Microbial quality and suitability of roof harvested rainwater in rural villages for crop irrigation and domestic use

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Abstract

The study aimed at assessing the microbiological quality and suitability of roof-harvested rainwater (RHRW) for crop irrigation and domestic use. In total, 80 rain water tanks (246 samples) across three rural villages (Ga-Molepane, Jericho and Luthngele) were visited. Culture based techniques were used to isolate bacterial microbes and identities were confirmed using matrix assisted laser desorption/ ionization time of flight (MALDITOF MS). Uncultured fungal populations were also identified using pyrosequencing. *Salmonella* spp. (3%), *L. monocytogenes* (22%), total coliforms (57.7%), *Escherichia coli* (*E. coli*) (30.5%), *Enterococcus* spp. (48.8%), *Pseudomonas* spp. (21.5%) were detected in RHRW samples after rainfall. Fungal sequences belonging to species known to cause fever, coughing and shortness of breath in humans (*Cryptococcus* spp.) were identified. This study indicates that RHRW quality can be affected by external factors such as faecal material and debris on the rooftops. The use of untreated RHRW could pose a potential health risk if used for irrigation of crops or domestic use, especially in the case of a relative high population of immunocompromised individuals. This study does not dispute the fact that RHRW is an alternative irrigation water source but it recommends treatment before use for domestic purposes or for watering crops.

Keywords: human pathogens, MALDITOF MS, microbial quality, pyrosequencing, roof harvested rainwater.

Introduction

Approximately 11% of the world's population is without access to quality clean water. Despite all the development and infrastructure, an estimated 1800 million people would still lack access to safe water by 2025 (UN 2012). Due to current water shortages, the use of potable municipal drinking water for irrigation in South Africa is not encouraged (Gemmell & Schmidt 2012). As a result, microbial quality of alternative water sources has received increased attention not only in South Africa but worldwide. Harvested rainwater is a realistic alternative source that can be used for irrigation and drinking purposes. Roof harvested rainwater (RHRW) has been used for potable and non-potable purposes in many countries (Ahmed *et al*. 2014). Despite public health perception, rain water poses a low risk to human health (Ahmed *et al*. 2014; Dobrowsky *et al*. 2014; Chidamba & Korsten 2015a). Various microorganisms, including human pathogens, could be present in dust, faeces of birds, insects and sometimes small mammals present on rooftops (Ahmed *et al*. 2010). During rainfall, faeces and other debris harbouring pathogens could be washed into rainwater harvesting tanks thus degrading its quality (Pachepsky *et al*. 2011). Pathogens have been reported in RHRW (Lye 2002; Lee *et al*. 2012; Ahmed *et al*. 2014) including bacteria, viruses, and parasites, many of which could lead to waterborne illnesses (Hageskal *et al*. 2009).

The most frequently studied group of microorganisms with respect to microbial quality of water is bacteria. Presence of potentially pathogenic microorganisms, such as *Listeria monocytogenes*,

Campylobacter spp., *Salmonella* spp., *Escherichia coli*, *Cryptosporidium* spp. and *Aeromonas* spp. in RHRW has been reported (Ahmed *et al*. 2012; De Kwaadsteniet *et al*. 2013).. Fungi are usually ignored with respect to microbial quality of water, mainly because many disease outbreaks related to use of contaminated water are caused by pathogenic bacteria (Hageskal *et al*. 2009). However, Many fungal species have been detected in water including *Fusarium oxysporum* Schltdl., *Fusarium moniliforme* Sheld., *Aspergillus flavus* Link, *Penicillium chrysogenum* Thom., *Penicillium corylophilum* Dierckx, *Acremonium strictum* Gams, *Phialophora bubakii* Schol. and *Phoma exigua* Sacc. (Monchy *et al*. 2011); some of these are pathogenic to humans or are known plant pathogens. Hageskal *et al*. (2009) stated that *Aspergillus fumigatus* Fresen is one of the most important fungal pathogens detected in water that could cause infections in immunocompromised individuals. Presence of pathogenic fungi in water has been considered as a chronic and possibly underestimated problem (Hageskal *et al*. 2009). Plant pathogens play an important role in infecting irrigated crops during the seedling stage (Bell *et al*. 2006), growing conditions or postharvestly (Prusky 1996). No study has reported on the link between RHRW and crop disease or decay potential due to presence of plant pathogens.

Reports on the role of irrigation water in the contamination of produce subsequently leading to foodborne illness outbreaks are increasing (Allende & Monaghan 2015). The transfer of any pathogen in water used for irrigation on to crops could be risky if produce is consumed fresh. Studies have linked presence of pathogens on fresh produce to contaminated water (Pachepsky *et al*. 2011; Du Plessis *et al*. 2015). Presence and subsequent transfer of fungal pathogens from irrigation water onto fresh produce has not been fully investigated.

Traditional culturing assays underestimate microbial diversity due to methodological constraints and limitations including the ones used for fungal populations. Previous studies on fungal analysis of water used only culturing methods. Disadvantages of culturing fungi include: 1) inability to grow non-sporulating fungi, and 2) diverse nutrient requirements (Hageskal *et al*. 2009). As a result, reliance on molecular methods has received global recognition. The 454 pyrosequencing platform is a recent type of next generation sequencing technology. This technique is a good diagnostic tool for detection and identification of microbial pathogens. Despite being an expensive technique, the 454 pyrosequencing technique is rapid and flexible with 99% accuracy (Liu *et al*. 2015). Therefore, this study aims to assess the microbiological quality of RHRW for irrigation and domestic purposes using culturable and non-culturable approaches for bacteria and fungi, respectively. In addition, presence of potential human pathogens (bacterial and fungal) was also assessed as part of the microbial population.

Materials and Methods

Study sites and sample collection

Sampling was conducted at three rural villages in South Africa; Ga-Molepane (Limpopo Province), Jericho (North West Province) and Luthengele (Eastern Cape Province). All villages experience serious water shortages hence the communities rely on RHRW. Ga-Molepane and Luthegele villages have no boreholes and households depend entirely on RHRW during the wet-season supplemented with stream water during dryer months. Domesticated animals use the stream as water source. Jericho village has boreholes often not adequate to supply the community with enough water for domestic use. At Luthengele village, leafy green vegetables

(mainly cabbage) are grown but RHRW not used for watering. In total, 246 RHRW samples were collected from water tanks during the 2013 – 2014 rainy seasons (September to May). The strategy was to sample before and after the onset of the main rainy season. Randomly selected households (80) were used for this study. Each site was visited twice in 2013 and repeated in 2014 following the same strategy, before and after onset of the rainy season and sampling from the same households. Sanitary factors i.e., overhanging trees, animal faecal material and other debris were identified on the rooftops. Samples were collected from the outlet taps located close to the base of the water tanks, in sterilized two litre (L) bottle containers. Prior to collecting water samples, the tap was sterilized with 70% ethanol, and water was allowed to run for 30 to 60s to flush out stagnant water from the tap (Ahmed *et al*. 2010). After collection, the samples were stored on ice in cooler boxes during transportation. All samples were brought back to Pretoria University Plant Pathology Laboratories, and processed within 24 hrs.

Enumeration and isolation of bacteria

Microbes in water were concentrated by filtering 750 ml of water through 0.45-µm-pore-sized (47mm diameter) nitrocellulose membranes (Sartorious Stedim Biotech, Gottingen, Germany) by vacuum filtration (Shi *et al*. 2013), and membranes aseptically transferred into 9 ml of sterile Tryptic Soy Broth (TSB) (Merck, Johannesburg South Africa). The tubes were vortexed vigorously for 2 to 5 minutes (min) to detach the bacteria from the membranes. After serial dilutions, 100 micro litre (µl) aliquots were plated out in triplicates onto Reasoner's 2A agar (R2A) (Merck) for heterotrophic plate counts. Membrane filters in 9 ml TSB were incubated at 37°C for 24 hrs with agitation (150 rpm). Afterwards, 0.1 ml of the pre-enriched broth was inoculated into 10 ml Rappaport-Vasidallis and Listeria enrichment broth for selective

enrichment of *Salmonella* (Gomba *et al*., 2016) and *L. monocytogenes*, respectively. Rappaport-Vasidallis was incubated at 42 °C for 24 hrs while *Listeria* enrichment broth was incubated at 37 °C for 18-24 hrs. A loopful of each selective enrichment culture with growth was streaked onto Xylose Lysine Deoxycholate (XLD) agar (for *Salmonella* spp.) and Oxford-*Listeria* selective agar (for *L. monocytogenes*) plates.

All media were incubated at 37 °C for 24-48 hrs except R2A plates (25 °C for 24-72 hrs) and *Salmonella* enrichment broth. All media used were obtained from Merck. After incubation, colonies recovered using R2A were counted, recorded and expressed as colony forming units per ml.

Indicator system

Densities of total coliforms, *E. coli*, *Pseudomonas* and *Enterococci* were determined with Colilert-18, Pseudolert-18 and Enterolert-18 chromogenic substrate tests kits and Quantitray 2000 trays (Idexx, Westbrook, Maine), as per the manufacturer's instructions. For highly contaminated samples, one ml was homogenized in 99 ml sterile water to satisfy the 100 ml requirement for quantification using Quantitray 2000 trays. The manufacturer's most probable number (MPN) data table was used to generate microbial density estimates per 100 ml.

After incubation (37 °C for 18-24 hrs), the backing material of each Quantitray was disinfected with 70% ethanol and a sterile razor blade used to pierce the backing material of three fluorescence positive wells per tray (Chidamba & Korsten 2015a). A loop full of well content was streaked onto *E. coli*, *Enterococcus* spp. and *Pseudomonas* spp. selective agar (Merck) as

described before (Chidamba & Korsten 2015a). All presumptive isolates were purified on nutrient agar (NA) (Merck) and confirmed with matrix-assisted laser desorption ionisation (MALDI-TOF-MS) analysis (MALDI Biotyper® system, Bruker Daltonics, Billerica, MA, USA).

Matrix assisted laser desorption ionisation identification

Presumptive isolates were purified on NA prior to MALDI-TOF-MS analysis. The automated process from MALDI-TOF-MS measurement to species identification occurs without user intervention (Pinto *et al*. 2011; Chidamba & Korsten 2015b). Using default settings, the manufacturer's MALDI Biotyper 3.0 software (Bruker Daltonics, Germany) analyzed spectra of the isolates and compares it with reference spectra in the database. A logarithmic score is generated corresponding to similarity of spectral patterns and interpreted as per manufacturer's instructions. Results are conveyed as log (score) values ranging from 0 to three levels. Scores designate high confidence level species identification (≥2.300), species identification (≥2.000), genus identification (1.700 –1.999) and no identification (<1.700).

DNA extraction

Genomic DNA (gDNA) was isolated directly from filter paper, with a ZM fungal/bacterial DNA miniprep™ kit (Zymo Research Corporation, Inqaba Biotech catalogue # D6005) as per manufacture's specifications. The DNA concentration was determined with the Qubit 2.0 Fluorometer (Lifescience Technology, Johannesburg). Isolated gDNA was stored at -20 °C and kept on ice during further experimental procedures.

Pyrosequencing analysis

DNA samples were sent to Inqaba Labs (Pretoria) for tag encoded pyrosequencing of a portion of the fungal internal transcribed spacer (ITS) region using the Roche GS FLX+ 454 pyrosequencer (GATC Biotech, Konstanz, Germany). The fungal internal transcribed spacer (ITS) region was amplified using primer set ITS1F (CTTGGTCATTTAGAGGAAGTAA) and ITS4R (TCCTCCGCTTATTGATATGC) (McHugh *et al*. 2014). Bacterial pyrosequencing is not presented in this paper and has been presented in another paper (Chidamba & Korsten 2015b). The MALDITOF MS database restricted fungi identification hence we relied on 454 fungal pyrosequencing to identify isolates.

Pyrosequencing data processing and analysis

Pyrosequencing data analysis for selected samples (Tanks 1-5) was performed using QIIME as established in previous studies (McHugh *et al*. 2014), with modifications. Selection of samples for analysis was based on population diversity. Sequences with less than 200 bp and quality score less than 25 were removed from the pyrosequencing-derived data sets using the UCLUST pyrosequencing pipeline initial processing (Cleary *et al*. 2012). The web-server SnoWMAn 1.15 (http://snowman.genome.tugraz.at) (Stocker *et al*. 2010) was used with the following settings for the taxonomic-based analysis: analysis type: UCLUST pipeline; chimera filtering reference database: Chimerarefdbs_09_Feb_2012_aligned; Mothur alignment reference database: fungalITS_24-Sep-2013-current_prokMSA_aligned; Mothur chimera checking reference database: chimerarefdbs_39-Feb-2012_16S_aligned and taxonomic reference database: greengenes_24-Mar-2010; rarefaction method: RDP; taxonomy: RDP; confidence threshold: 80%; include taxa covering more than 1%.

Operational taxonomic units (OTUs) for rarefaction analysis, were clustered at 97% (species level), 95% (genus level) and 80% (phylum level) similarity cut-offs (Yang *et al*. 2014). The calculation of ACE, Evenness, Shannon, Chao1 and Species Richness diversity indices and the generation of principal coordinate analysis (PCoA) plots were generated using the open source software package QIIME (http://qiime.sourceforge.net), designed for combining heterogeneous experimental datasets, analysis of high throughput community sequencing data and obtaining new insights about various microbial communities (Caporaso *et al*. 2010).

Statistical analysis

Statistical analyses were conducted with SAS statistical software (version 9.3). Analysis of variance (ANOVA) tests were performed and the least significance difference (LSD) test used to derive statistical differences ($P< 0.05$) of microbial loads among all sampling areas.

Results

Microbial quality of roof harvested rain water

Out of 246 samples average total coliforms (57.7%), *E. coli* (30.5%), *Enterococcus* spp. (48.8%), *Pseudomonas* spp. (21.5%) and total aerobic bacteria (100%), were detected after rainfall. Prevalence of total coliforms, *Enterococcus* and *Pseudomonas* from all sites was not significantly different before and after rainfall. Before the rainy season, total coliforms were more prevalent in water from Ga-Molepane (43%), followed by Luthengele (35%) and Jericho (31%). Total coliforms were more prevalent in water samples from Ga-Molepane (73.7%) while the least prevalence was detected in samples from Luthengele after rainfall (Table 1).

Table 1. Average microbial loads in roof harvested rain water before and after rain[α]

Season	Site	Sample number	APC (cfu/ml)	Total coliforms*	*Escherichia coli*	*Enterococci*	*Pseudomonas*
1 (Before rainfall)	Ga-Molepane	38	5.32 ± 0.68 (100%)	1.2 ± 1.2 (43%)	0.52 ± 0.87 (23.5%)	1.45 ± 1.53 (27.1%)	0.45 ± 1.05 (31.3%)
	Jericho	89	5.91 ± 1.13 (100%)	1.22± 1.1 (31%	0.02 ± 0.1 (5%)	2.03 ± 0.98 (13.6%)	0.48 ± 1.11 (14%)
	Luthengele	119	5.61 ± 0.71 (100%)	1.11 ± 1.34 (35%)	0.35 ± 0.67 (18.7%)	1.52 ± 1.47 (45.8%)	0.19 ± 0.62 (17%)
2 (After rainfall)	Ga-Molepane	38	5.26 ± 0.69 (100%)	1.62 ± 1.25 (73.7%)	0.82 ± 1.02 (31.6%)	0.44 ± 0.7 (42.1%)	0.98 ± 1.1 (36.8%)
	Jericho	89	5.67 ± 1.03 (100%)	1.47 ± 1.14 (74%)	0.66 ± 0.85 (29.2%)	0.13 ± 0.35 (48.3%)	0.8 ± 1.27 (21.3%)
	Luthengele	119	5.36 ± 0.73 (100%)	2.19 ± 1.13 (40.3%)	0.26 ± 0.49 (31.1%)	0.62 ± 0.72 (51.2%)	0.52 ± 1.08 (16.8%)

APC: aerobic plate counts, ( ): prevalence percentages, [α] Values are log mean ± standard deviation, * Values are MPN estimates unless otherwise stated.

For season 1, concentrations of *E. coli* isolated from Ga-Molepane were significantly different from *E. coli* isolated from Jericho, but not significantly different from Luthengele *E. coli* isolates. Higher levels of *E. coli* were detected in water samples from Ga-Molepane (31.6%), followed by Luthengele (31.1%) and Jericho (29.2%) after rainfall. At least 45 and 51% of water samples from Luthengele represented *Enterococcus* spp. before and after rainfall respectively, while 21.3% accounted for *Pseudomonas* spp. in RHRW from Jericho after rainfall. In general, water samples from Ga-Molepane had the highest concentrations of faecal coliforms, *E. coli*, *Enteroccoccus* spp. and *Pseudomonas* spp. (Table 1). Total aerobic bacteria ranged from a geometric mean of 5.26 (Ga-Molepane) to 5.91 (Jericho) log CFU/ml (Table 1). Means for Aerobic plate counts from Ga-Molepane, Jericho and Luthengele were significantly different from one another with samples from Jericho recording the highest and samples from Ga-Molepane recording the lowest.

Table 2. Prevalence of human pathogenic bacteria in roof harvested rainwater

	Number of positive samples/total number of samples (%)			
	Before rain		After rain	
Site	*Salmonella*	*L. monocytogenes*	*Salmonella*	*L. monocytogenes*
Ga-Molepane	0 (0)	2 (10.5)	2 (10.5)	4 (21.1)
Jericho	0 (0)	8 (18.2)	1 (2.2)	14 (31.1)
Luthengele	2 (3.4)	19 (32.2)	2 (3.4)	25 (41.6)

Salmonella spp. and *L. monocytogenes* were present in RHRW from Ga-Molepane, Jericho and Luthengele villages. In total, 2 and 29 (before rainfall); 5 and 43 (after rainfall) RHRW samples tested positive for *Salmonella* spp. and *L. monocytogenes* respectively (Table 2). Generally, the prevalence of *L. monocytogenes* and *Salmonella* spp. increased after rainfall at all sampling sites (Table 2). The prevalence of *L. monocytogenes* was 18.2 (before rainfall) and 31.1% (after rainfall) in RHRW samples from Jericho. *Salmonella* spp. was not detected before rainfall in water from Ga-Molepane and Jericho but was present after rainfall. The number of *Salmonella* positive samples increased (from 0 (before rainfall) to 2 (after rainfall)) in RHRW samples from Ga-Molepane.

Table 3. Frequency of selected significant dominant bacterial isolates from roof harvested rainwater as identified by matrix assisted laser desorption time of flight mass spectrometry[α]

Species name	Ga-Molepane	Jericho	Luthengele
Aeromonas caviae	1	1	2
Aeromonas jandaei	3	1	2
Aeromonas veroni	2	2	2
Bacillus cereus	5	1	3
Bacillus pumilus	2	5	3
Enterobacter amnigenus	2	2	7
Enterobacter asburiae	5	7	1
Enterobacter cancerogenus	1	0	3
Enterococcus faecalis	4	0	0
Enterococcus faecium	5	15	9
Enterococcus durans	9	7	12
Enterococcus mundtii	6	9	9
Escherichia vulneris	7	1	11
Escherichia coli	15	11	9
Klebsiella pneumonia	4	5	3
Listeria monocytogenes	9	4	6
Morganellamorganii	3	2	1
Pantoea agglomerans	6	2	0
Pseudomonas otidis	2	1	1
Pseudomonas taetrolens	1	6	5
Salmonella spp.	3	4	6
Staphylococcus auricularis	3	12	4
Staphylococcus capitis	1	2	1

[α]: values are in percentages calculated from the total isolates identified.

Matrix assisted laser desorption time of flight

Bacterial isolates of from all sampling sites as identified by MALDITOF MS were tabulated (Table. 3). Of the total, opportunistic pathogens (*Staphylococcus* spp. (Niazi *et al*., 2010) (13%), *Enterococci* spp. (Shioya *et al*., 2011) (7%), and *Pseudomonas* spp. (de Bentzmann & Plésiat 2011) (3%)) and known potential human pathogens (*L*. *monocytogenes* (6%) and *Salmonella* spp. (4%)) were identified.

Of the total bacteria isolated from Ga-Molepane, *E. coli* prevalence was 15%, the highest compared to other sites. Various species belonging to *Enterococci* were identified. These included *E. faecalis* (only Ga-Molapane village) and *E. faecium* (all sites) (Table 3). Non-human pathogenic environmental bacterial isolates among them, *Acinetobacter* spp. and *Anaerococcus* spp., were also detected (results not shown).

Fungal Pyrosequencing

At phylum level classification, fungal sequences were classified into five phyla (Figure 1). These include *Ascomycota*, *Basidiomycota*, *Chytridiomycota*, *Glomeromycota and Zygomycota*. *Ascomycota* dominated the data set (32.1%), while 45.7% of the sequences were not classified to any known fungal phyla.

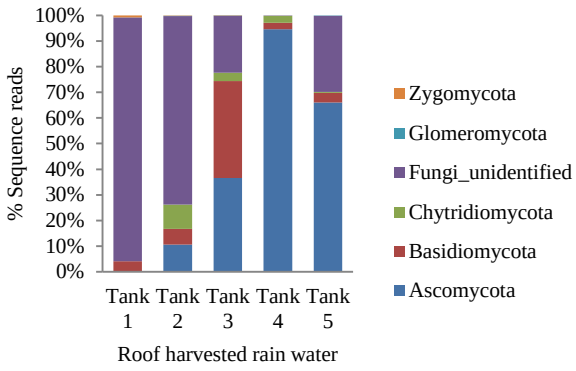

Figure 1. Phylum level pyrosequencing classification of selected roof harvested rainwater samples.

Of the identified sequences 17, 4.8 and 0.09% were classified to *Basidiomycota*, *Chytridiomycota* and *Glomeromycota* respectively (Figure 1). There were notable differences in fungal diversity in the selected RHRW samples. Fungal sequences (95%) from tank 1 were not identified while all sequences in tank 4 were identified. Ascomycota was identified in four out of the five tanks (Tank 2, 3, 4 and 5). Highest concentration of *Chytridiomycota* (70%) was identified in tank 2. Only two fungal phyla (*Basidiomycota* and *Zygomycota*) were identified in

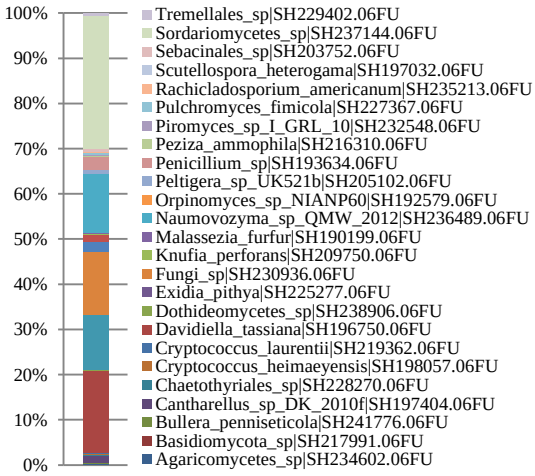

Figure 2. Species level relative abundance profiles using 18S rRNA sequence classifications of a water tank with roof harvested rainwater.

tank 1 RHRW (Figure 2). Tank 2 and 3 had more diverse fungal populations with five identified sequences represented in both RHRW tanks.

Classification at species level revealed a diverse fungal population. *Sordariomycetes* species and *Davidiella tassiana* were more predominant in tank 3 water samples (29.3% and 18.1% respectively) than other water samples (Figure 2). Sequences of species in tank 3 (13.9%) remained unclassified. Other species also identified included *Cryptococcus* spp., *Dothideomycetes* spp., *Tremellales* spp. and *Knufia perforans* Sterflinger.

Discussion

In this study, microbial quality of RHRW was tested for the presence of bacterial indicators (total coliforms, *E. coli* and *Enterococci*), opportunistic (*Pseudomonas* spp.) and potential pathogenic microorganisms (*L. monocytogenes* and *Salmonella* spp.), and fungal organisms. To assess the suitability of RHRW for irrigation and domestic use conventional culture based methods were used. There were significant variations of *E. coli* and aerobic plate counts between the three rural villages sampled. A previous study reported similar variations in the microbiological quality of RHRW (Evans *et al*. 2006a). The study further reported extensive disparities in the level of water pollution and bacterial composition between sites, and over time. Results of our study indicate presence of potential human pathogenic bacteria in RHRW from all the sites tested.

Salmonella spp. (3%) and *L. monocytogenes* (22%) were detected in RHRW samples from all three sampling areas. Ahmed *et al*. (2008) detected *Salmonella* spp. but not *L. monocytogenes* in RHRW. A previous study indicated that drinking of RHRW containing *Salmonella* spp. caused

diarrhoea and abdominal pains (De Kwaadsteniet *et al*. 2013). Based on observations during this study, RHRW was being contaminated with rooftop faecal material and dust. The source of this contamination was not specifically studied in this paper but it was observed and previously described in other studies (Evans *et al*. 2006b; Ahmed *et al*. 2012).

Rainfall patterns of Jericho and Ga-Molepane are similar and the rainfall events occur seasonally. Hence, *E. coli* concentration in RHRW is significantly higher immediately after rainfall events. Luthengele area on the other hand, experiences rain events more frequently. As a result, there is little time for accumulation of faecal material and other debris on rooftops. This could partially explain *E. coli* concentrations not being significantly different before and after rain events. The low coliforms and *E. coli* counts detected in RHRW before the start of the rainy season, may suggest that the rooftops are more consistently washed down by pouring rainwater and are therefore not consistently highly contaminated. However, the presence of *Enterococci* spp., *L. monocytogenes* and *Salmonella* spp. suggests otherwise. The mere presence of *L. monocytogenes* in water does not mean infection is possible upon consumption. However, the pathogen can proliferate in storage tanks leading to high quantities suitable for onset of listeriosis (Morobe *et al*., 2009; Golberg 2015).

In a recent study, low counts of *E. coli* were observed but detection of faecal indicator bacteria (*Enterococci* spp.) suggested the water may be contaminated (Chidamba & Korsten 2015a). Although the concentration of *E. coli* most likely decreased with time, *Enterococci* and other pathogens persisted owing to their resilience (Ahmed *et al*. 2012). This study therefore finds RHRW a potential hazard if used for domestic purposes or to irrigate crops due to the presence

of well-known human pathogens. However, it is worth noting that using polluted irrigation water does not always result in contaminated crops.

Basic systems of roof-water harvesting are used in the sampling sites visited. The system is deprived of first flush diverters and a filtration apparatus (Chidamba & Korsten 2015a). Due to this deprivation, microbiological quality of the RHRW is compromised and treatment prior to use is thus necessary. Absence of these first flush diverters contribute to increased chances of contamination of RHRW, hence faecal material and other debris on the roof and/or gutter washes into rainwater tanks. Chidamba & Korsten (2015a) established a link between indicator bacteria and pathogens isolated from rooftop surfaces and RHRW. The presence of such pathogens could be transmitted to leafy green vegetables when RHRW is used for irrigation purposes. Several studies have linked irrigation water with pathogens isolated from fresh produce (Allende & Monaghan 2015; Gelting *et al*. 2015), therefore substandard irrigation water is a contamination risk factor of fresh green leafy vegetables. The presence of human pathogens on irrigated crops due to the use of contaminated irrigation water is well described (Pachepsky *et al*. 2011).

In this study heterotrophs were isolated in RHRW at all sampling sites in concentrations higher than recommended for domestic and irrigation purposes according to the Department of Water Affairs standard, South Africa (DWAF 1996). Concentrations higher than 1000 CFU/ml in water used for drinking indicate contamination or after-growth in the water and pose an increased risk of infectious disease transmission if consumed (DWAF 1996). Even though heterotrophic bacterial counts indicate the general microbial quality of water, they do not indicate possible faecal pollution (DWAF 1996). However, DWAF (1996) recommends that water with high

concentrations of heterotrophs requires treatment prior to use. Prevalence of *Enterococci* was higher than *E. coli* in this study. This is consistent with other reports (Spinks *et al*. 2006; Ahmed *et al*. 2011). These results suggest that RHRW from these sampling sites is not fit-for-irrigation of fresh products consumed raw unless treated prior to use according to the South African irrigation water standards (DWAF 1996).

The reliance of total coliforms and indicator bacteria for water quality assessment has come under scrutiny in the recent past (Chidamba & Korsten 2015a), as findings emerged that pathogenic *E. coli* could not be isolated from water samples positive for indicator bacteria of faecal origin. However, the use of total coliforms and indicator bacteria to evaluate water quality is by far the most widely used method in laboratories. The simultaneous use of *E. coli* and *Enterococci* for potential faecal contamination assessment is recommended (Savichtcheva & Okabe 2007; Ahmed *et al*. 2011; Chidamba & Korsten 2015a), and our study also supports this approach.

Although presence of human bacterial pathogens have been widely reported in RHRW (Ahmed *et al*. 2011), the same cannot be said about human fungal pathogens. Gikas & Tsihrintzis (2012) mentioned that water harvesting systems could transmit microorganisms able to affect human health. Conventional culture techniques underestimate microbial populations (Ahmed *et al*. 2011) hence utilization of molecular methods is increasingly used to provide a better understanding of spp. present. Pyrosequencing of the ITS 1 and ITS 2 hypervariable regions of the 18S rRNA was used to investigate the fungal communities in RHRW. Results of this study indicated a diverse fungal community in RHRW.

Pencillium and *Cryptococcus* spp. were mostly detected in RHRW. Some *Penicillium* spp. are pathogenic to plants and others can affect human health such as *P. digitatum* (Pers.) Sacc.) (Hageskal *et al*. 2009; Oshikata *et al*. 2013). Further, mycotoxins and other metabolites that are produced by these organisms have been detected in storage water tanks (Paterson *et al*. 1997). Prolonged water storage may therefore lead to increased mycotoxin concentration, and could pose a potential risk to consumers with persistent consumption (Hageskal *et al*. 2009). In such a case immuno-compromised individuals are at a higher risk due to their diminished immune system. HIV prevalence in rural communities in South Africa has been reported (Shishana *et al*. 2014) and is considered high (39.4%) by world standards.

Human pathogenic yeasts, *Cryptococcus* spp., belonging to a diverse filamentous fungal division *Basidiomycota*, were also detected in RHRW. *Cryptococcus* spp. is an opportunistic human pathogen of global importance (Loftus *et al*. 2005), which has been isolated in bird faeces (Soltani *et al*. 2013). Effects of continuous exposure to *Cryptococcus* spp. include fever, coughing and shortness of breath.

Monchy *et al*. (2011) highlighted the significance of further characterization of human pathogenic microbes and understanding their effects on human health due to persistent exposure. The risk posed by the presence of sequences belonging to phylotypes with a known pathogenic history cannot be underestimated. Presence of unidentified sequences signify their absence in cluster analysis databases, hence the need for research to identify more environmental fungal populations and collection of curated sequences for fungal identification (Liu *et al*. 2015). The use of RHRW with human pathogenic isolates for domestic use and irrigation purposes without

any prior treatment could therefore be a cause of concern if pathogens persist up to the point of consumption. Future studies should focus on the link between fungal spp. in RHRW and crop disease or decay.

Conclusion

This study concluded that RHRW collected in three rural villages were of poor microbiological quality, therefore not fit for domestic use or irrigation purposes unless treated prior to use as per national guidelines (DWAF 1996). The presence of known human pathogenic microorganisms and faecal indicators in RHRW indicate a potential risk if the crops are also irrigated with this water and the product is consumed raw. Better maintenance of RHRW systems are required to reduce contamination levels. Development of multiple detection systems using faecal indicators, *E. coli*, *Enterococci* and next generation sequencing techniques could holistically improve water quality assessment.

Acknowledgements

This study was part of a solicited research project (K5/1875/4: Evaluation of the risks associated with the use of rain-water harvested from rooftops, for domestic use and homestead food gardens; and groundwater for domestic use and livestock watering) funded by the Water Research Commission and co-funded by Department of Agriculture, Forestry, and Fisheries, Republic of South Africa. We acknowledge the editorial input by Mrs. Annancietar Gomba and Dr. Erika du Plessiss. We thank the assistance of Dr. Lizyben Chidamba, Sphumelele Mkhize and Dennis Makuwa.

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