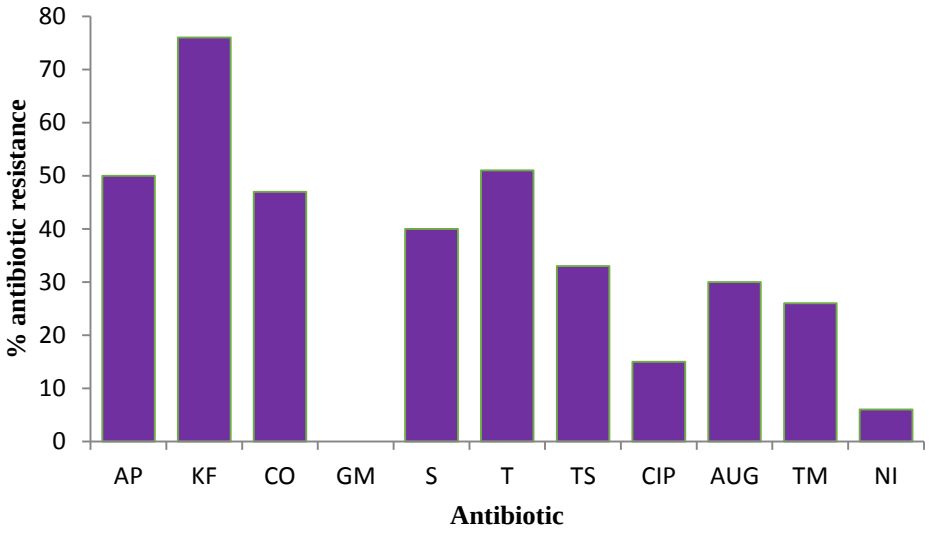

Figure 5.2: *Percentage antibiotic resistance of E. coli isolates to selected antibiotics. Full meaning of antibiotic abbreviations is shown in table 5.1*

The bacterial resistance rate in individual tanks is shown in Table 5.4. Resistance to Nitrofurantoin was only observed in T1 and T2, while resistance to Augmentin was seen in all the tanks studied. Some of the selected isolates showed the presence of multiple-antibiotic resistance (MAR) where simultaneous resistance ranged from 3 to 9 antibiotics.

Table 5.4: *Antibiotic resistance among E. coli strains isolated from various rainwater tanks*

Tank ID	*n*	% Resistance										
		AP	**KF**	**CO**	**GM**	**S**	**T**	**TS**	**CIP**	**AUG**	**TM**	**NI**
T1	11	72	91	63	0	45	72	39	27	27	63	9
T2	11	72	81	36	0	27	45	45	9	54	45	18
T3	11	27	36	27	0	45	18	27	18	9	18	0
T4	11	36	90	54	0	36	36	27	0	9	27	0
T5	11	36	100	54	0	0	54	36	27	18	45	0
T6	11	45	100	45	0	18	36	36	0	9	27	0
T7	10	30	30	20	0	100	20	30	20	30	30	0
T8	10	30	100	10	0	20	10	0	0	40	0	0
T9	7	75	87	75	0	62	100	87	0	75	50	0
T11	7	0	0	0	0	0	0	0	0	40	0	0

5.3.3.2 Prevalence of multiple-antibiotic resistance

The presence of MAR was also observed for most isolates. Multiple-antibiotic resistance in this study was defined as the resistance of bacterial strains to three or more antibiotics (Abia *et al*., 2015). Of the 100 isolates tested, more than half (52%) were MAR (Table 5.5). Ten of the 52 MAR isolates demonstrated simultaneous resistance to up to nine antibiotics. A total of 24 different MAR phenotypes were identified in this study.

Table 5.5: *Multiple-antibiotic-resistant phenotypes of E. coli isolated from different rainwater tanks*

T1		**T2**	
MAR Phenotype	**Number of Isolates**	**MAR Phenotype**	**Number of Isolates**
AP-KF-CO-T	1	KF-T-NI	1
AP-KF-CO-T-TM	1	AP-KF-AUG	1
AP-KF-CO-S-T-TS-TM	1	AP-KF-NI	1
AP-KF-CO-S-T-TS-CIP-AUG-TM	1	AP-KF-S-T-TM	1
AP-KF-CO-S-T-TS-CIP-TM	1	AP-KF-CO-T-AUG	1
KF-CO-S-T-TS-AUG-TM	1	AP-KF-CO-S-T-TS-TM	1
AP-KF-CO-S-TS-CIP-TM	1	AP-KF-CO-S-T-TS-CIP-AUG-TM	1
AP-KF-CO-S-T-TS-AUG-TM	1	AP-KF-CO-T-TS-CIP-AUG-TM	1
		AP-KF-CO-S-T-TS-AUG-TM	1
T3		**T4**	

MAR Phenotype	Number of Isolates	MAR Phenotype	Number of Isolates
AP-KF-CO-S-TS-CIP-TM	1	KF-ST-AUG	1
AP-KF-CO-S-T-TS-TM	1	KF-T-NI	1
AP-KF-CO-S-T-TS-CIP-AUG-TM	1	AP-KF-CO-S-T-TS-TM	2
		AP-KF-CO-S-T-TS-CIP-TM	1
		AP-KF-CO-S-T-TS-CIP-AUG-TM	2
T5		**T6**	
MAR Phenotype	**Number of Isolates**	**MAR Phenotype**	**Number of Isolates**
AP-KF-CO-S-T-TS-CIP-TM	1	AP-KF-AUG	1
AP-KF-CO-T-TS-TM	1	KF-CO-S-T-TS-AUG-TM	1
AP-KF-CO-S-T-TS-CIP-AUG-TM	1	AP-KF-CO-S-TS-TM	1
KF-CO-T-TS-AUG-TM	1	AP-KF-CO-T-TS-TM-NI	1
AP-KF-CO-S-T-TS-CIP-AUG-TM	1	AP-KF-CO-S-T-TS-CIP-AUG-TM	2
T7		**T8**	
MAR Phenotype	**Number of Isolates**	**MAR Phenotype**	**Number of Isolates**
AP-KF-CO-S-T-TS-CIP-AUG-TM	1	AP-KF-T	1
AP-KF-CO-S-TS-AUG-TM	1	KF-CO-S-TS	1
AP-KF-CO-S-T-CIP-AUG-TM	1	AP-KF-CO-S-T-TS-CIP-AUG-TM	2
		AP-KF-CO-S-T-TS-AUG-TM	1
T9		**T11**	
MAR Phenotype	**Number of Isolates**	**MAR Phenotype**	**Number of Isolates**
AP-KF-CO-S-T-TS-CIP-AUG-TM	1	AP-KF-CO-S-T-TS-AUG-TM	2
AP-KF-CO-S-T-TS-AUG-TM	2	AP-KF-CO-S-T-TS-TM	1

5.4 DISCUSSION

5.4.1 Concentration of *E. coli* in Harvested Rainwater

Faecal coliform bacteria such as *E. coli* have been widely used as indicator organisms to assess the possibility of pathogen presence in water (Hachich *et al*., 2012). Therefore, the presence of *E. coli* in roof-harvested rainwater in the Eastern Cape, South Africa, was monitored. All the 11 tanks monitored in this study were contaminated with varying concentrations of *E. coli* (0.85 ± 0.26–3.02 ± 0.21 MPN/100 mL). Other scholars have previously reported on the high detection of *E. coli* from roof-harvested rainwater (2 to 986 CFU/100 mL; 1 to 99 MPN/100 mL and 0 to 41 CFU/100 mL) (Ahmed *et al*., 2011; Spinks *et al*., 2006; Sazakli *et al*., 2007). None of the tanks monitored in this study met the guidelines for drinking-water quality, as the *E. coli* amounts exceeded the South African drinking-water quality guidelines of 0 CFU/100 mL. The considerable amounts of *E. coli* in the harvested rainwater samples indicate possible faecal contamination. The variations in the number of *E. coli* contamination

in different HRW systems could be attributed to the fact that some of the HRW systems (Rhodes University) had a constant presence of birds which could have landed and dropped faecal matter on the roof, thereby contaminating tank water. Bird faecal droppings may negatively impact roof-harvested rainwater quality due to the presence of zoonotic pathogens (Ahmed *et al*., 2012). A study conducted in South Africa investigated antibiotic resistance in *E. coli* isolates from roof-harvested rainwater tanks and urban pigeon faeces as the likely source of contamination and concluded that urban pigeons, the most likely source of HRW contamination, are also reservoirs of multiple antibiotic-resistant bacteria (Chidamba and Korsten, 2015). The findings of the South African study on bird faeces and antibiotic-resistant *E. coli* have a similar conclusion to our study where bird faecal matter was suspected to contribute to the contamination of HRW. In cases where the sources of faecal pollution in rainwater tanks are suspected to be from birds, the application of bird faecal markers may have the potential to confirm the sources of faecal contamination in a rainwater tank (Ahmed *et al*., 2012). In another study to identify the likely sources of potential clinically significant *E. coli* in rainwater tanks, a source-tracking approach was used where a biochemical-fingerprinting method for typing of *E. coli* strains revealed that of the 43 strains from rainwater tank samples, 14 (from 7 tanks) and 9 (from 6 tanks) had identical biochemical phenotypes to those found in bird and possum faecal samples, respectively (Ahmed *et al*., 2016). Furthermore, five strains from 4 rainwater tanks were identical to those isolated from both bird and possum faecal samples (Ahmed *et al*., 2016).

The rainwater tanks in the current study are used for various purposes such drinking and toilet flushing (for tanks situated at Rhodes University). Tanks situated at Grahamstown west were mainly used for gardening and sometimes drinking, depending on the availability of the municipal supply, while Kenton-on-sea tanks were used for indoor potable uses such dish-washing and laundry. In order to reduce or limit the risk of pathogenic and antimicrobial resistant *E. coli*, constant cleaning and maintenance of the catchment area may significantly improve the quality of the HRW, as the catchment area is suspected to contribute largely to the deterioration of the HRW in the Eastern Cape due to birds landing on the roof. Installation of first flush diverters may also help to improve the quality of the HRW.

A study conducted in South Africa on the quality of HRW reported that 100% of the samples tested for *E. coli* exceeded the recommended standard of 0 CFU/100 mL (Dobrowsky *et al*., 2014). Their results were similar to the ones observed in this study where all of the samples showed high levels of *E. coli*. In the Eastern Cape, where harvested rainwater is used for various household purposes including drinking, the presence of *E. coli* in the rainwater tanks is a major health concern as the presence of *E. coli* could imply the presence of other bacterial pathogens which may be detrimental to the health of rainwater users. The findings of the current study are of significant health concern as antibiotic-resistant pathogenic *E. coli* isolates may cause diseases if the users of the HRW consume the water without treatment. Furthermore, resistance of the isolated pathogenic *E. coli* to commonly used antibiotics in

South Africa may lead to antibiotic treatment failure with serious public health implications for the population and the country.

5.4.2 Identification of virulence genes among *E. coli* isolates

Pathogenic *E. coli* strains are a major cause of infections worldwide, the most common of which are diarrhoeal diseases. All the 100 *E. coli* isolates from the tanks tested positive for one or more VGs. The most detected pathotype was the NMEC (*ibeA*; 28%) which is responsible for neonatal meningitis and endothelial cell invasion (Johnson *et al*., 2012). The *ibeA* gene is also reportedly found in avian pathogenic *E. coli* (APEC) and causes avian colibacillosis, which is the most significant infectious bacterial disease of poultry worldwide (Johnson *et al*., 2012). The detection of the *ibeA*-positive strains in this study possibly indicates that the observed pathotype may be due to the presence of birds around the HRW systems. Although the present study did not investigate whether the *ibeA* gene detected was of human or avian origin, the presence of *ibeA*-positive isolates in the HRW systems is still of health concern given that there could be a possibility of zoonotic infections arising from the consumption of untreated rainwater containing these strains.

Genes pertaining to other pathotypes of public health concern were also detected in the present study. For example, the *flicH7* (22%) and *Stx2* (14%) genes of EHEC were also detected in the isolates. Members of the EHEC group have been involved in many diarrhoeal disease outbreaks around the world, and they are known to cause hemorrhagic colitis and hemolytic uremic syndrome in humans (Hamilton *et al*., 2010). The EHEC pathotype showed high prevalence across all the sampling sites except for the sites located in Grahamstown west. Both T1 and T6 which yielded a high percentage in VGs detection were situated at Rhodes University. Prevalence of the virulence gene *ipaH* (26%) (pathotype EIEC) was also noticeable in 4 tanks; 3 of the tanks were situated on campus and 1 in Kenton-on-sea. A previous study conducted in Cape Town, South Africa, reported that EPEC and EHEC (3% each) were detected in lower numbers, whereas EIEC was not identified in any of the rainwater tanks tested in their study (Dobrowsky *et al*., 2014). The results differ from the findings of the current study where EIEC (26%) was the second most detected pathotype. This shows that the location of the tank could affect the pathotypes detected. Due to the detection of *E. coli* pathotypes in the current study, there is a great need to create awareness on household treatment technologies among users of HRW. Available treatment options which have proven to be successful in the treatment of HRW such as boiling, closed-couple solar pasteurizer, and solar disinfection can be used to decontaminate HRW (Spinks *et al*., 2003; Dobrowsky *et al*., 2015; Amin *et al*., 2014). In this study, all the rainwater tanks did not have any treatment option fitted, such as first-flush diverters and filters, except for T5 which had a chlorinator. However, due to limited maintenance of the rainwater-harvesting systems, the chlorinator in T5 was clogged in the middle of the sampling season and the *E. coli* counts

increased going forward. The interruption of the treatment option observed in this study is also a clear indication of lack of proper maintenance of the HRW systems.

5.4.3 Detection of antibiotic-resistant *E. coli* in harvested rainwater

Results of the antibiotic-resistance profiling of the isolates from harvested rainwater analyzed in the current study revealed that most of the *E. coli* isolates were resistant to the commonly prescribed antibiotics in South Africa. In areas such as the Eastern Cape where most of the population rely on harvested rainwater, exposure to antibiotic-resistant bacteria can further increase the health risk, particularly to children, the elderly and immune-compromised individuals. Antibiotic resistance is on the increase worldwide as most microorganisms now exhibit resistance to a large number of known antibiotics. The *E. coli* isolates from harvested rainwater in this study revealed resistance to Cephalothin (76%), Tetracyclines (51%), Colistin sulphate (47%), Ampicillin (50%) and Streptomycin (40%). The antibiotics most used in South Africa are the penicillins (Cephalothin) and fluoroquinolones, (Ciprofloxacin and glycopeptides) (Essack *et al*., 2011). Tetracyclines and trimethoprim are also extensively used in the treatment of bacterial infections in both human and animals (Ruhe and Menon, 2007).

Cephalothin belongs to the β-Lactam class of antibiotics which are characterised by a β-lactam ring in their molecular structure (Beceiro *et al*., 2013). Resistance to beta-lactam antibiotics has been highly documented as bacterial strains that produce extended-spectrum beta-lactamases have become more common (Parteson and Bonomo, 2005). Extended-spectrum beta-lactamase (ESBL)-producing *E. coli* are highly resistant to an array of antibiotics and infections by these strains are difficult to treat (Parteson and Bonomo, 2005). Furthermore, genes for ESBLs are most often encoded on plasmids, which can readily be transferred between bacteria (Haenni *et al*., 2014). Given that most of the isolates carrying virulence genes, especially the *ibeA* gene, were also resistant to Cephalothin, this could suggest that most of the isolated *E. coli* strains may carry the ESBL genes with the possibility of transfer to related organisms within the rainwater tanks. However, it is necessary to conduct further studies to ascertain such ARGs' transfer within harvested-rainwater systems. Results of such studies would highlight the need for implementation of appropriate treatment options and better policies for the safe use of harvested rainwater, especially where such water is the main source of water for personal and household uses, thus protecting the lives of users of harvested rainwater. In the current study, the tested *E. coli* isolates showed resistance to one or more antibiotics with the highest *E. coli* resistance recorded against Cephalothin, Ampicillin and Tetracyclines. Also, there was evidence of MAR *E. coli* in almost all the HRW systems with some isolates showing simultaneous resistance to a panel of up to nine antibiotics. These results indicate that in the case of infections occurring due to the consumption of contaminated

harvested rainwater, treatment may fail because of the persistent resistance of the *E. coli* isolates detected in the HRW systems.

A similar study carried out in Pretoria and Johannesburg, South Africa, showed that the resistances most encountered were against Ampicillin, Gentamicin, Amikacin and Tetracyclines (Chidamba and Korsten, 2015). These results were not in agreement with our findings, where *E. coli* isolates were resistant to Cephalothin and 100% susceptible to Gentamicin, although the same method and concentration was used for Gentamicin in both studies. The difference in antibiotic resistance results from the two studies could be attributed to the fact that roof-harvested rainwater samples were collected from different locations (Gauteng and Eastern Cape). Our findings were, however, similar to those of Chidamba and Korsten (2015) in that the authors also reported a substantial prevalence of MAR. All the isolates tested in this study showed a MAR index greater than 0.2, suggesting that a greater proportion of the isolates were likely to be from a high-risk source such as faecal material. These results and the differences observed with other studies could inform those implementing antibiotic-resistance surveillance schemes that would address different geographical locations. Also, the presence of MAR *E. coli* in harvested rainwater could pose a severe health risk to the public in general, as antibiotic resistance decreases the efficiency of antibiotics used in the treatment of infections. These findings are of major concern, as more households are now reported to be using harvested rainwater for their daily water needs.

5.5 CONCLUSIONS

Rainwater samples tested in this study showed contamination with varying concentrations of pathogenic *E. coli* strains. The outcome of the study further demonstrates that HRW tanks could serve as reservoirs for not only pathogenic but also antibiotic-resistant *E. coli* strains including MAR strains. These findings suggest that the tested harvested rainwater was not fit for human consumption and, therefore, should not be used for potable purposes without appropriate treatment. Furthermore, routine monitoring and treatment are essential to ensure that harvested rainwater is fit for intended use as well as to stimulate the need for strategies (e.g., maintenance of HRW systems, constant cleaning of the roof, and installation of first-flush diverters to minimize faecal contamination) that would prevent the spread of antibiotic-resistant bacteria.

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CHAPTER SIX: OCCURRENCE OF *LEGIONELLA*, ZOONOTIC AND FUNGAL PATHOGENS IN HARVESTED RAINWATER

6.1 Introduction

Onsite rooftop rainwater has gained popularity worldwide and employed not only in areas where water supply is limited by climate or infrastructure, but recently also in well-developed, water-ample regions (Friedler *et al*., 2017). Uses of harvested rainwater range from toilet flushing, garden irrigation, laundry, car washing, etc. (Friedler *et al*., 2017). Although roof harvested rainwater is used in many areas as an alternative water source, there are reports of poor quality of such water from literature (Simmons *et al.*, 2001). The location of the rainwater harvesting system (RWHS) is an important aspect to consider when assessing the level of contamination in harvested rainwater. In urban areas, rainwater might be already contaminated before it reaches the catchment area (Sánchez *et al*., 2015). This is regarded as the first stage of contamination which occurs when rainfall washes out and scavenges aerosols, gases and thin volatile particles from the urban atmosphere (Sánchez *et al*., 2015). High levels of heavy metals have been isolated in harvested rainwater from urban areas as compared to rural areas (Azimi *et al*., 2005). Air quality can also be affected by organic pollutants derived from fuel leakage of vehicles, petrochemical and plastic-chemical industries which may in turn contaminate the harvested rainwater (Huston *et al*., 2012).

Pathogens which are often present in faeces of animals that have access to the roof may be transported to rainwater tanks during rainy seasons (Ahmed *et al*., 2009). Pathogens may also enter into the tanks through aerosol deposition and plant litter (Hamilton *et al.*, 2017). In addition, the microbial quality of tank water differs with geographical location, climatic conditions, roof and tank maintenance practices, tank hydraulics and surrounding environment (Vialle *et al*., 2011). Consumption of contaminated harvested rainwater may lead to gastrointestinal illness caused by variety of different microbes which present symptoms such as diarrhoea, nausea, vomiting, fever and abdominal pain (Pandey *et al*., 2014). Microorganisms such as *Shigella, Salmonella, Vibrio cholerae* and *Legionella* have been isolated in varying densities from collected roof rainwater samples (Lye, 2009). The diseases caused by all of these microorganisms could be serious, resulting in death. *V. cholerae* causes cholera, a disease with endemic or pandemic potential characterized by watery diarrhea and vomiting, leading to severe and rapidly progressing dehydration and shock (Akoachere and Mbuntcha, 2014). Shigellosis and salmonellosis are caused by *Shigella* spp. and *Salmonella spp*., respectively. These organisms are likely to be the common cause of diarrhea worldwide (Mulatu *et al*., 2014). *Shigella spp*. are the causative agents of

inflammatory diarrhea and dysentery, thus presenting a serious challenge to public health authorities worldwide (Mulatu *et al*., 2014). Kim *et al*. (2016) reported that potential human pathogens such as *L. pneumophila* and fungal pathogens such as *Aspergillus flavus*, *Aspergillus fumigatus*, and *Aspergillus niger* were detected with DNA-based methods in cisterns and in treated rainwater delivered at the tap. A study by Jongman and Korsten (2016) conducted in South Africa on microbial quality and suitability of roof harvested rainwater in rural villages for crop irrigation and domestic use has reported on the identification of fungal species known to cause fever, coughing and shortness of breath in humans (*Cryptococcus* spp.) and penicillium spp from harvested rainwater. Other fungal species isolated from harvested rainwater include *Fusarium moniliforme, Aspergillus flavus, Penicillium chrysogenum, Penicillium corylophilum* and *Phoma exigua Sacc*. (Monchy *et al*., 2011). One of the frequently identified fungal pathogens detected in water that could cause infections such as bronchopulmonary aspergillosis in immunocompromised individuals is *Aspergillus fumigatus* (Hageskal *et al*., 2009). The presence of fungal flora in harvested rainwater was also stated by Nishihara *et al*. (1989) who detected 29 fungal genera from roof harvested rainwater and 17 genera from atmospheric samples, while Czeczuga and Orłowska (1997) identified 33 fungus species of the Hyphomycetes in the rainwater falling from six different roof types.

6.2 Methodology

6.2.1 Study area

The study was carried out in three locations namely; Grahamstown/Makhanda (33°18′36″S; 26°31′36″E, situated in the Eastern Cape Province), Johannesburg (Wits University 26.1924° S, 28.0316° E, situated in Gauteng Province) and lastly Pretoria (CSIR 25.7530° S, 28.2768° E, situated in Gauteng Province). Figure 6.1 shows the three sampled locations.

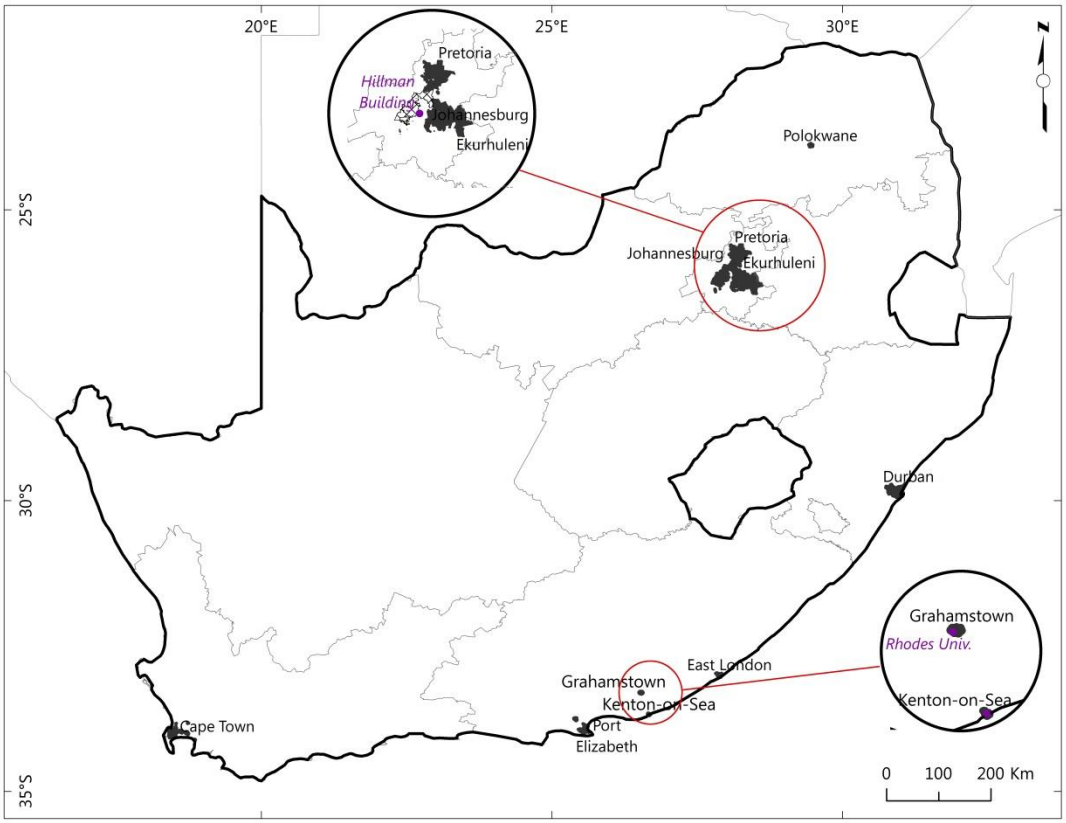

Figure 6.1: *Map showing the three sampling locations (Pretoria, Johannesburg and Grahamstown)*

6.2.2 Seasonal information

In Grahamstown/Makhanda the average midday temperatures for Grahamstown/Makhanda range from 18.9°C in July to 26.8°C in February. The winter season in Grahamstown usually receives constant showers accompanied by colder humid temperatures. The region is the coldest during July when the mercury drops to 5.6°C on average during the night. Furthermore, the area had frequent showers and at times warmer temperatures. Mean annual rainfall is 650 mm (Zengeni *et al*., 2016), with bimodal peaks in October–November and again in March–April. The winter season in Johannesburg is a dry season, with rare and sporadic rains. During the coldest months (June and July), the minimum temperature is near freezing (0 °C or 32 °F), especially in the outskirts of the city and in the countryside, where light frosts can occur. In the daytime, however, when the sun usually shines, the air is mild, and the maximum temperature is around 18/20 °C (64/68 °F). Johannesburg normally receives about 604 mm of rain per year, with most rainfall occurring during summer; it receives the lowest rainfall (0 mm) in July and the highest (113 mm) in January. The winter season in Pretoria is usually dry with an average temperature

of 12°C (54°F). Pretoria normally receives about 573 mm of rain per year, with most rainfall occurring during summer. It receives the lowest rainfall (0 mm) in June and the highest (110 mm) in January.

6.2.3 Roofing materials and site characteristics

Eleven RWHS were sampled in the Eastern Cape and five had galvanized roofing material and a further five was made of tiled roof. Only one RWHS had asbestos roof in the Eastern Cape. The roofing material from the RWHS in Johannesburg was made of a paved roof while in Pretoria two of the RWHS had tiled roof. Each of the RWHS monitored in this study from the three locations (Grahamstown, Johannesburg and Pretoria) had distinct characteristics. The RWHS in Grahamstown were situated in both semi-urban and urban areas. The RWHS from Johannesburg and Pretoria were situated in an urban area. Overhanging tree branches and flying birds characterized the Grahamstown and Pretoria RWHS while the Johannesburg system had no overhanging trees and birds**.** All the RWHS were made of the catchment area, gutters and storage tank.

6.2.4 Sample collection

Sampling was performed as mentioned in section 3.2.2. Sampling in all of the RWHS was done over a three-month period (July-September 2016 for the Grahamstown RWHS and July-September 2017 for the Johannesburg and Pretoria RWHS). A total of 123 samples were tested where 67 samples were selected from the Grahamstown area, 28 from Pretoria and a further 28 samples from Johannesburg. Samples were then transported to Rhodes University's Microbiology laboratory (for the Grahamstown RWHS) while samples from the Johannesburg and Pretoria RWHS were transported to the Council of Scientific and Industrial Research laboratory on ice for microbial analysis within 6 hours.

6.2.5 Identification of bacterial pathogens from harvested rainwater

6.2.5.1 Enrichment of harvested rainwater and DNA extraction

The presence of four pathogenic (*Salmonella, Shigella, Legionella and V. cholerae*) bacteria in harvested rainwater samples was tested using specific polymerase chain reaction (PCR). Fifty millilitres (50 mL) of harvested rainwater samples were transferred to 100 ml sterile bottles containing 50 mL double strength selenite broth for *Salmonella* spp., peptone water for *Shigella* spp. and alkaline

peptone water for *V. cholerae* and incubated at 35.0 ± 0.5 °C for 24 hours. All culture media were purchased from Merck (South Africa). After incubation, 1 mL of the overnight broth culture was then transferred into a centrifuge tube and centrifuged at 13,000 g for 3 min. DNA was extracted from the harvested cells using the Instagene™ Matrix (Bio-Rad Laboratories, South Africa) following the manufacturer's instruction. The supernatant from the tubes was then used as a source of template DNA for the real-time PCR reactions. Analysis of *Legionella* spp. was carried out by pipetting 100 µl of the rainwater sample onto BCYE agar plates, incubated at 35.0 ± 0.5 ˚C for 48 hours. Presumptive *Legionella* colonies were selected and transferred to 1.5 ml Eppendorf tubes containing 100 µl nuclease free water and vortexed until the colony has dissolved. DNA was then extracted using Instagene™ Matrix.

6.2.5.2 Oligonucleotide primers and PCR amplification

The primer sets used in this study are presented in Table 6.1. All the primers have been published previously and are commonly used in many studies. Primer sets were designed and synthesized on the basis of the available nucleotide sequence data.

***Table 6.1**: Selected primer sets used in this study*

Target gene	Strain	Primer sequence	Orientation	Source
ipaH	*Shigella*	GTTCCTTGACCGCCTTTCCGATACCGTC' GCCGGTCAGCCACCCTCTGAGAGTAC	Forward Reverse	Vidal et al. (2005)
Inva	*Salmonella*	GTGAAATTATCGCCACGTTCGGGCAA TCATCGCACCGTCAAAGGAACC	Forward Reverse	Rahn et al. (1992)
ompW	*Vibrio cholerae*	CACCAAGAAGGTGACTTTATTGTG GAACTTATAACCACCCGCG	Forward Reverse	Nandi et al. (2000)
ctxAB		GCCGGGTTGTGGGAATGCTCCAAG GCCATACTAATTGCGGCAATCGCATG	Forward reverse	Goel et al. (2005)
JFP *JRP*	*Legionella*	AGGGTTGATAGGTTAAGAGC CCAACAGCTAGTTGACATCG	Forward reverse	Jonas et al. (1995)

PCR amplification in a final volume of 20 µl was used. The reaction mixture for *Salmonella* consisted of 1 µl Inva (forward and reverse primer sets), 10 µl sensiFAST™ HRM (Bioline GmbH, Germany), 3 µl nuclease free water and 5 µl template DNA. For *Vibrio cholerae* the reaction mixture consisted of 0.5 µl ompW (forward and reverse primers), 0.5 µl ctxAB (forward and reverse primers), 10 µl sensiFAST™ HRM, 3 µl nuclease free water and 5 µl template DNA. *Shigella* reaction mixture consisted of 10 µl sensiFAST™ HRM, 1 µl ipaH (forward and reverse primers), 3 µl nuclease free water and 5 µl template DNA. For *Legionella*, the reaction mixture consisted of 1 µl JFP and JRP

(forward and reverse primers), 10 µl sensiFAST™ HRM, 3 µl nuclease free water and 5 µl template DNA.

6.2.5.3 Analysis of the PCR products

The PCR analysis for *Salmonella*, *Shigella* and *Vibrio cholerae* were performed using Corbett Life Science Rotor-Gene™ 6000 cycler (Qiagen, Hilden, Germany) under the following conditions: Heat denaturation at 94 ˚C for 2 min, followed by 35 cycles of heat denaturation at 94 ˚C for 1 min, primer annealing at 62 ˚C for 1 min and DNA extension at 72 ˚C for 2.5 min. This was followed by incubation at 72 ˚C for 10 min and cooling at 4 ˚C. PCR amplification for *Legionella* was also performed in a Corbett Life Science Rotor-Gene™ 6000 cycler (Qiagen, Hilden, Germany) under the following conditions: Initial incubation of 37 ˚C, for 10 minutes, followed by 38 cycles of initial denaturation at 95 ˚C, 20 min, heat denaturation of 94 ˚C, 45 seconds, annealing at 57 ˚C, 45 seconds, initial elongation of 72 ˚C, 45 seconds and final elongation of 72 ˚C, 1 hr. All PCR reactions included a positive (genomic DNA of a reference strain) and a negative control (made of the PCR reaction mixture without the template DNA). Reference strains used were *Salmonella ser Typhimurium* (ATCC 14028), *Shigella flexneri* (ATCC 12022), *V. cholerae* O1 (NCTC 5941) and *L. pneumophila* (ATCC 33152).

6.2.6 Identification and phylogenetic analysis of cultured fungal isolates in harvested rainwater

To detect the presence of fungal isolates in harvested rainwater, 100 µl of the harvested rainwater samples was spread onto prepared Potato Dextrose agar plates and Rose Bengal agar plates (Lasec, South Africa). The plates were allowed to dry and incubated at 25 ˚C for 72 hours. Following incubation colonies suspected of being fungal isolates were clearly marked and the agar plates sent to Inqaba for sequencing. Phylogenetic analysis was performed to check if there was any relationship between the fungal isolates from both Gauteng and Grahamstown. Sequencing of the fungal isolates was performed using the Sanger sequencing method and raw sequences were visualised, edited and assembled into contiguous sequences using DNA baser version 5.15 (http://www.dnabaser.com). Default parameters were applied in DNA baser program for trimming of poor-quality sequence ends to retain only high-quality bases. A total of 37 sequences were generated from the sequence assembly step and used in a BLAST search to identify various fungal species. MEGA7 was used to carry out the phylogenetic tree reconstruction and the test of phylogeny was achieved by applying bootstrapping for 1000 replicates. The Newick tree generated was visualized in Figtree software and clustering pattern annotated. Further annotations were conducted to identify clades composed of fungal strains isolated from different sites

within South Africa. The individual predicted clades were indicated in specific colors suggesting close relationships.

6.2.7 Statistical analysis

Data analysis was done using the Statistical Package for the Social Sciences (SPSS) version 16.0 (Norusis, 2008). One-way Analysis of Variance (ANOVA) was used to determine if there was any significant difference between the number of pathogens observed in the tanks located in the Grahamstown, Johannesburg and Pretoria.

6.3 RESULTS

6.3.1 Roofing materials and rainwater quality

Salmonella and *Legionella* were present in all the RWHS made of different roofing materials situated in different regions (Table 6.2). *Vibrio cholerae* was isolated from RWHS in Pretoria and Johannesburg made of tile and paved roofs while *Shigella* was isolated from roofing materials made of Galvanized, tile and asbestos situated in Gauteng and Grahamstown.

Table 6.2: *Pathogens isolated from different roofing materials in harvested rainwater from the Eastern Cape and Gauteng*

Location of the RWHS	Roof type	Pathogen isolated
Grahamstown	Galvanized	*Salmonella, Shigella, Legionella*
Grahamstown	Tile	*Salmonella, Shigella, Legionella*
Grahamstown	Asbestos	*Salmonella, Shigella, Legionella*
Pretoria	Tile	*Salmonella, Shigella, Legionella, V. cholerae*
Johannesburg	Paved	*Salmonella, Legionella, V. cholerae*

6.3.2 Identification of bacterial pathogens from harvested rainwater

Polymerase chain reaction was used to detect selected pathogenic organisms from harvested rainwater samples. A total of 123 samples inclusive of all sites were tested for the presence of pathogens and results indicate the presence of pathogens distributed among the 3 locations tested in this study (Figure 6.2). Pathogens such as *Salmonella*, *Shigella* and *Legionella* were mostly detected from RWHS situated

in Grahamstown area with the highest *Salmonella* detection observed in Grahamstown (73%). Furthermore, all the pathogens tested were detected in samples from Pretoria.

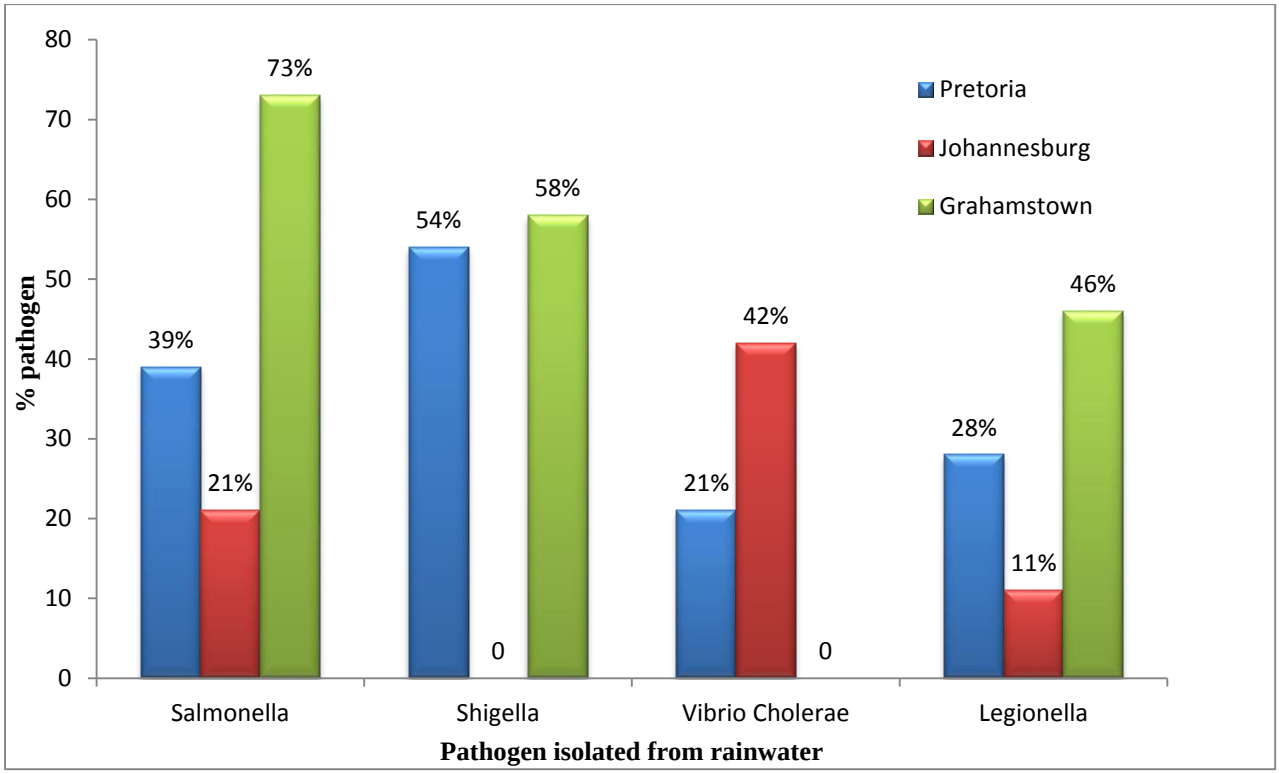

Figure 6.2: *Detection of pathogenic bacteria in harvested rainwater from different locations*

One-way ANOVA was performed to determine possible significant differences at a *P* level of <0.05 between the number of pathogens observed in the various locations (Johannesburg, Pretoria and Grahamstown). Statistical analysis revealed a significant difference of 0.000 when *P*<0.05 between the number of *Salmonella* as well *V. cholerae* observed in the various locations. There were no further significant differences observed between the location of the RWHS and the presence of pathogens. Rainwater tanks situated in the Gauteng province (Pretoria and Johannesburg) were only 58 km apart, ANOVA was performed to test for significant difference in the occurrence of pathogens detected from rainwater tanks. Results showed a statistical significance difference between the amount of *Legionella* from Johannesburg and Pretoria (0.026, *P*<0.05). A further significant difference was observed between the amount of *Salmonella* (0.022, *P*<0.05) obtained in Johannesburg and Pretoria.

6.3.3 Identification and phylogenetic analysis of cultured fungal isolates in harvested rainwater

Rainwater samples from the Eastern Cape and Gauteng province were tested for the presence of pathogenic fungi. Results from Pretoria and Johannesburg (referred as Gauteng going forward) were pooled when identifying the fungal isolates. The most frequently found fungi genera during the sampling period in the Eastern Cape was Cladosporium (100%) while Penicillium (100%) species were mostly identified in Gauteng (Figure 6.3). Some genera of fungi were found in both locations while others were either identified in the Eastern Cape or in Gauteng. All the fungal isolates were identified at the species level to show the presence of pathogenic fungi. Pathogenic fugal strains such *Aspergillus fumigatus* were identified in both Gauteng and in the Eastern Cape. Another rare fungal pathogen *Cryptococcus laurentii* was identified in the Eastern Cape and not present in Gauteng. Other identified human pathogenic fugal isolates included *Aureobasidium pullulans* (found in both study areas) and *Mucor circinelloides* (only Eastern Cape).

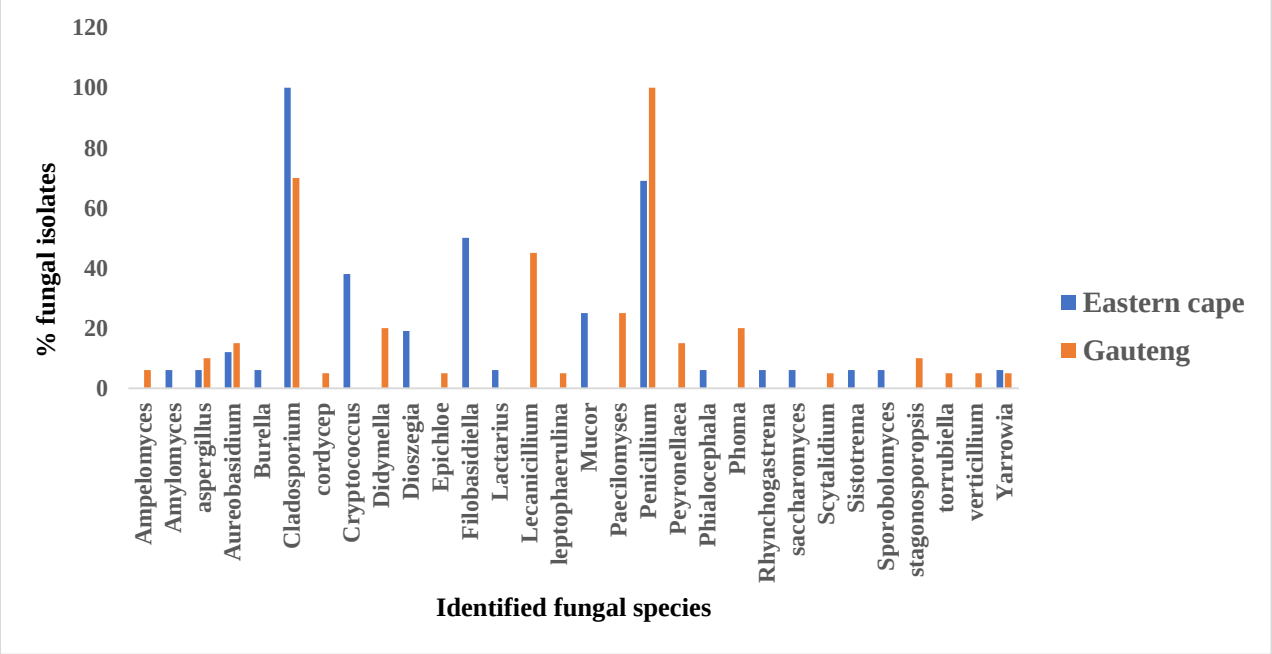

Figure 6.3: *Fungal isolates identified in the Eastern Cape and Gauteng*

Multiple fungal species were identified in roof harvested rainwater sampled in two provinces of South Africa. This diversity is reflected in the BLAST results in which significant hits to known fungal species were obtained. Three clusters were identified that composed of sequences retrieved from samples

collected from Grahamstown and Gauteng sites. This clusters suggested that the fungal species circulating in both sites were related. The observed results further imply that rain harvested water may contain fungal impurities that might be circulating within the environment and therefore antifungal treatment interventions may improve the safety of water for human consumption (Figure 6.4).

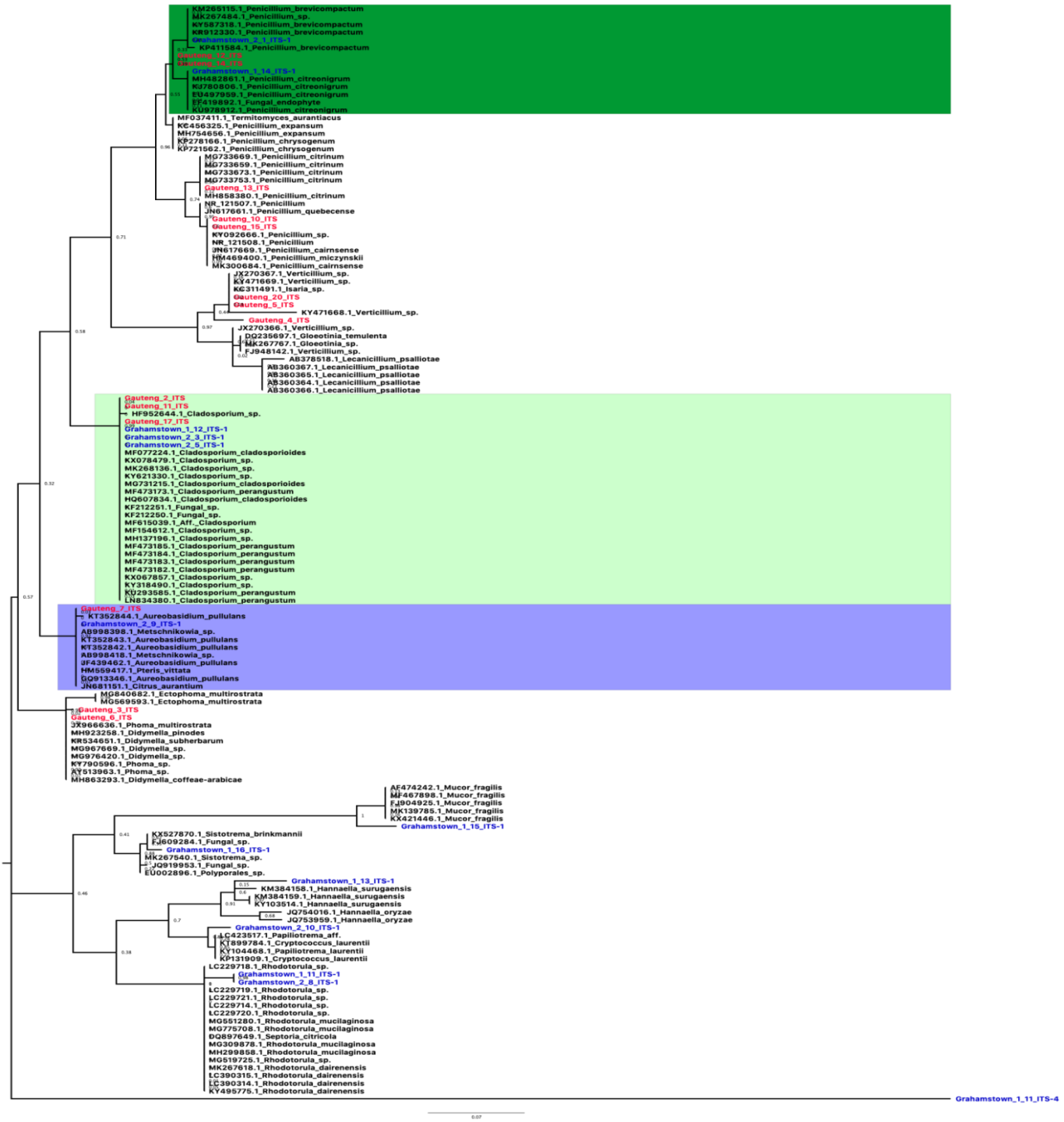

Figure 6.4: *Phylogenetic tree generated from PCR sequences of fungal isolates identified in Gauteng and Grahamstown. The highlighted colours show that the fungal isolates circulating in both Gauteng and Grahamstown were related*

6.4 DISCUSSION

6.4.1 Roofing materials and rainwater quality

Rainwater quality is dependent on various factors such as the catchment area, the location of the RWHS and even the storage tanks. The study evaluated if roofing materials from different regions can affect the quality of harvested rainwater by harbouring the same pathogens or rather different pathogens depending on the location of the RWHS. Results showed that pathogens such as *Salmonella* and *Legionella* can be detected from various roofing materials irrespective of the location of the RWHS (Table 6.2). However, *V. cholerae* was not detected in samples from Grahamstown even with the various roofing materials used which may imply that *V. cholerae* may be dependent on the location of the RWHS. *Shigella* was isolated in harvested rainwater made of different roofing materials except from paved roof surfaces which may also suggest that either location of the RWHS or the catchment area play an important role. While roof surfaces are mostly used in rainwater harvesting, some drawbacks have been reported. Birds and small animals can access the roof and drop faecal matter which will be washed in to the storage tank during the rainy season (Ahmed *et al*., 2009). Rooftop runoff quality is dependent on both the roof type and the environmental conditions (local climate and atmospheric pollution) (Farreny *et al*., 2011).

6.4.2 Identification of bacterial pathogens from harvested rainwater

Results of the current study shows that all the rainwater samples from the three locations were contaminated with *Salmonella* and *Legionella*. Samples from the Grahamstown area had higher levels of *Salmonella* (73%), *Shigella* (58%) and *Legionella* (46%). *V. cholera* was detected in rainwater samples from Johannesburg (42%) and Pretoria (21%) and not detected in Grahamstown samples. Furthermore, *Shigella* was present from samples obtained in Pretoria (54%) and Grahamstown (58%) while it was not observed from samples in Johannesburg. All the tested pathogens (*Salmonella, Shigella, V. cholerae and Legionella*) were detected from rainwater samples in Pretoria. The observed results suggest that the location of the RWHS may have an influence on the type and number of contaminants detected in rainwater tanks. Rainwater tanks situated in EC are used for various purposes such drinking, toilet flushing and gardening. Therefore, results of the current study are of concern as pathogens such as *Legionella, Salmonella and Shigella* were isolated from tanks in Eastern Cape. The RWHS in Johannesburg is used for toilet flushing and the isolated pathogens included *Legionella,*

Salmonella and V. cholera. In cases where rainwater is used for non-potable use such as toilet flushing the water must be appropriately disinfected. The presence of *Legionella* in rainwater used for toilet flushing may pose a health risk when aerosols containing *Legionella* are formed during toilet flushing and then inhaled (Schets *et al*., 2010). Furthermore, an extensive literature review on toilet plume aerosols and subsequent study indicated that potentially infectious aerosols may be produced in substantial quantities during flushing and that aerosolization can continue through multiple flushes to expose subsequent toilet users (Johnson *et al*., 2013). The risk associated with the presence of *Legionella* in harvested rainwater is gaining popularity around the world. *Legionella* causes disease through inhalation of aerosols containing *Legionella*. Rainwater tanks have been proposed as potential sources of organisms that could be amplified in hot water systems in buildings (Chapman *et al*., 2008). Simmons *et al*. (2008) reported on the outbreak of Legionnaires' disease in New Zealand that may have been associated with rainwater fed hot water tanks. Ahmed *et al*. (2008) reported that contaminated rainwater may harbour various pathogens such as *Salmonella, Shigella, V. cholera* and *Legionella*. The presence of these pathogens in rainwater may introduce diseases that differ in severity from mild gastro-enteritis to severe and sometimes fatal dysentery, cholera, or typhoid (Dobrowsky *et al*., 2014). The results of the current study are similar to a study done in Queensland Australia where *Salmonella* was isolated in harvested rainwater samples using PCR (Ahmed 2008, 2009).

6.4.3 Identification and phylogenetic analysis of cultured fungal isolates in harvested rainwater

Identification of fungal isolates was performed using fungal sequencing technique. Though the main portal of entry of fungi is inhalation, several studies have indicated that exposure from water can occur (Warris *et al*., 2001). Results showed that samples from Gauteng had higher detection rate of the Penicillium fungi while Cladosporium species were mostly detected in the Eastern Cape Province. The isolates were identified to species levels to show the pathogenic fungal strains. Pathogenic strains such as *Cryptococcus laurentii* and *Mucor circinelloides* were identified in Eastern Cape while *Aspergillus fumigatus* was identified in Gauteng. Fungal pathogen *Aureobasidium pullulans* was detected in both the Eastern Cape and Gauteng. *Aspergillus fumigatus* is a major cause of aspergillosis which mainly affects immune compromised people (Cloherty, 2012). The identification of the pathogenic fungal strains in both Gauteng and Grahamstown is of serious concern especially taking into consideration that the RWHS situated in Grahamstown (semi-urban area) are sometimes used for potable purposes such as drinking and dish washing due to frequent water shortages in the Eastern Cape. The findings of this study imply that the consumption of the untreated harvested rainwater may results in water-borne infections especially for people with a compromised immune system such as the elderly, children under

5 years and pregnant women. Furthermore, the study highlights the importance of documenting the type of contaminants from different locations as the results suggests that the location of the harvested rainwater system may have a significant impact on the type and number of contaminants. Some *Aspergillus* species cause serious disease in humans and animals (Bozkurt *et al*., 2008). The most common pathogenic species are *A. fumigatus* and *A. flavus*, which produce aflatoxin which is both a toxin and a carcinogen (Cloherty, 2012). About 40 of the 250 species of *Aspergillus* have been reported as human pathogens (Klich, 2006) but the majority of cases are associated with *A. fumigatus, A. niger*, *A. flavus, A. nidulans,* and *A. terreus* whose normal portal of entry is respiratory system. The clinical spectrum of *Aspergillus* infections includes aspergilloma, allergic bronchopulmonary aspergillosis, fungal ball and invasive aspergillosis (Stevens *et al*., 2000). Infections due to *Aspergillus* are considered as an emerging disease and several reports are available (Lat *et al*., 2010).

Penicillium sp. are well known for positive (fermentation and drugs production) and negative impacts (production of mycotoxins; stimulating hypersensitivity reactions and human infections like asthma, extrinsic allergic alveolitis, fungal ball) (Lyratzopoulos *et al*., 2002; Chen *et al*., 2013). The genus *Paecilomyces* was detected in Gauteng samples and it is involved in various diseases such as prepatellar bursitis, cutaneous mycosis, non-invasive sinusitis, endocarditis and endophthalmitis, especially among immunocompromised individuals (Das *et al*., 2000; Jahromi and Khaksar, 2004). Penicillium spp may cause hypersensitivity pneumonitis, asthma, and allergic alveolitis in susceptible individuals (Henk *et al*., 2012). *Cryptococcus laurentii* is known to occasionally cause moderate-to-severe disease meningitis in human patients with compromised immunity (Cheng *et al*., 2001). Mucor circinelloides infections are associated with cutaneous, rhinocerebral, and pulmonary infections (Lazar *et al*., 2014) while chronic human exposure to *Aerobasidium. pullulans* via humidifiers or air conditioners can lead to extrinsic allergic alveolitis (Gostinčar *et al*., 2014). The phylogenetic analysis revealed that some of the fungal species found in Grahamstown and Gauteng are related while others are not related. The observed relationship suggest that the fungal species identified in harvested rainwater could be from the same source. This observation may further imply that some of the species are circulating both in Gauteng and Grahamstown.

6.5 CONCLUSIONS

The current study showed evidence of pathogenic microorganisms isolated from RWHS situated in different regions of South Africa. Knowledge on the type of contaminants from various locations may help in the development of proper and efficient treatment technologies. Furthermore, results showed that pathogens such as *Salmonella* and *Legionella* can be isolated from various roofing materials irrespective of the location of the RWHS. To ensure successful implementation or installation of the

RWHS factors such as location need to be taken into consideration as users of harvested rainwater may be at risk of contracting water-borne diseases. The results obtained in this study must be used to improve the quality of harvested by taking precautions when installing new RWHS and implementing proper monitoring programmes. Proper maintenance of the RWHS coupled with the correct treatment technologies will ensure that users of harvested rainwater consume good quality water.

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CHAPTER SEVEN: EFFICIENCY OF A NANO COMPOUND QUATERNARY IMIDAZOLIUM MODIFIED MONTMORILLONITE IN INACTIVATING BACTERIA ISOLATED FROM HARVESTED RAINWATER[5]

7.1 INTRODUCTION

Access to water of adequate quantity and quality is essential to sustain life. However, lack of access to safe water due to population growth, contamination of fresh water resources and climate change has led to increased water demand (Becker, 2013). Depletion of water sources such as river and groundwater, coupled with low water use efficiency are increasingly threatening the security of urban, agricultural and environmental water needs (Rathnayaka *et al*., 2016). Furthermore, the cost of providing safe water is rising because of increasing energy costs, growing populations, climatic changes and other environmental issues (Grey *et al*., 2013). The individual daily water requirements under average conditions are estimated at 2.5 L for males and 2.2 L for females (WHO, 2005). South African standards relating to a 'basic' level of water supply is defined as 25 L per capita per day, which is a level sufficient to promote healthy living (DWAF, 2005). Alternative water supplies such as harvested rainwater, grey water and storm water have been explored in many parts of the world (Rathnayaka *et al*., 2016). Rainwater harvesting has received increased attention as a potential alternative drinking water supply source worldwide. Advantages of using harvested rainwater include that it could be used to augment water supply, prevent flooding, help in controlling climate change impacts and contribute to the storm water management (Eroksuz and Rahman, 2010). However, the quality of harvested rainwater from the rooftop catchments is often polluted and consumers of collected and stored rainwater may be at considerable risk to a variety of infectious diseases around the world (Lye, 2014). Waterborne diseases such as cholera, typhoid fever and gastroenteritis caused by harmful microorganisms affect many people globally and originate from untreated water sources such as harvested rainwater, ground water and river

[5] Malema, M.S., Abia, A.L.K., Tandlich, R., Zuma, B., Mwenge Kahinda, J., Ubomba-Jaswa, E., 2018. Efficiency of a nano compound quartenary imidazolium modified montmorillonite in inactivating bacteria in harvested rainwater. Manuscript submitted to the Journal of Environmental Sciences.

water (WHO, 2003). Household water treatment technologies such as boiling, chlorination and solar water disinfection (SODIS) are used to reduce pathogens in water sources before consumption. Although these technologies are typically user-friendly, low cost, low maintenance, and grid-independent, they all have disadvantages. Boiling requires high costs fuel consumption; often traditional fuel (firewood, kerosene/gas) that contributes to deforestation, indoor air pollution, potential user taste objections and high burn incidences (Skinner and Shaw, 2004). Moreover, water heating is responsible for 32% of household energy consumption in South Africa, where water is predominantly heated with horizontally-oriented cylindrical electric water heaters (Stone *et al*., 2019). Stone *et al*. (2019) further indicated that during water heating, in which the lower surfaces of the heater remain at temperatures below 45 °C, an ideal environment for *Legionella* growth is created and that it is likely that only biofilm-associated *Legionella* survive inside a heater tank. Chlorination may lead to the formation of disinfection by-products (Lalley *et al*., 2014). Limitations of SODIS include the small volumes of water that can be treated and the dependence on suitable climate and weather conditions (Polo-López *et al*., 2011). The increasing demand for water mostly in developing countries will likely lead to simultaneous use of water sources, which then will drive the need to explore treatment methods which will be able to treat water from various sources. For example, during the rainy season, communities might resort to water harvesting methods but switch to another water source such as ground water during dry periods, this might lead to a mixture of rainwater and ground water in the tank or storage material. Therefore, treatment methods must also be able to handle the various water mixes that might occur when communities are facing water shortages.

With various available water treatment methods, communities must be educated about choosing the correct treatment method especially if they use a combination of water sources. For example, harvested rainwater and borehole water may have different water quality problems which may have a significant impact on the suitability and effectiveness of the chosen water treatment method. As alluded above, household treatment options must be simple, cost-effective and capable of treating daily, to the required standards, sufficient quantities of water. With the disadvantages of household treatment technologies discussed above, it is evident that emerging treatment technologies should be able to treat water from various sources as well as be able to remove all the potential pathogens. The use of nanomaterials in the treatment of water is on the rise with various natural or engineered nanoparticles developed as antibacterial agents (Qin *et al*., 2018). Nano-adsorbents used at the household level are available in powder form, coated onto a substrate or in a filter (Prathna *et al*., 2018). Modification of clay minerals in nanotechnology to increase their adsorbent capacity to efficiently remove contaminants from drinking water has been reported (Srinivasan, 2011). When compared to other low-cost adsorbents, the contaminant adsorption capacity of clays and their modified composites are either equivalent or better (Srinivasan, 2011). Montmorillonite (MMT), kaolinite and illite are widely used because of their high specific surface area, chemical and mechanical stability, a variety of surface and structural properties

and cost effective (Krishna *et al*., 2000; Lin and Juang, 2002). Montmorillonite is a layered alumosilicate mineral most often present in clays and most often used in polymer nanocomposite preparation (Huskić *et al*., 2008). The price of clay ranges from $0.005–0.46/kg and the price of montmorillonite is about $0.04–0.12/kg (Babel and Kurniawan, 2003). Although few studies have reported on the effectiveness of using clay based nano materials-organoclays, information on the cost of this emerging technology is still very scarce. This is partly due to the emerging nature of the field as well as limited commercialization of the clay-based nanotechnology techniques. Nanomaterials such as silver nanoparticles, titanium dioxide, carbon nanotubes, zinc oxide, copper and copper oxide nanomaterials and quaternary modified montmorillonite have been tested for their antibacterial properties to a broad spectrum of microorganisms (Dankovich and Gray, 2011; Raghupathi *et al*., 2011; Li *et al*., 2012; Kleyi et *al*., 2016). The use of nanomaterials for disinfection of harvested rainwater have been reported previously using silver impregnated nanomaterials (Nawaz *et al*., 2012; Adler *et al*., 2013). The use of silver nanoparticles in water treatment was effective against microorganisms such as *E. coli* and *P. aeruginosa* (Dankovich and Gray, 2011). Kleyi *et al*. (2016) reported on the success of the quaternary imidazolium modified MMT in both river and borehole water, but such a test has not been tested as a suitable option for harvested rainwater. Therefore, this study aims to report on the disinfection properties of quaternary imidazolium modified MMT in harvested rainwater with the inclusion of river and borehole water.

7.2 METHODOLOGY

7.2.1 Sample collection

Rainwater samples were collected from the Council for Scientific and Industrial Research (CSIR)'s rainwater harvesting systems (25.7539° S, 28.2784° E). River water was collected from the Olifants River, Witbank (25°5825.5 S, 29°1707.5°E). Borehole water was collected from a privately-owned borehole in Pretoria (25°742.58 S, 28°306.18 E). Samples were collected once a week between April and June 2018 in sterile 5 L bottles and transported to the CSIR's Microbiology laboratory on ice for microbial analysis within 6 hrs. A total of 72 samples were analysed during the study period inclusive of all the water sources i.e. harvested rainwater, river water and borehole water. An aliquot of 1 L was preserved from each sample and was used to analyse the physicochemical parameters.

7.2.2 Preparation of quaternary imidazolium modified montmorillonite

The nanomaterial used in this study was obtained from the CSIR's material science and manufacturing unit (Figure 7.1). The preparation, synthesis, modification and characterisation of the MMT nanomaterial- organoclay have previously been reported by Kleyi *et al*. (2016).

Figure 7.1: *Quaternary imidazolium modified montmorillonite*

7.2.2.1 Synthesis of quaternary imidazolium salts

Bromooctane (1 mol equiv.) was added to a solution of *N*-octylimidazole (1 mol equiv.) in acetonitrile (20 mL). The mixture was heated under reflux for 12 hrs. The unreacted starting reagents were removed by extracting several times with hexane. Removal of acetonitrile under vacuum resulted in a product of brown oil or gel.

7.2.2.2 Modification of the nanoclay (Montmorillonite - Cloisite-Na)

Approximately 20 g Cloisite-Na (CNa) was fully dispersed by vigorously stirring in 400 mL deionised water for 4 hrs. Approximately 100 mL of ethanol solution containing an appropriate mass of the surfactant (quaternary imidazolium salt) was added to the dispersed CNa. The mixture was continuously stirred overnight for 12 hrs and the surfactant-modified CNa was filtered under suction, washed several times with a (50:50 v/v) ethanol/water mixture and dried in an oven at 100 °C. It was then ground to pass through a 250 μm sieve and stored in a closed container. A 200 mg of the modified quaternary imidazolium MMT was chosen for the *E. coli* inoculation studies and 500 mg for the total bacterial experiments. Initially, the water samples for the inoculation studies were subjected to 50 mg and 100 mg of the treatment material but due to poor *E. coli* removal, the treatment was increased to 200 mg.

similarly a treatment dosage of 200 mg and 300 mg yielded poor results with the total bacterial experiments and therefore increased to 500 mg.

7.2.3 Determination of physicochemical parameters in unsterilised water sources

Conductivity, temperature and pH were measured using a multi-parameter water analyzer (Hach, HQ40d, Colorado, USA), Turbidity was measured using a portable turbidity meter (Hach, 2100Q, Colorado, USA). Alkalinity, aluminium, ammonia, bicarbonates, carbonates, chloride, COD, lead, nitrate, ortho Phosphates, sulphate and zinc were analysed by the CSIR's environmental laboratory, Pretoria.

7.2.4 Water disinfection analysis

7.2.4.1 *E. coli* inactivation of sterilised harvested rainwater using 200 mg quaternary imidazolium modified montmorillonite

Rainwater samples (1 and 2 L) were first sterilised at 121°C for 15 minutes and inoculated with *E. coli* (ATCC® 25922, Quantum biotech, Johannesburg) to a final concentration of 10^7 CFU/mL. Approximately 200 mg of the quaternary imidazolium modified MMT was weighed and transferred to the inoculated harvested rainwater samples. A sample was collected immediately after inoculation (time 0) and thereafter every hour for 5 hrs. *E. coli* concentrations in the samples were analysed using the spread plate method on nutrient agar (Merck, Johannesburg). The plates were incubated at 37 °C for 24 hrs. Following incubation, *E. coli* concentrations were counted and expressed as colony forming unit (CFU) and recorded. Harvested rainwater samples with *E. coli* and without the nanomaterial were used as controls. To test if other parameters such as turbidity could affect the treatment efficiency of harvested rainwater using the nanomaterial, unfiltered river water and borehole water samples were included simultaneously with harvested rainwater. The disinfection experiments using river and borehole water samples were carried out as previously described for harvested rainwater samples.

7.2.4.2 Total bacterial inactivation of unsterilised harvested rainwater using 500 mg quaternary imidazolium modified montmorillonite

Unsterilised harvested rainwater samples (1 and 2 L) were collected and analysed for bacterial concentrations before treatment. *E. coli* and total coliforms were measured using the Colilert-18® / Quanti-tray®/2000 (IDEXX Laboratories, Inc., Johannesburg South Africa) as previously described (Malema *et al.*, 2018). Water samples were then treated with 500 mg quaternary imidazolium modified MMT to check the efficiency of the treatment material in water with a higher microbial load. Treated samples were collected immediately after treatment (time 0) and after that every hour for 8 hrs. Bacterial concentrations in the samples were analysed using the spread plate method on nutrient agar (Merck, Johannesburg) and incubated at 37 °C for 24 hrs. Following incubation, colonies were counted to express the bacterial concentrations in CFU/mL. Unsterilised river and borehole water samples were included in the analysis and disinfection experiments carried out as previously described for harvested rainwater samples.

7.2.4.3 Evaluation of *E. coli* and total bacterial regrowth

To examine whether there was any possible regrowth of *E. coli* and total bacteria after disinfection with the modified quaternary imidazolium MMT, the treated water samples (harvested rainwater, river water and borehole water) were kept for 24 hrs and 48 hrs at room temperature, and the spread plate technique was used to enumerate viable *E. coli* and total bacterial cells on nutrient agar.

7.3 RESULTS

7.3.1 Determination of physicochemical parameters in unsterilised water sources

All the measured physicochemical parameters in the harvested rainwater samples fell within the prescribed South African drinking water quality standards (DWAF, 1996) (Table 7.1). Borehole and river water samples had higher levels of turbidity; 10.12 NTU and 5.39 NTU, respectively. Conductivity was also higher in both river water (380.67 µS/cm) and borehole water (298.33 µS/cm) samples. All the other parameters such as pH, nitrate, chloride, COD, ammonia and sulphates were within the South African drinking water quality standards (DWAF, 1996) for all the water sources.

Table 7.1: *Mean values of physicochemical parameters of water sources (rainwater, river and borehole) before treatment*

Parameter	Rainwater	Borehole	River	SA guideline	Unit
Alkalinity	0.39	3.45	2.46	NA	mg/L
Aluminium	0.06	Below LOD	0.01	<300	mg/L
Ammonia	0.23	0.15	0.01	<1000	mg/L
Bicarbonates	5.0	5.0	Below LOD	NA	mg/L
Carbonates	Below LOD	Below LOD	0.03	NA	mg/L
Chloride	2.6	21	17	<300	mg/L
COD	Below LOD	Below LOD	1.00	<75	mg/L
Conductivity	56.8	**298.33**	**380.67**	<170	µS/cm
Lead	0.02	Below LOD	Below LOD	<10	mg/L
Nitrate	2.4	1.0	Below LOD	<10	mg/L
Orthophosphate	0.23	0.26	Below LOD	<5	mg/L
pH	5.75	6.0	7.84	5.0-9.7	pH units
Turbidity	0.73	**10.12**	**5.39**	<5	NTU
Temperature	16.27	18.3	18.2	NA	°C
Sulphate	2.04	Below LOD	186.13	<500	mg/L
Zinc	2.57	0.02	**Below LOD**	**<5**	mg/L

Note: NA= not applicable, LOD= limit of detection

7.3.2 Inactivation of *E. coli* from sterilised harvested rainwater using 200 mg quaternary imidazolium modified montmorillonite

The complete inactivation of *E. coli* in sterilised harvested rainwater was achieved in 3 hrs for the 1 L samples while for the 2 L samples complete inactivation was achieved in 2 hrs (Figure 7.2a). For sterilised river water, complete inactivation of *E. coli* was achieved in 4 hrs for the 1 L and 5 hrs for the 2 L samples (Figure 7.2b). Inactivation of *E. coli* from sterilised river water with 200 mg of quaternary imidazolium modified MMT required a longer time period when compared to harvested rainwater. However, more time was required for complete *E. coli* inactivation in sterilised borehole water samples where complete *E. coli* inactivation was achieved in 5 hrs (2 L) and 6 hrs for the 1 L samples (Figure 7.2c).

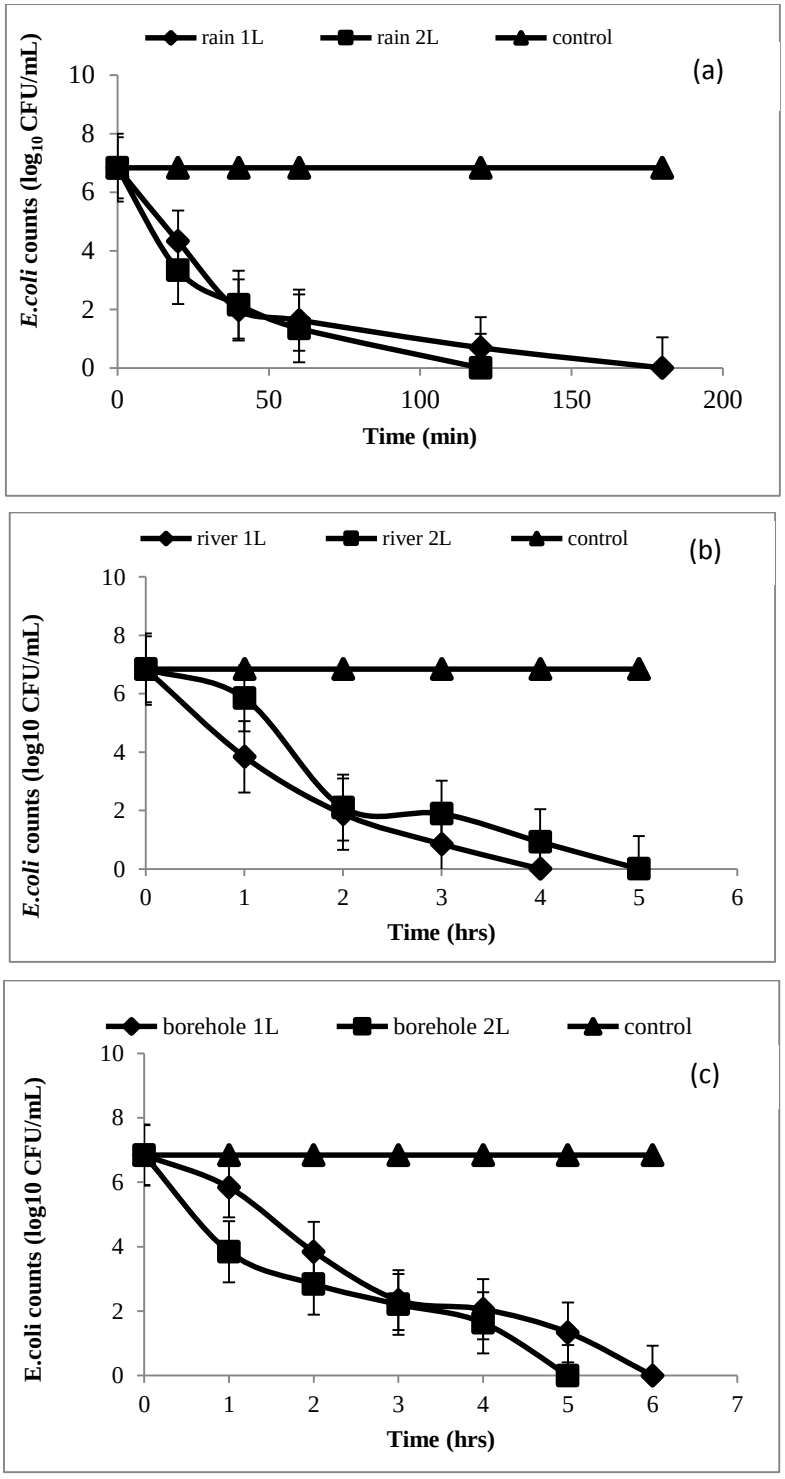
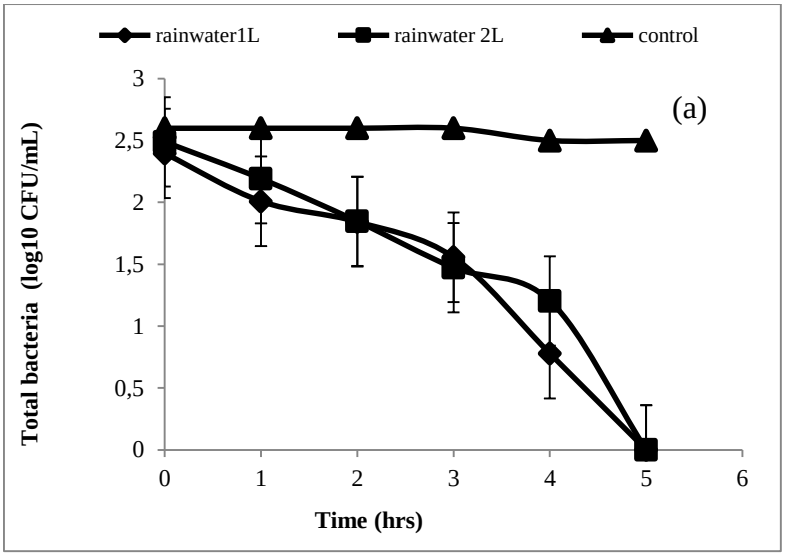
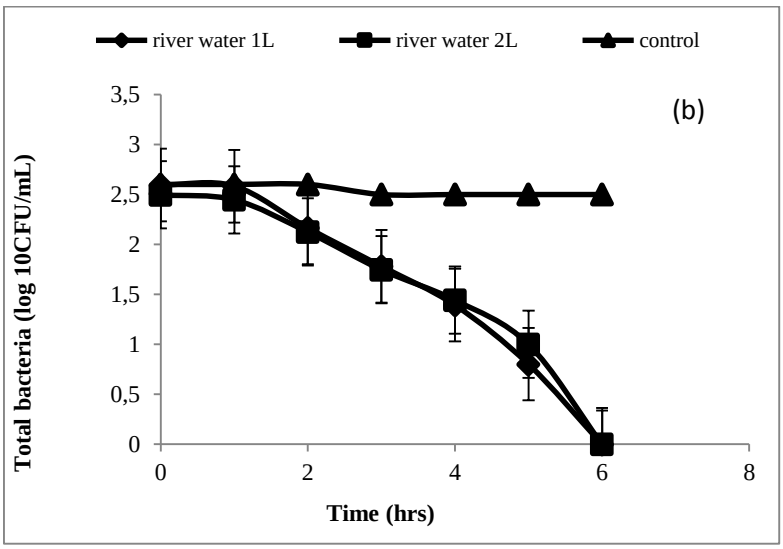


Figure 7.2: *Inactivation of E. coli using 200 mg of quaternary imidazolium modified MMT from sterilised (a) harvested rainwater, (b) river water and (c) borehole water. Error bars indicate standard error of triplicate measurements*

7.3.3 Total bacterial inactivation of unsterilised harvested rainwater using 500 mg quaternary imidazolium modified montmorillonite

The *E. coli* and total coliforms evaluated using Colilert-18/Quanti-tray/2000 before treatment of raw water was 1MPN/100 mL and >2419.6 MPN/100 mL respectively for harvested rainwater. The treatment dosage was initially 200 mg but increased to 500 mg due to poor efficiency in the inactivation of total bacteria in the water samples. Complete bacterial inactivation was observed in 5 hrs for both the 1 and 2 L harvested rainwater samples (Figure 7.3a). The initial *E. coli* count in unsterilised river water was 1MPN/100 mL and 141 MPN/100 mL for total coliforms. Complete bacterial inactivation from unsterilised river water samples was achieved in 6 hrs (both 1 and 2 L) (Figure 7.3b). For borehole water the initial *E. coli* and total coliform count was >2419.6 MPN/100 mL. Complete total bacterial inactivation from borehole water was observed after 8 hrs in both the 1 and 2 L water samples (Figure 7.3c).





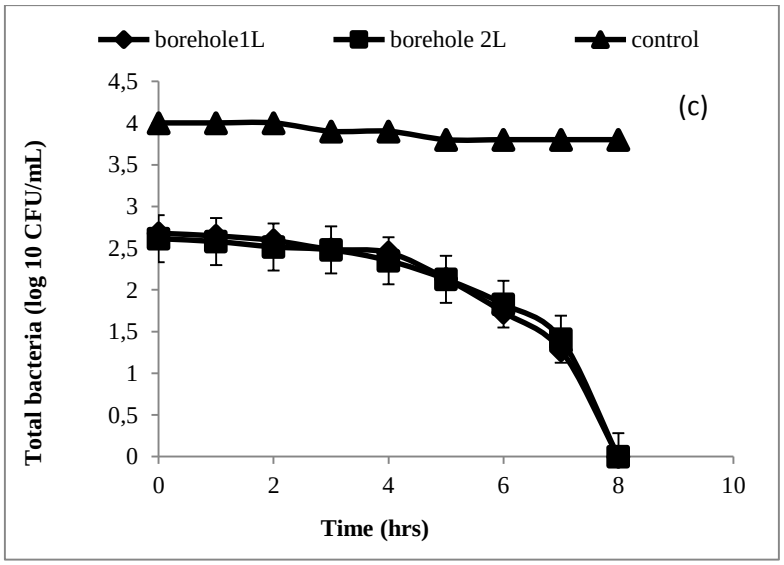


Figure 7.3: *Inactivation of total bacteria using 500 mg of quaternary imidazolium modified MMT from unsterilised (a) harvested rainwater, (b) river water and (c) borehole water. Error bars indicate standard error of triplicate measurements*

7.3.4 Evaluation of bacterial regrowth in disinfected water samples

7.3.4.1 Regrowth analysis from the *E. coli* inoculated water samples

No regrowth was observed from the *E. coli* inoculated harvested rainwater samples (both 1 and 2 L) after 24 hrs and 48 hrs of inactivation. However, in the inoculated river water samples, there was an observed regrowth of 3 CFU/mL in the 2 L samples after 48 hrs while the 1 L river water samples did not show any regrowth after both 24 and 48 hrs. Inoculated borehole water samples had no observed regrowth in the 1 L water samples while a regrowth of 4 CFU/mL was observed in the 2 L water samples after 48 hrs. No *E. coli* regrowth was observed in the 1 L water samples of the various water sources.

7.3.4.2 Regrowth analysis from unsterilised water samples

Harvested rainwater samples did not exhibit any bacterial regrowth after both 24 and 48 hrs. In the river water samples, bacterial regrowth of 9 CFU/mL and 12 CFU/mL (1 and 2 L, respectively) were observed after 24 hrs and a further regrowth of up to 16 CFU/mL (1L) and 19 CFU/mL (2 L) in 48 hrs. In the borehole water samples, bacterial regrowth of 22 CFU/mL in the 1 L and 35 CFU/mL in the 2 L were observed after 24 hrs and 32 CFU/mL (1 L) and 55 CFU/mL (2 L) in 48 hrs.

7.4 DISCUSSION

7.4.1 Determination of physicochemical parameters in unsterilised water sources

Physicochemical parameters of the harvested rainwater, such as pH, nitrate and COD were within the South African drinking water quality recommended limits. Turbidity was higher in both river water (5.39 NTU) and borehole water (10.12 NTU) and exceeded the recommended South African drinking water quality guideline of <5 NTU as compared to 0.73 NTU in harvested rainwater. A study by Keegan *et al*. (2012) reported that turbidity above 1–2 NTU reduces the efficacy of chlorination by increasing chlorine demand and potentially shielding microorganisms from inactivation. Similarly, turbidity can reduce the effectiveness of ultraviolet (UV) light disinfection by reducing UV light transmission or by shielding microorganisms from inactivation (Kollu and Ormeci, 2012). Furthermore, high levels of turbidity in water sources may limit the effectiveness of household treatment methods; for example, by overloading and clogging filters (WHO, 2011). The high turbidity in both river and borehole water observed in the current study (Table 7.1) could have contributed to the slower treatment of the water. Therefore, if the turbidity in harvested rainwater was increased by the presence of decaying leaves for example, it would be necessary to include a prefiltration step of the water before and form of treatment.

Conductivity was also higher in river and borehole water (380.67 µS/cm and 298.33 µS/cm, respectively) while in harvested rainwater conductivity was measured at 56.8 µS/cm. The recommended limit for conductivity as set out by South African drinking water quality standard is <170 µS/cm. Conductivity indicates the amount of total dissolved constituents in water (Yilmaz and Koc, 2014).

7.4.2 *E. coli* inactivation in sterilised harvested rainwater using 200 mg quaternary imidazolium modified montmorillonite

The inactivation efficiency of the modified MMT nanomaterial was determined, and the results indicate that although the treatment material proved to be more effective in harvested rainwater samples, it was also capable of treating other water sources. The results also indicate that more time or a higher treatment dosage may be required for complete inactivation of *E. coli* in both river water and borehole water. This observation could be attributed to the fact that borehole and river water had higher turbidity measurements which could have slowed the treatment process. Kleyi *et al*. (2016) modified montmorillonite using quaternary imidazolium salts with alkyl chains, (octyl, decyl, dodecyl, tetradecyl, hexadecyl octyl and decyl) and used them for disinfection of drinking water (distilled, borehole and river water inoculated with *E. coli*). They reported a disinfection contact time of 15 min for borehole water and 25 min for river water using 50 mg of quaternary imidazolium modified MMT and 20 mL of water. Furthermore, Kleyi and colleagues reported that the mechanism of inactivation of *E. coli* on this modified clay mineral was suggested to be by rupturing of the bacteria cell membrane with subsequent leaking out of the cytoplasm, resulting in cell death. Differences in the contact times between the study by Kleyi *et al*. (2016) and the current study may be attributed to the volume difference of water samples; 1 and 2 L in this study compared to 20 mL. Therefore, the total disinfection was 5 hrs for river water and 6 hrs for borehole water. It was also noted that where parameters such as turbidity exceeded the South African recommended maximum allowable limits, treatment may be slow as it was observed on river and borehole water samples. It is, therefore, advisable to remove parameters such as turbidity from the water before treatment. Furthermore, the results further emphasize the importance of monitoring both the microbial and the physicochemical parameters of the water before deciding on the treatment option. The volume of water used in this study was based on the fact that South Africa recommend an intake of least 2 L per day of safe drinking water for the proper functioning of the body's metabolic system.

7.4.3 Total bacterial inactivation in unsterilised harvested rainwater using 500 mg quaternary imidazolium modified montmorillonite

Treatment of unsterilised water proved to be longer as compared to the inoculation experiments. Complete bacterial inactivation was 5 hrs (harvested rainwater), 6 hrs (river water) and 8 hrs (borehole water). The results were not surprising as the unsterilised water sources may contain broader microbial contamination than the *E. coli* inoculation experiments. Thus, a higher bacterial population would likely result in lower bacterial deactivation efficiency. Furthermore, analyses conducted before treatment showed that the contamination in both harvested rainwater and river water was likely due to microbes other than *E. coli*. The different rate of inactivation observed shows that various water sources may require different treatment methods or if the same treatment method is used concentration of the treatment method should be carefully applied. Furthermore, initial bacterial counts before treatment revealed that borehole water was the more contaminated (>2419.6 MPN/100 mL for both total coliforms and *E. coli*) as compared to harvested rainwater and river water, hence a slower treatment process was observed. Results observed in this study indicate that water high in turbidity coupled with higher contamination levels would likely render the chosen treatment method ineffective. The quaternary imidazolium modified MMT used in the current study shows potential as a household water treatment method. However, more studies must be carried out concentrating on various volumes of water samples as well as various concentrations in order to establish the correct treatment dosages and also to document the time needed for complete inactivation of microorganisms.

7.4.4 Evaluation of bacterial regrowth in disinfected water samples

7.4.4.1 Regrowth analysis from the E. coli inoculated water samples.

There was no *E. coli* regrowth observed in all the tested *E. coli* inoculated harvested rainwater samples using 200 mg dosage after 24 and 48 hrs. This confirms the efficacy of the quaternary imidazolium modified MMT in harvested rainwater. Furthermore, there was no observed regrowth in the 1 L water while a regrowth of 3 CFU/mL for the river (2 L) and 4 CFU/mL borehole water (2 L) was observed after 48 hrs suggesting that the treatment material for the 2 L water may not have been enough or needed more time to prevent regrowth or to offer residual treatment after 24 and 48 hrs.

7.4.4.2 Regrowth analysis from unsterilised water samples

No bacterial regrowth was observed in unsterilised harvested rainwater with the 500 mg dosage after 24 and 48 hrs. However, in river and borehole water samples, both the 1 L and 2 L water samples

showed bacterial regrowth which indicates the lack of residual treatment which would have prevented the observed regrowth. A study by Nawaz *et al.* (2012) investigated silver disinfection of *P. aeruginosa,* and *E. coli* in rooftop harvested rainwater and reported an insignificant re-growth at 0.01mg/l and 0.02 mg/L while at a higher dosage of 0.04 mg/L of silver no *E. coli* regrowth was observed. They further noted that turbidity might lead to regrowth of microorganisms in water as it serves as a source of nutrients for water-borne bacteria. A similar observation was made in this study; *E. coli* regrowth was observed for the water samples with high turbidity (river and borehole) while no regrowth was observed for harvested rainwater samples.

7.5 CONCLUSION

The quaternary imidazolium modified MMT proved to be an efficient modified treatment option for various water sources especially harvested rainwater. The results suggest that turbidity is a critical aspect to consider before implementing treatment. Harvested rainwater was efficiently treated with less contact time compared to river and borehole water because all the physicochemical parameters were within the recommended South African standards. Furthermore, microbial regrowth was observed in water with high turbidity such as river and borehole water while harvested rainwater had no regrowth after both 24 and 48 hrs. Thus, using the quaternary imidazolium modified MMT to treat rainwater with turbities as those recorded for river or borehole water, it would be essential to include a prefiltration step to maximise the treatment efficiency. Prefiltration may also eliminate the chance for microbial re-growth after treatment and therefore ensure that users of the water are protected from any harmful microorganisms. Although the current study shows the potential of using the nano compound-organoclay as a potential household water treatment method, further studies must be carried out to check the efficiency of the treatment method on various pathogens such as *Salmonella, Shigella, vibrio cholera*, viruses and fungal isolates. This will further ascertain that the nano compound-organoclay used in this study is an efficient treatment method which can be applied to various water sources containing various microorganisms because an effective treatment method must be able to remove all pathogens and produce safe water for human consumption.

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CHAPTER EIGHT: CONCLUSIONS, RECOMMENDATIONS AND FUTURE WORK

8.1 CONCLUSIONS

The aim of the current study was to determine the quality of harvested rainwater from different areas in South Africa and to further explore a suitable treatment method. The findings of the study suggested that harvested rainwater from all the monitored areas is of poor quality and should not be used for potable purposes without any treatment. The treatment material used in this study further showed potential as a household water treatment option which will also cater for harvested rainwater as results revealed that it was more efficient in the treatment of harvested rainwater. In South Africa, Drinking water quality guidelines stipulated by DWAF (1996) and the South African Bureau of Standards are used to assess the quality of harvested rainwater. The stipulated guidelines states that indicator organisms such as *E. coli*, total coliforms and enterococci should be used on regular basis to test for potential possible contaminants in drinking water. Currently there are no international or national guidelines for monitoring and routine analysis of harvested rainwater in South Africa. Pathogens such as S*almonella, Shigella, Legionella, Vibrio cholera* and pathogenic fungal isolates were found in various RWHS made of different roofing materials and situated in different areas of South Africa. The existence of these pathogenic strains is of serious concern as consumers of harvested rainwater may be at higher risk of contracting water-borne infections. To overcome the problems associated with contamination of the RWHS, there is a need to explore simple, cost-effective and easy to use household water treatment methods capable of delivering safe water to communities using harvested rainwater. Results from the current study also indicated that physicochemical analysis such as turbidity, COD and nitrates also exceeded the recommended South African drinking water quality guidelines, therefore new household water treatment methods should also be able to address the chemical quality of harvested rainwater.

8.2 RECOMMENDATIONS

The risk of infections due to consumption of contaminated harvested rainwater is very high as the quality of harvested rainwater deteriorates rapidly and affected by various factors. The implementation of proper RWH guidelines in South Africa will help minimise the various contamination pathways that currently contribute to the poor quality of harvested rainwater. The installations of first flush diverters and pre-filtration methods such as gutter guard and leaf screen will help reduce contamination in

harvested rainwater. Furthermore, proper maintenance of the RWHS coupled with the correct treatment technologies will ensure that users of harvested rainwater consume good quality water. Rainwater samples tested in this study showed contamination with varying concentrations of pathogenic *E. coli* strains and that HRW tanks could serve as reservoirs for not only pathogenic but also antibiotic-resistant *E. coli* strains including MAR strains. It is therefore recommended that in areas where the harvested rainwater is used for potable purposes, the water be properly treated to eliminate chances of contracting water-borne diseases. Monitoring the quality of harvested rainwater is another important tool and should be carried out in a manner that is easy and cost effective. The current study showed the potential of a household monitoring water quality tool (H_2S test kit) which is efficient in detecting faecal contamination in harvested rainwater. The use of the H_2S test method in monitoring the quality of harvested rainwater was successful according to a study by Tandlich *et al.* (2014). Therefore, raising awareness on the importance of water quality monitoring and household water treatment options are highly recommended and will ensure that communities relying on harvested rainwater are empowered to take charge of their own water thereby reducing diarrhoeal diseases caused by contaminated harvested rainwater. Routine monitoring and treatment are essential to ensure that harvested rainwater is fit for intended use as well as to stimulate the need for strategies (e.g., maintenance of HRW systems, constant cleaning of the roof, and installation of first-flush diverters to minimize faecal contamination) that would prevent the spread of antibiotic-resistant bacteria. Community participation is also an integral part of ensuring a diseases free community. If consumers of harvested rainwater are included and educated on the proper use of household water treatment methods and home-based water quality monitoring methods, it will be easier to implement and maintain adherence of the new methods among community members. It is also recommended that results obtained in this study be used to improve the quality of harvested rainwater by taking precautions when installing new RWHS and implementing proper monitoring programmes.

8.3 FUTURE WORK

Although the current study shows the potential of using the clay-based nano compound as a potential household water treatment method, further studies must be carried out to check the efficiency of the treatment method on various pathogens such as *Salmonella, Shigella, Vibrio cholera*, *Legionella*, viruses and fungal isolates. This will further ascertain that the treatment method used in this study is an efficient treatment method which can be applied to various water sources containing various microorganisms because an effective treatment method must be able to remove all pathogens and produce safe water for human consumption. Emerging pathogens such as *Legionella* were also isolated in the RWHS tested in this study and have the potential to spread through the harvested rainwater. Further studies are needed in order to understand the real significance and dimension of the diseases

caused by water contaminated with these emerging bacteria and the ecology of these pathogens. Future studies should also include the investigations of simple household water treatment methods which are highly effective in treating harvested rainwater or improving the existing methods in order to maximise treatment efficiency. Household water quality methods serve as an early warning tool and alert users of harvested rainwater when there is contamination. More studies should also be directed towards developing efficient water quality monitoring tools which will be used in remote areas and in areas which lack proper laboratory facilities. Currently domestic rainwater harvesting in South Africa is the most common method of water harvesting and future research may also include upscaling RWH to a central water supply in South Africa. Upscaling RWH to a central supply will also minimise contamination of the RWHS as the central supply will be managed and monitored to ensure communities receive water of high quality. Quantitative microbial risk assessment (QMRA) is of importance in determining the risk of infection associated with harvested rainwater. Due to time constrains and budget, the current study did not assess the QMRA and further studies should assess the potential risk of infection associated with consuming contaminated harvested rainwater. Biofilm formation in RWH tanks is another essential element to consider when implementing monitoring programmes as biofilm may also shield microorganisms from treatment.