Comparison of biofilm formation and water quality when water from different sources was stored in large commercial water storage tanks

Venessa van der Merwe, Stacey Duvenage and Lise Korsten

ABSTRACT

Rain-, ground- and municipal potable water were stored in low density polyethylene storage tanks for a period of 90 days to determine the effects of long-term storage on the deterioration in the microbial quality of the water. Total viable bacteria present in the stored water and the resultant biofilms were enumerated using heterotrophic plate counts. Polymerase chain reaction (PCR) and Colilert-18® tests were performed to determine if the faecal indicator bacteria *Escherichia coli* was present in the water and in the biofilm samples collected throughout the study. The municipal potable water at the start of the study was the only water source that conformed to the *South African Water Quality Guidelines for Domestic Use.* After 15 days of storage, this water source had deteriorated microbiologically to levels considered unfit for human consumption. *E. coli* was detected in the ground- and potable water and ground- and potable biofilms periodically, whereas it was detected in the rainwater and associated biofilms at every sampling point. Imperfections in the UV resistant inner lining of the tanks were shown to be ecological niches for microbial colonisation and biofilm development. The results from the current study confirmed that long-term storage can influence water quality and increase the number of microbial cells associated with biofilms on the interior surfaces of water storage tanks.

Key words | biofilm, rainwater harvesting, water-borne pathogens, water storage

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INTRODUCTION

A great proportion of rural communities in South Africa lack access to clean potable water (Momba & Notshe 2003; DAFF 2010). Due to the lack of efficient potable water delivery systems in communities, people have to travel vast distances to collect water, making use of small plastic-based water transport devices (Momba & Kaleni 2002; Jagals *et al.* 2003; Momba & Notshe 2003). Water storage is mainly achieved through rainwater harvesting or collecting surface or groundwater which is either used directly or retained in small volumes (Momba & Notshe 2003; WHO 2008). Water within water storage tanks can be contaminated via: storm-water run-off, faulty septic systems, contaminated soil, run-off from manure in the vicinity or livestock/wildlife faeces (Beuchat 2002; Cessford & Burke 2005). In some cases, communities have access to street taps installed by the municipality to provide potable water, but families still have to collect and temporarily store the water (Nala *et al.* 2000).

The conditions under which the water is stored often affects the quality of the water, as stored water is more susceptible to environmental influences and contamination than if the water were still in its natural habitat (Jagals *et al.* 2003). It is therefore a concern that the collection and storage of untreated water supplies, such as roof catchments (rainwater harvesting), surface and groundwater, which may be contaminated with pathogens, can provide an ideal environment for microbial proliferation. Numerous studies have been done to monitor the microbial quality of water that is transported and stored in small household containers (Momba & Mnqumevu 2000; Jagals *et al.* 2003;

doi: 10.2166/wh.2012.014

Momba & Kaleni 2002; Momba & Notshe 2003; Maraj *et al.* 2006). Many of the studies have shown that the transport and storage of water after collection from the source results in microbial deterioration of the water, which often leads to levels of heterotrophic bacteria that are unsuitable for human consumption.

Studies have shown that water storage containers made of plastic-based materials, such as polyethylene, are able to support more bacterial incorporation into biofilms on their interior surfaces than those made of metal-based materials (Momba & Kaleni 2002; Momba & Notshe 2003). In addition, studies have shown that plastic-based water storage containers have a greater tendency to support the incorporation of faecal coliforms into biofilm structures (Momba & Kaleni 2002; Momba & Notshe 2003). This is concerning as these biofilms can act as reservoirs for pathogenic microorganisms that can, through growth and detachment, be responsible for the majority of the planktonic cells found in the aqueous environment (Van der Wende *et al.* 1989; Percival *et al.* 1998; Chang *et al.* 2003).

In the current study, a comparison of the water quality of three different water sources (rain-, ground- and potable water) was conducted to determine the effect of storage on water quality as well as the resultant development of biofilms. Water quality and biofilm biomass changes were followed through heterotrophic plate counts (HPCs) and scanning electron microscopy. The detection of *Escherichia coli* and total coliforms was also performed for all samples through polymerase chain reaction (PCR) and Colilert-18® analysis.

MATERIALS AND METHODS

Water storage tank design

Three 750 l water storage tanks were set up in the same vicinity at the University of Pretoria's Experimental Farm (Pretoria, South Africa; S25°45′10″; E28°14′46′) after being washed and sterilised with 70% ethanol. The tanks were made from food-grade low density polyethylene and the interior was lined with UV-resistant carbon black lining to prevent algal growth. All the tanks had a green exterior and were filled with water from different sources, i.e. ground-, rain- and municipal potable water. The rainwater that was harvested was the first rain of the season; a first-flush apparatus was not utilised. The tank containing the municipal potable water served as the control as the water is municipally treated.

The water storage tanks used in this study were specifically modified for the monitoring of biofilm formation and the collection of water from different levels within the tanks (Figure 1). The tanks were horizontally divided into three non-partitioned layers: the top level which represented the most aerobic environment; the bottom level which was considered the most anaerobic and had the most sedimentation; and the middle level which had intermediate properties. When positioned in the field, the tanks were all orientated in the same manner so as to ensure that the one side received the morning sunlight and the other the

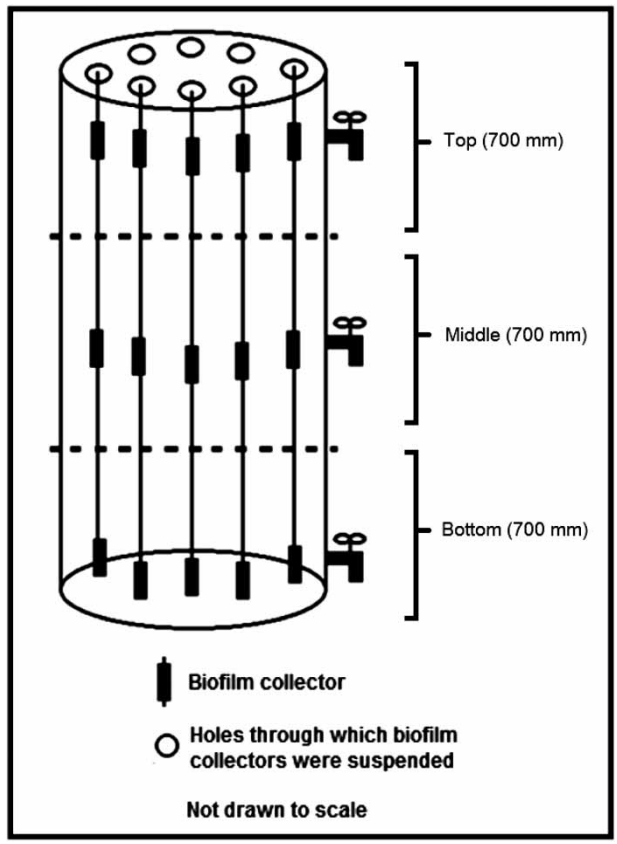

Figure 1 | Schematic presentation of the experimental set up of water storage tanks used in the current study. Tanks were horizontally divided into three non-partitioned layers (700 mm each): Top, Middle, and Bottom. Biofilm collectors were positioned so that collectors attached to a single fishing line were suspended in the middle of each of the horizontal layers. Water and biofilm samples were collected from each layer at various time intervals.

afternoon sunlight. Taps were placed on the 'afternoon sun' side of the tanks to enable water collection from the different horizontal layers at the various testing intervals.

The top of the tank was also modified to allow the suspension of biofilm collectors inside the tanks (Figure 1). The biofilm collectors were cut-outs of a tank not used in the current study. Ninety biofilm collectors, each with a surface area of ±140 mm², were suspended in each tank. Three collectors were attached to a sterile fishing line at different heights and suspended from the top of the tanks so that each collector was placed within a specific region (Figure 1). The experiment was performed on two occasions separated by 7 days. Day 0 was the start of the experiment when water was added to the tanks.

Water analysis

At day 0, 1 l water samples were collected from the source waters that were used to fill the tanks on that same day. One litre water samples were collected in triplicate from each horizontal level of the tank at day: 15, 30, 60 and 90. Water analysis of day 0 samples was therefore used to determine the background heterotrophic bacteria and *E. coli* in all the water sources at the start of the study. The water samples were filtered through a 0.45 μm pore size cellulose nitrate filter (Sartorious, Johannesburg, South Africa). The material on each filter was dislodged in to 9 ml 0.1% peptone buffered water (Merck, Pretoria, South Africa), serially diluted and used to perform viable plate counts on Standard 1 Nutrient Agar (Merck) supplemented with 0.1% cycloheximide (Sigma Aldrich, Johannesburg). Samples were incubated at 25 °C for 48 h after which colonies were recorded and transformed to $\log_{10}(x+1)$ CFU ml^{-1}.

Biofilm analysis

Biofilm collectors were installed at different positions within the tanks (Figure 1). Three 'strings' of collectors were removed from the tanks at each sampling point so that a total of nine replicates were obtained. Biofilm formation on biofilm collectors was followed for 90 days with collectors being retrieved from the tanks at the following intervals: day 1, 3, 5, 7, 9, 11, 20, 30, 60 and 90. Samples were transported to the laboratory in sterile Petri dishes for analysis.

As the biofilm collectors were cut-outs of an existing tank, one side consisted of low density polyethylene whilst the other consisted of UV-resistant carbon black lining. The side of the biofilm collector that was made of the low density polyethylene was swab sterilised with 70% ethanol to remove all biofilm formation to allow for quantification of biofilm biomass that developed on the UV-resistant carbon black lining only as this represented the inside of the tank. Cells not associated with the surface were removed by rinsing the collectors with double-distilled water before biofilm cells were removed. Attached cells were removed from biofilm collectors in a modified version of the Lehtola *et al.* (2006) protocol; mechanical shaking with 5 g 4-mm glass beads in 1 ml 0.1% peptone buffered water for 10 min at 12 Hz was used to detach biofilm cells. The bacterial content of the biofilms was then analysed through serial dilutions in 0.1% peptone buffered water and HPCs on Standard 1 Nutrient Agar (Merck) supplemented with 0.1% cycloheximide (Sigma Aldrich). Samples were incubated at 25 °C for 48 h after which colonies were recorded and transformed to $\log_{10}(x+1)$ CFU cm^{-2}.

Nucleic acid extraction

Filters from the water samples with the material collected on them (see Water analysis in Materials and methods) and biofilm biomass removed from collectors (see Biofilm analysis in Materials and methods) were enriched in tryptone soy broth (Merck) for 24 h at 37 °C. DNA was extracted from each sample using an optimised version of the Triton-X100 method (Wang & Slavik 2005). One millilitre of the samples was centrifuged for 5 min at 6,000 g respectively. Once large enough pellets were obtained, they were resuspended in double-distilled water and centrifuged for 5 min at 16,000 g; this was performed three times for each sample. The pellets were then resuspended in 50 μl 1% (v/v) Triton X-100 (Sigma Aldrich) and boiled for 10 min followed by a 10 min incubation on ice. The solution was then centrifuged at 16,000 g for 5 min and the supernatant removed. Three microlitres RNase (Roche, Johannesburg) was then added to the supernatant which was subsequently incubated at 37 °C for 2 h. The DNA extracted was used as a template for PCR.

PCR for *E. coli* detection

PCR for the detection of *E. coli* was performed using the primers: Eco1 5′-GACCTCGGTTTAGTTCACAGA-3′, Eco2 5′-CACACGCTGACGCTGACCA-3′ (585 bp) (Schippa *et al.* 2010). The PCR amplification was carried out in a GeneAmp 2400 PCR system (Applied Biosystems, Foster City, USA) with a PCR reaction mixture containing: 16.1 µl sterilised Sabax water (Adcock Ingram, Johannesburg), 0.3 µl primer Eco1 (10 pM), 0.3 µl primer Eco2 (10 pM) (Whitehead Scientific, Cape Town, South Africa), 1.5 µl template DNA (~25 ng/µl), 2.5 µl PCR buffer, 1.5 µl $MgCl_2$ (10×), 1.25 µl DMSO, 0.75 µl BSA, 0.5 µl dNTPs (10 mM of each) and 0.3 µl Taq polymerase (5 U/µl) (all from Celtic Molecular Diagnostics, Cape Town). The samples were initially incubated for 2 min at 95 °C to denature the template DNA. This was followed by 35 cycles under the following conditions: 30 sec at 94 °C, 45 sec at 61 °C and 1.5 min at 72 °C with an additional extension at 72 °C for 7 min. The products of the amplification were then analysed by electrophoresis in a 2% (w/v) agarose gel containing 0.01% ethidium bromide (Merck).

Enumeration of total coliforms and *E. coli* by Colilert-18®

Triplicate 100 ml water samples were collected at day 0 from the source waters that were used to fill the tanks on the same day. Triplicate 100 ml water samples were collected from each horizontal division of each of the water storage tanks at day 45 and 90. Colilert-18® tests (Dehteq, Johannesburg) were performed on each sample according to the manufacturer's instructions. Positive (*E. coli* inoculated sterile water) and negative (sterile water) controls were also included. All Quanti-Tray®/2,000 trays were then incubated at 37 °C for 18 h. MPN/100 ml values were recorded according to a tabulation of 95% confidence intervals provided by the manufacturer (IDEXX, Maine, USA).

Scanning electron microscope examination of biofilm collectors

The formation of biofilms within the water storage tanks was followed throughout the 90 days that the study ran via scanning electron microscopy. Samples were collected in triplicate from each region of the tank (Figure 1) at days 15, 30, 60 and 90. The biofilm collectors were fixed in 2.5% glutaraldehyde in 0.075 M phosphate buffer (pH 7) from being harvested until the completion of the field study. The fixed samples were then rinsed three times in 0.075 M phosphate buffer for 10 min each followed by three rinses in distilled water. Samples were then dehydrated in a graded ethanol series of 30, 50, 70 90, 100, 100 and 100% for 10 min each. This was followed by critical point drying with liquid CO_2 and sputtering with gold before being viewed with a Jeol JSM-840 Scanning Electron Microscope (Jeol, Tokyo, Japan) at 5 KV.

Statistical analysis

Data obtained from water ($\log_{10}(x+1)$ CFU ml^{-1}) and biofilm ($\log_{10}(x+1)$ CFU cm^{-2}) samples were analysed using an analysis of variance (ANOVA) with SAS-9.2 software (SAS Institute Inc., Cary, USA). Means obtained were compared by the Fisher's protected least significant difference (LSD) test at a 5% ($p = 0.05$) level of significance. Repeats were considered as blocks. A significant difference was observed between the blocks and this was accounted for when the two repeats were averaged for data analysis.

RESULTS

Water analysis

The heterotrophic bacterial deterioration of the different water sources is presented in Table 1. Significant interactions occurred between the different water sources and time ($F = 13.06$; $p < 0.0001$) and therefore this was considered for data analysis. No significant difference was observed between water samples that were collected from different positions within the tanks (data not shown).

All the water sources tested contained viable heterotrophic cells throughout the study ranging from 3.98 to 5.33 $\log_{10}(x+1)$ CFU ml^{-1}; 1.79 to 3.13 $\log_{10}(x+1)$ CFU ml^{-1}; and 0.22 to 3.73 $\log_{10}(x+1)$ CFU ml^{-1} for the rain-, ground- and potable water, respectively (Table 1). The rainwater showed significantly higher HPCs throughout

Table 1 | Number of heterotrophic bacterial plate counts ($\log_{10}(x+1)$ CFU ml^{-1}) in different waters stored in low density polyethylene water storage tanks

	Water source		
Time interval (days)	**Rain**	**Ground**	**Potable**
0	5.33 (0.75)^{a}	2.26 (0.11)^{f}	0.22 (0.15)^{h}
15	5.11 (0.29)^{a}	3.13 (0.75)^{de}	3.61 (0.41)^{bc}
30	5.17 (0.22)^{a}	1.88 (0.73)^{g}	3.73 (0.29)^{b}
60	5.29 (0.66)^{a}	2.00 (0.32)^{fg}	3.30 (0.38)^{cd}
90	3.98 (0.34)^{b}	1.79 (0.55)^{g}	2.81 (0.39)^{e}

All means obtained from 18 replicates with standard deviations shown in parentheses. All means followed by the same letter are not significantly different ($p < 0.05$). An analysis of variance indicated a highly significant difference between the water sources ($F = 664.03$; $p < 0.0001$) as well as over time ($F = 51.12$; $p < 0.0001$). As the interactions between the two variables were also highly significantly different ($F = 13.06$; $p < 0.0001$), this relationship was used to analyse data.

the study with significantly similar values found only for the potable water at day 15 and 30. The rainwater HPC values decreased gradually over the 90-day period; however, the only significant decrease was observed between day 60 and day 90. The groundwater HPC values increased significantly between the source water and day 15. The groundwater also showed an overall decrease in HPC values, although the only significant decrease was observed between day 15 and day 30. The potable water showed the most significant increase in HPC values between the source water (day 0) and the water that was stored over the 90-day period. The potable water HPC values remained steady between day 15 and 30 after which significant decreases occurred.

Biofilm analysis

All of the biofilm collectors analysed showed the association of heterotrophic bacterial cells with the surface as early as day 1; biofilm heterotrophic bacterial numbers are presented in Table 2. Significant interactions were observed between the biofilms that developed from the different water sources over time ($F = 7.13$; $p < 0.0001$) and therefore this was considered for data analysis. An ANOVA between the positions within the tanks from which the collectors were collected (Figure 1) showed a significant difference between the various non-partitioned sectors ($F = 12.78$; $p < 0.0001$). The bottom sector of the tank showed significantly less bacterial incorporation into

Table 2 | Heterotrophic plate counts ($\log_{10}(x+1)$ CFU cm^{-2}) of bacteria that were incorporated into biofilms on the surfaces of storage tanks containing rain-, ground- and potable water

	Water source		
Time interval (days)	**Rain**	**Ground**	**Potable**
1	5.16 (0.86)^{d}	3.72 (0.82)^{efgh}	3.66 (0.90)^{efgh}
3	5.49 (0.71)^{bcd}	4.16 (0.19)^{e}	3.70 (0.23)^{efgh}
5	5.77 (0.38)^{b}	4.16 (0.51)^{e}	3.25 (0.49)^{h}
7	5.85 (0.72)^{b}	3.92 (1.08)^{efg}	2.14 (1.80)^{i}
9	5.98 (0.42)^{b}	4.02 (1.50)^{ef}	3.89 (0.55)^{efg}
11	5.77 (0.40)^{b}	2.56 (2.12)^{i}	3.77 (1.00)^{efgh}
20	5.86 (0.51)^{b}	3.49 (0.59)^{fgh}	3.67 (0.29)^{efgh}
30	5.69 (0.72)^{bc}	3.42 (0.61)^{gh}	3.64 (0.53)^{efgh}
60	6.54 (0.74)^{a}	3.29 (0.32)^{h}	3.97 (0.71)^{efg}
90	5.12 (1.00)^{d}	3.67 (0.64)^{efgh}	3.98 (0.45)^{efg}

All means obtained from 18 replicates with standard deviations shown in parentheses. All means followed by the same letter are not significantly different ($p < 0.05$). An analysis of variance indicated a highly significant difference between the water sources ($F = 381.54$; $p < 0.0001$) as well as over time ($F = 3.79$; $p = 0.0001$). As the interactions between the two variables were also highly significantly different ($F = 7.13$; $p < 0.0001$), this relationship was used to analyse data.

biofilm structures than the top and middle sectors (data not shown).

The HPC values obtained for biofilms that developed from the rainwater were significantly higher than for the biofilms that developed from the other water sources for the entire duration of the study. The HPC values of the rainwater biofilm increased non-significantly until day 30 when there was a significant increase in the amount of heterotrophic bacterial incorporation into the biofilm until day 60. The HPC values then decreased significantly again until the end of the study when a final HPC value of 5.12 $\log_{10}(x+1)$ CFU cm^{-2} was obtained. The final HPC value recorded for the rainwater biofilm was non-significantly different from the HPC value obtained at day 1. The groundwater and potable water biofilms showed very similar heterotrophic bacterial incorporation patterns. The HPC values for the two biofilms were not significantly different for the greater part of the study. Significant differences were observed on day 5, 7, 11 and 60. Between day 9 and 11, the potable water biofilm reached HPC values that were higher than the groundwater biofilms which had had more bacterial incorporation into biofilms until this point.

Prevalence of total coliforms and *E. coli* in water and biofilm samples

E. coli was detected through PCR in all the water sources (Table 3) and biofilm samples (Table 4) at some point during the study. The rainwater showed the highest prevalence of *E. coli* as it was detected on all water sampling days throughout the study. The rainwater biofilms also showed high prevalence of *E. coli* as it was detected on every sampling day except for day 90 of the second experiment. The groundwater showed the second highest prevalence of *E. coli* in both the water samples and biofilms, with the least *E. coli* being detected in potable water and biofilm samples.

Colilert-18® analysis of water samples revealed the presence of total coliforms in the potable, ground and rain source water (Table 5). Total coliform levels in the rain source water were so high that they could not be counted through Colilert-18® analysis; this pattern prevailed throughout the duration of the study with countable levels only being detected at day 90 of the first experiment. Total coliforms were periodically detected over the duration of the study in both experiments of the potable water with faecal coliforms only being detected at day 45 in the second experiment despite no *E. coli* being detected in the source water. As was observed with PCR analysis, the groundwater showed the second highest prevalence of total coliforms in both experiments throughout the duration of the study; however, *E. coli* was only detected in the second experiment (Table 5).

Table 3 | Presence (+)/absence (–) of *E. coli* in stored rain-, ground- and potable water detected via PCR

		Time (days)				
Water source	**Repeat**	**0**	**15**	**30**	**60**	**90**
Rainwater	1	+	+	+	+	+
	2	+	+	+	+	+
Groundwater	1	–	+	+	–	–
	2	+	+	+	+	–
Potable water	1	–	+	–	–	–
	2	–	–	–	–	+

Scanning electron microscope examination of biofilm collectors

Scanning electron micrographs of the different biofilms correspond with the HPC values (Figure 2(a) and (b)). The rainwater biofilms which had the highest heterotrophic bacterial incorporation also appeared to be larger in size, i.e. covering more surface area, than the other biofilms (data not shown). The groundwater biofilms appeared to be thicker than the potable water biofilms, although the groundwater biofilm had predominantly more fungal incorporation compared to the predominant appearance of bacteria in the potable water biofilms. Microscopic imperfections in the UV-resistant carbon black lining were commonly encountered. Biofilm formation within the imperfections was also commonly observed (Figure 2(c) and (d)).

Table 4 | Presence (+) / absence (–) of *E. coli* in biofilms that developed from stored rain-, ground- and potable water detected via PCR

		Time (days)									
Water source	**Repeat**	**1**	**3**	**5**	**7**	**9**	**11**	**20**	**30**	**60**	**90**
Rainwater	1	+	+	+	+	+	+	+	+	+	+
	2	+	+	+	+	+	+	+	+	+	–
Groundwater	1	+	+	+	+	–	–	–	+	–	+
	2	–	+	–	–	–	+	+	–	–	+
Potable water	1	–	+	+	–	–	+	+	–	–	+
	2	–	+	–	–	–	–	–	–	–	–

Table 5 | Total and faecal coliform detection in water samples from the potable-, ground- and rainwater storage tanks through Colilert-18® analysis

		Time (days)		
Water source	**Repeat**	**0**	**45**	**90**
Potable water	1	0.00 (0)	2.31 (0.44)	45.06 (0)
	2	0.67 (0)	0.00 (0)	76.41 (0)
Groundwater	1	3.43 (0)	0.00 (0)	45.06 (0)
	2	28.50 (3.47)	139.53 (23)	164.38 (1.26)
Rainwater	1	>2,419.6 (1,624.65)	>2,419.6 (>2,419.6)	41.61 (7.19)
	2	>2,419.6 (>2,419.6)	>2,419.6 (126.16)	>2,419.6 (24.13)

Tabulation of total coliforms recorded with faecal coliforms shown in parentheses.

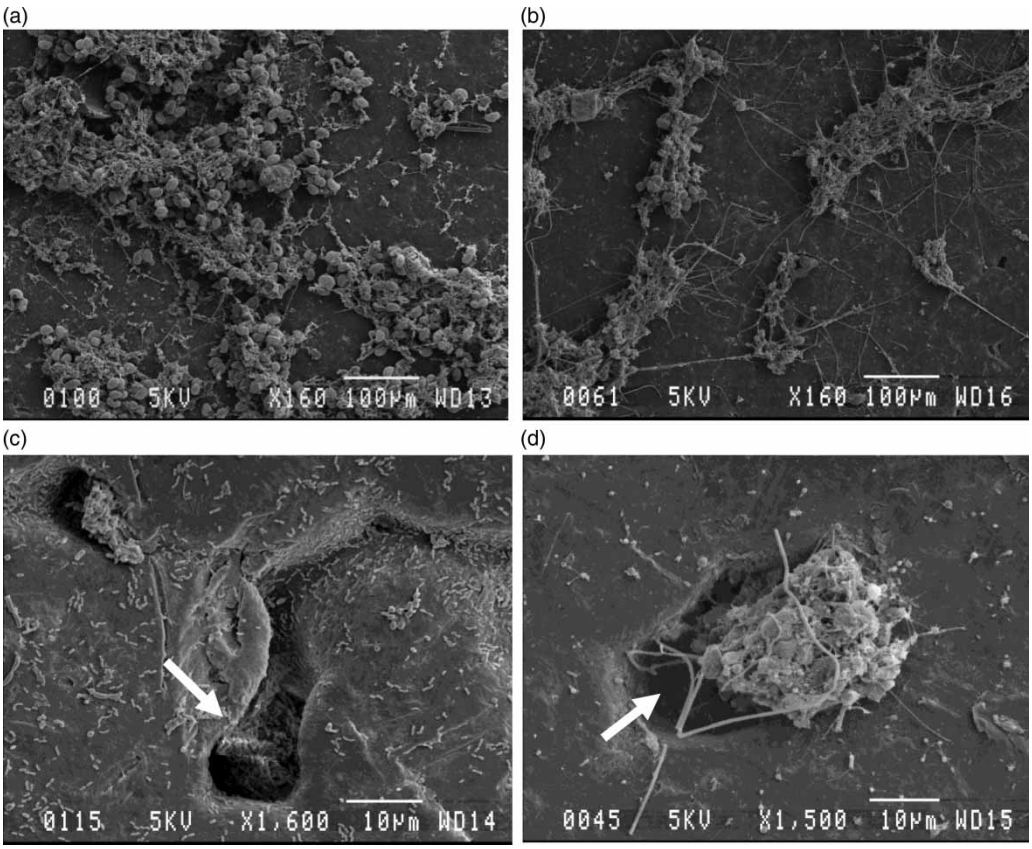

Figure 2 | Scanning electron microscopy images of microorganisms associated with the interior surfaces of water storage containers: (a) rainwater; (b) groundwater; (c) and (d) imperfections in the UV resistant carbon black lining from the rainwater tanks. Note the same was observed in all of the tanks.

DISCUSSION

The presence of coliforms and *E. coli* has been reported in stored rainwater (Zhu *et al.* 2004; Evans *et al.* 2006; Ahmed *et al.* 2008, 2010), groundwater (Momba & Mnqumevu 2000; Momba & Notshe 2003) and potable water (Momba & Kaleni 2002). Direct PCR analysis of the water samples in the current study showed the presence of *E. coli* in the bulk liquid phase of all the stored water sources tested; this was confirmed through the Colilert-18® test. In

addition to direct PCR analysis, alternate PCR technologies such as qPCR have been used before as a diagnostic test to identify *E. coli* in stored water (Ahmed *et al.* 2010, 2012). Colilert-18® analysis used in the current study has also been efficiently used in the past (Juhna *et al.* 2007; Fremaux *et al.* 2009). To our knowledge, no studies have used PCR analysis coupled with Colilert-18® tests to study the microbial quality of stored water.

Direct PCR analysis and Colilert-18® tests of the rainwater samples in the current study showed the presence of *E. coli* in all rainwater samples tested throughout the study period. As a result, the rainwater failed to meet water quality guideline standards (DWAF 1996; SABS 2006). According to SANS 241, the permissible number per 1% of samples for total coliforms and *E. coli* is 10 per 100 ml and 1 per 100 ml, respectively (SABS 2006). HPC bacteria are also used as indicators of the general microbial quality of water (DWAF 1996). The permissible target range for HPC values in water for drinking purposes is 0–100 counts ml^{-1} and was only met in the case of the potable water before it was stored.

Harvested rainwater is generally considered of good quality but is dependent on atmospheric microbial levels as well as the surface from which the water is collected (Zhu *et al.* 2004; Helmreich & Horn 2010; Ahmed *et al.* 2012). Handia *et al.* (2003) found that the collection of rainwater with the use of a first flush device yielded water that was safe for human consumption without prior treatment. However, the majority of studies have found that water collected through rainwater harvesting is, in fact, not fit for human consumption due to levels of faecal coliform contamination (Zhu *et al.* 2004; Ahmed *et al.* 2008, 2010). In the current study, the building from which rain was harvested was situated next to cattle pens which contributed towards dust generation and deposition on building roofs. The dry deposits on the building from which the rain was harvested contained large amounts of heterotrophic bacteria, in particular *E. coli*, since the resultant water that was collected showed the highest HPC and *E. coli* values. The overall microbial quality of the rainwater was not considered ideal and although there were no drastic increases in the HPC values as was seen with the potable water, the water was still considered to have deteriorated.

The potable water had the lowest and rainwater the highest HPC values at the start of the study, and the potable water was the only water source to comply with water quality standards at the start of the study (DWAF 1996; SABS 2006). However, the quality of the potable water deteriorated so rapidly that by day 15 it displayed HPC values that were above the acceptable limits for potable water and were significantly higher than the groundwater HPC values. Water that is stored often stagnates and as a result, disinfectant residuals in potable water may dissipate to levels low enough to lead to increases in microbial growth (Maraj *et al.* 2006). Although the potable water did not have the highest HPC values, it showed the greatest increase in heterotrophic bacterial growth out of the three stored water sources.

The stored groundwater also did not conform to drinking water standards (DWAF 1996; SABS 2006) due to the high HPC values obtained and the presence of *E. coli*. Momba & Notshe (2003) found that the quality of stored groundwater within plastic-based containers could deteriorate within 24 h after storage and gradually deteriorate over the next 72 h period when the water was tested. In the current study, the HPC values showed a significant increase in the first 15 days of storage; however, the HPC values decreased from this point to a value lower than the starting HPC value. The decrease in HPC values could be attributed to unfavourable conditions, such as nutrient depletion (Momba & Notshe 2003) or the possibilty that many of the planktonic cells became incorporated into the biofilm.

The majority (99.9%) of microorganisms present in water-related environments are attached to surfaces exposed to water (Donlan & Costerton 2002; Juhna *et al.* 2007; Huq *et al.* 2008). Despite this, HPCs of routine water samples, and not biofilm samples, are still highly regarded in determining the microbial safety of different water sources (DWAF 1996). This underestimation of the amount of microorganisms present in the water and its surrounding environment can often be misleading and result in water quality being miscalculated. In the current study, a comparison of biofilms that developed in the different storage tanks with the water contained within them, confirmed that there was just as much, if not more bacterial cells attached to the surface compared to planktonic cells in the bulk water.

E. coli was found to have been incorporated into biofilm structures in this study within 24 h for the rain- and groundwater biofilms and after 3 days for the potable water biofilms. This was also observed by Momba & Kaleni (2002) who showed that biofilm formation from ground- and potable water on polyethylene material could occur within 24 h after initial exposure and that the indicator organisms had already adhered to the surfaces within that time frame. The occurrence and survival of *E. coli* in the bulk liquid phase of stored water facilitates the incorporation of the pathogen into biofilms that develop on the interior surfaces of the water storage tanks (Momba & Kaleni 2002).

E. coli incorporation into biofilms that develop from rainwater has not been demonstrated as frequently as incorporation into biofilms supported by other untreated water sources (Momba & Mnqumevu 2000; Momba & Kaleni 2002; Banning *et al.* 2003; Momba & Notshe 2003). The detection of *E. coli* in the ground- and potable water samples of the current study through PCR analysis was more sporadic and no relationship between the appearance of *E. coli* in the water and in the biofilms could be deduced. As the water storage tanks used in the current study were sealed to prevent unnecessary introduction of contamination (Maraj *et al.* 2006), the presence of *E. coli* indicates contamination prior to storage. This was evident in the water samples taken at day 0 direct from the water sources (Tables 3 and 5). Other studies have reported that contamination of collected water can be as a result of dust deposits, leaves from trees or bird droppings (Zhu *et al.* 2004; Kahinda *et al.* 2007; Ahmed *et al.* 2012).

In addition to microbial colonisation and biofilm formation on the interior surface of water storage tanks, the current study revealed microbial association with microscopic imperfections in the UV-resistant carbon black lining of the tanks. Scanning electron micrographs revealed whole microcolonies developing within these imperfections. The protection afforded to the biofilms within these imperfections in the current study prevented complete removal of surface-associated microorganisms with the removal method employed. This would have therefore resulted in an underestimation of the number of heterotrophic bacteria associated with the surface. Microbial growth within imperfections, such as those found in the current study, may act as a mode of survival for microorganisms as they would not be removed during routine cleaning of the tanks. As biofilms naturally protect the cells from antimicrobial agents such as antibiotics, disinfectants or germicides (Webb *et al.* 2003), growth within the storage tank imperfections can further decrease the efficiency of antimicrobial agents in biofilm control.

In light of the findings of the current study, future research should investigate the ability of microbial biofilm formation within water storage container imperfections to protect the cells from removal and disinfection activities thereby providing more information on how to combat their formation. Methods should also be devised to attempt to remove these microbial growths from the surface. Future research should also focus on the mechanism of *E. coli* survival in water storage tanks.

CONCLUSIONS

The findings of the current study revealed that both untreated and municipally treated water sources were able to support biofilm formation on the interior of low density polyethylene water storage tanks as early as 1 day after collection. It was also found that the storage period and the microbial quality of the source water could influence water quality deterioration in terms of water HPC values and the rate of biofilm formation. Imperfections in the interior surface of storage tanks were also found to provide an ecological niche for biofilm formation and persistence. To our knowledge, this has not been shown before. Due to the widespread use of water storage tanks, similar to those employed in the current study, suitable information should be given to the public about the potential risks associated with the storage of water (especially first seasonal rainwater) and the potential for water deterioration in the absence of disinfectant applications or periodic cleaning of the water storage containers/tanks.

ACKNOWLEDGEMENTS

We herewith acknowledge financial support for solicited research by the Water Research Commission for the

project No. 2175: *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*. In addition, student support is gratefully acknowledged from the Jo Jo water tank company and the National Research Foundation. The authors would like to thank Amanda Redmond, Erika du Plessis, Tsedal Ghebremarium, Rene Jacobs, Alan Hall and Chris van der Merwe for their invaluable assistance.

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First received 11 January 2012; accepted in revised form 21 October 2012. Available online 3 December 2012