Bacterial Water Quality of Rainwater Fed Domestic Hotwater Systems

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Abstract Rainwater harvesting within the urban environment is being increasingly recognised for its important role in reducing pressures on mains water supply and reducing stormwater runoff. The extent to which rainwater tanks can improve our management of the urban water cycle depends on the level of usage of the rain-harvested water. By incorporating the hotwater service into the suite of uses of harvested rainwater, substantial systemic benefits can be realised. This paper discusses some of the water quality implications of using harvested rainwater through hotwater systems. Field sampling of hot and cold water taps supplied by rainwater tanks was conducted over a 12 month period, together with laboratory conducted thermal destruction experiments on enriched rainwater samples. The field results showed substantial reductions (85-100%) of *E. coli*, total coliform, *Pseudomonas*, and Heterotrophic Plate Count concentrations in harvested rainwater passing through hotwater systems maintained between 50-70°C. The results of the laboratory thermal destruction experiments indicated that the time required to reduce a heterogeneous bacterial population at 55°C over each of the first three log reductions were 10, 190, and 400 seconds, respectively. The lengthening of times required for inactivation demonstrated a pronounced shift in population. The hygienic quality of harvested rainwater was greatly improved after passing through hotwater systems maintained at adequately high temperatures.

Introduction

The sustainability of the urban water cycle has come under increasing pressure over the last century. Freshwater supplies, including surface waters, rivers, and groundwater reservoirs, face increasing pressures from growing urban populations demanding increasing rates of water consumption. Environmental degradation of receiving waterways such as creeks and lakes caused by increasing volumes of poor quality stormwater runoff have resulted from the expansion of impervious surface area. However, the practice of rainwater harvesting within the urban environment has the potential for substantial mitigation of these pressures. The installation of rainwater tanks in new developments and retrofitting existing houses may result in both environmental and economic savings (Coombes et al., 2000). The extent of benefits provided by rainwater tanks is determined by the extent to which the harvested rainwater is used. The use of rainwater for hotwater applications has been shown to be a significant factor in maximising the potential impact of rainwater tanks on urban water cycle sustainability (Coombes et al., 2002). The constant drawing down of the rainwater tank through hotwater use provides larger available tank capacity for rainwater harvesting in subsequent rain events and provides substantial reductions to mains water demand and peak demand.

However, heated rainwater must be of sufficiently high quality before the widespread recommendation to incorporate rainwater into hotwater systems can be made. Heating water as a means of disinfection is an ancient practice. Widespread recommendations to boil water are still given by water suppliers during times of potential contamination. However, domestic hotwater systems operate at sub-boiling temperatures, and the acceptability of the quality of hotwater supplied from rainwater tanks is under debate as only limited published data is available. The aims of this study were, firstly, to investigate the water quality in five urban rainwater tanks and associated hotwater systems operating at a variety of temperatures, and secondly, to examine the thermal resistance capacities of heterogeneous populations of bacteria found in rainwater tanks.

Methods

Rainwater Tanks & Hotwater Samples

Cold and hot water samples were collected from rainwater tanks from five houses within the Hunter Region/Central Coast areas of New South Wales, Australia. System 1 was comprised of two joining concrete tanks with combined capacity of 35KL located in a low density semi-urban suburb. The system included a drown pipe, which remained submersed at all times, allowing collection of rainwater from all roof surfaces. No screens or meshes were installed to any of the tank inlets. The tank water was used for all domestic purposes and no connection to mains water was available at the site. Site 2 was a 9KL polyethylene tank, including a drown pipe, located in an urban area in close proximity to major roads. The tank water was used for all domestic purposes with mains water trickle top-up occurring when tank capacity fell below approximately 10%. Sites 3 and 4 were located in a leafy urban area near a major bush reserve. Site 3 included a 9KL galvanised iron tank with first flush device. Tank water was used for all household purposes and was topped-up with mains water during low capacity. Site 4 was a 5.4KL galvanised iron tank used for outdoor, toilet, and hotwater uses. A leaf diverter was used but no first flush device was installed, with trickle top-up from mains water. Site 5 comprised two Aquaplate tanks joined to give combined capacity of 9.06KL, located in close proximity to a heavily industrial area. The tank water was used for outdoor, toilet, and hotwater purposes with trickle top-up from mains water.

Bacteriological samples were collected in sterile 500mL polyethylene jars and analysed within 6 hours of collection. Hotwater samples were quenched in cold water immediately upon collection and kept in the dark during transport. Samples were analysed using Membrane Filtration (Millipore) with enumeration of *E. coli* and Total coliform bacteria on m-ColiBlue24® broth (Prod.# M00PMCB24) by filtering 100mL and 10mL, *Pseudomonas spp.* with Pseudomonas Selective broth (Prod.# M00000P2P) by filtering 100mL and 1mL, and Heterotrophic Plate Counts (HPC) on m-Heterotrophic Plate Count Medium (Prod.# M00000P2S) by filtering 1mL and 0.1mL.

Thermal Destruction Experiments

Laboratory experiments were conducted to examine inactivation rates of a heterogeneous bacterial population at 55°C. A 200mL cold water sample was taken from Tank 2 and enriched for 48 hr followed by thermal destruction experiments. Enrichment was necessary to produce bacterial concentrations high enough to allow thermal destruction characteristics to be assessed over several log reductions. The enrichment step involved splitting the sample into two 100mL portions with the addition of 100mL Brain Heart Infusion (BHI – Oxoid) broth followed by incubation of the split portions at 24°C and 37°C for 24 hr before re-combining the samples and incubating at 24°C for a further 24 hr. This was designed to allow the culturing of aerobic species of both environmental and enteric bacteria into the stationary growth phase, which has previously been demonstrated as the growth phase in which bacteria possess maximal heat resistance. Cells were harvested by centrifugation and re-suspended in 1.5mL sterile water.

200mL of sterile deionised water was heated to 55°C before inoculation with 1mL of sample. A magnetic stirring bar was set to produce a slight vortex to allow rapid mixing, with sampling occurring at pre-determined intervals thereafter. Temperature was recorded using two Resistance Temperature Detectors (RTDs) which logged directly into a Dataker DT50 data logger connected to a laptop computer providing temperature profiles at one-second intervals using the DeTransfer software. Surviving bacteria were enumerated by spread-plating appropriate dilutions onto R2A agar (Oxoid) and incubating at 37°C for 48 hr. The surviving bacteria from the hotwater samples and from the thermal destruction experiments were identified using Polymerase Chain Reaction (PCR) and sequencing of the 16S rRNA gene.

Results

All cold and hotwater samples were taken concurrently on the same day so that a direct assessment of hotwater system performances could be made. Physicochemical properties of the hotwater changed slightly, with increases in pH between 0.5 and 1.5 pH units. The results show substantial reductions in bacterial concentrations when water is passed through hotwater systems ranging between 50-70°C. In three of the systems *E. coli* reductions were 100%, although these systems generally had low initial concentrations of *E. coli*, as seen in Table 1.

Table 1 Average *E. coli* concentrations in Cold and Hot water samples

System	N	Cold Water	Hotwater	Hotwater Temperature	Reduction %
1	10	0.3	0	66.3°C	100
2	8	1429	0.1	50.7°C	99.991
3	8	113	1.3	65.4°C	98.893
4	4	7	0	60°C	100
5	5	8	0	69.2°C	100

Large variations in bacterial concentrations of the harvested rainwater were found in some of the systems. On three occasions Tank 2 had *E. coli* concentrations above 1000 CFU/100mL (maximum 6800 CFU/100mL) while two samples had less than 10 CFU/100mL and four samples contained less than 60 CFU/100mL. In total, 17 (49%) cold water samples were negative for *E. coli*. After passing through the associated hotwater systems, 31 (89%) of the samples were negative for *E. coli*. Three of the four positive samples were from the same system, and contained 1, 1, 2, and 7 CFU/100mL. Interestingly, system 3 was slightly less effective at reducing E. coli concentrations than system 2, despite significantly higher temperatures in the hotwater system.

All rainwater tank samples were positive for Total coliform bacteria, ranging between 1 – 26600 CFU/100mL. All hotwater systems averaged over 99% inactivation of Total coliform bacteria as seen in Table 2, although only 10 (29%) hotwater samples were negative for Total coliforms, ranging between 0 – 90 CFU/100mL. System 2 contained the highest number of Total coliforms in both the cold and hot water samples. *Bacillus spp.* were positively identified within the Total coliform counts on the chromogenic mColiBlue24® media, an issue currently under further investigation.

Table 2 Average Total Coliform concentrations in Cold and Hot water samples

System	N	Cold Water	Hotwater	Hotwater Temperature	Reduction %
1	10	1417	1.8	66.3°C	99.873
2	8	7269	29.8	50.7°C	99.591
3	8	718	4.3	65.4°C	99.408
4	4	679	3	60°C	99.558
5	5	787	1.6	69.2°C	99.797

Pseudomonas spp. appeared highly sensitive to thermal stress and all hotwater systems achieved greater than 99.8% reduction. Hotwater system 2, operating at around 50°C, achieved reductions in Pseudomonas comparable to the other four systems operating between 10-20°C higher. Temperature appeared to be more crucial to the rates of inactivation of HPC, as seen in Table 4. The residence time in the hotwater system was not measured but may be an important factor. Spore-forming bacteria are known to have significant thermal resistance capacities, and therefore, the profile of the initial bacterial community entering the hotwater system is also likely to be a significant factor in the efficacy of hotwater system performance.

Table 3 Average *Pseudomonas* concentrations in Cold and Hot water samples

System	N	Cold Water	Hotwater	Hotwater Temperature	Reduction %
1	10	11630	0.2	66.3°C	99.998
2	8	14868	19.6	50.7°C	99.868
3	8	4306	0.4	65.4°C	99.991
4	4	5693	4.5	60°C	99.921
5	5	5773	0.5	69.2°C	99.991

Table 4 Average HPC concentrations in Cold and Hot water samples

System	N	Cold Water	Hotwater	Hotwater Temperature	Reduction %
1	10	207	15.6	66.3°C	92.456
2	8	2640	395	50.7°C	85.038
3	8	396	3.5	65.4°C	99.117
4	4	483	14.6	60°C	96.96
5	5	1045	0.3	69.2°C	99.976

Inactivation of the enriched heterogeneous population at lethal temperature 55°C for the first log reduction (90%) required 10 seconds. The second log reduction (90-99%) took approximately 190 seconds, the third log reduction (99-99.9%) taking a further 400 seconds, and the fourth log reduction (99.99% inactivation predicted from extrapolation) taking approximately 1400 seconds.

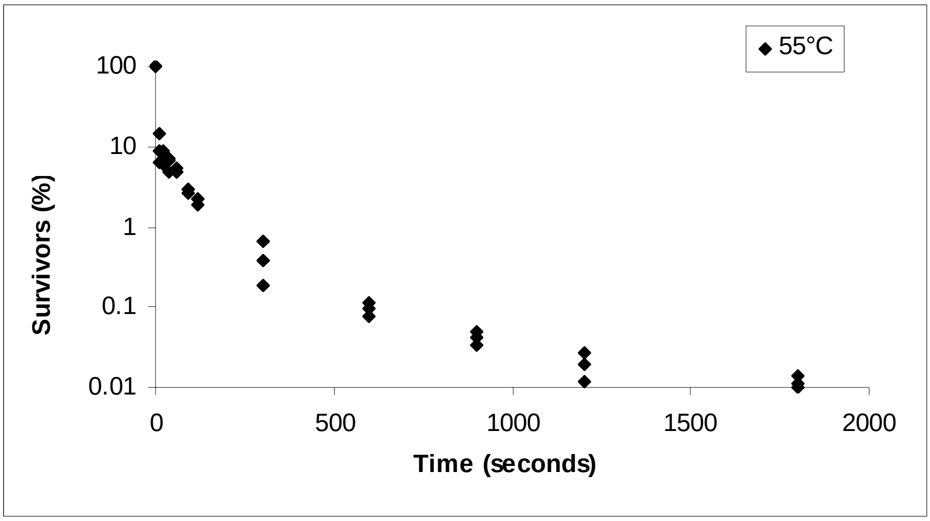

Figure 1 Inactivation of Heterogeneous Bacterial Population at 55°C

Discussion

The quality of stored rainwater varied significantly between systems and over time. System 2 was consistently more contaminated than the other systems with none of eight water samples complying with ADWG for *E. coli*. The presence of a large tree overhanging the roof catchment which was known to house bats may have been a significant contributor to contamination. Colour and odour complaints had previously been made regarding the aesthetic quality of the water during the study period, and felling of the tree post-study appeared to improve water quality. System 1 maintained relatively high quality rainwater with 80% of cold water samples complying with ADWG for *E. coli* despite having employed no water quality protection measures such as first flush devices, leaf diverters, or screens on inlets. Two lizard carcases were also recovered from the tank sludge and several overhanging trees were present, although the owners did not attribute any gastrointestinal complaints to the consumption of the untreated tank water. Four of the five systems relied on mains water to top-up the tanks when capacity fell below approximately 10%. The input of chlorinated mains water may have had an impact on tank water quality although this was not captured in the present study.

The hotwater systems did achieve substantial reductions in the numbers of viable bacteria in the heated rainwater. All hotwater systems monitored in this study were storage hotwater tanks. The timing of sampling within the cycle of the hotwater systems was likely to be an important factor, as longer residence time in hotwater results in greater reductions of bacteria. The level of inactivation was not only associated with the temperature of the hotwater system. The initial composition of bacterial communities within the rainwater tank may also significantly influence the degree of reduction. The results of the thermal destruction experiments indicated that significant levels of reduction are achieved upon initial exposure to heat, presumably due to inactivation of heat-sensitive species, but that the rate of population reduction is significantly slowed after a period due to the residual presence of spore-forming bacteria. Spore-forming bacteria, such as *Bacillus* and *Clostridium spp.*, are known to possess heat resistance capacities beyond that which could reasonably be imparted by domestic hotwater services. However, while some species of spore-forming bacteria are pathogenic, these are rarely transmitted through water and consequently are not addressed as bacterial species of concern in the Australian Drinking Water Guidelines (ADWG).

Previous studies have also noted reductions in bacterial loads of harvested rainwater passing through domestic hotwater systems. In Newcastle, monitoring of the Figtree Place water sensitive urban design project showed that in the electric hotwater systems used on these premises total coliform counts were reduced from an average 112CFUs/100mL in the rainwater tanks to 0CFU/100mL from the hotwater system tap, while *Pseudomonas* were reduced from 29500CFUs/100mL to 0CFUs/100mL (Coombes et al., 2000). Substantial reductions in bacterial loads through instantaneous hotwater systems have also been noted. The results of monitoring a single instantaneous hot water system set to 55°C demonstrated a 4-log reduction in *Pseudomonas* concentrations in less than 1 minute of exposure (Coombes et al., 2003).

Table 5 Occurrence of *Legionella*-like Isolates within Cistern Systems

System	1		2		3		4		5	
	Hot	Cold	Hot	Cold	Hot	Cold	Hot	Cold	Hot	Cold
Water Temp. (°C)	52	21	63	22	52	22	52	21	63	21
Total CFU	$2x10^3$	$1x10^6$	$2x10^3$	$8x10^2$	$2x10^3$	$1x10^2$	$1x10^5$	$6x10^3$	$1x10^2$	$5x10^5$
Total Coliforms	2	3	0	0	13	36	0	300	0	0
CFU on BCYE medium	190	34	0	15	225	7	18	25	0	0
Possible Isolates of Legionella	YES	NO	NO	NO	YES	YES	YES	NO	NO	NO

All counts in CFU/mL (Lye, 1991)

One of the major threats to public health associated with hotwater systems is that of infection caused by *Legionella pneumophila*. *L. pneumophila* is the aetiological agent of Legionnaires disease, an acute form of pneumonia, which most commonly infects the respiratory tract of immuno-compromised individuals. *L. pneumophila*-associated infections occur as a result of the inhalation of contaminated aerosols. Ingestion of high concentrations of *L. pneumophila* does not cause harm. A limited amount of research has been conducted which investigated the heat tolerance of *L. pneumophila* in a water medium (Stout et al., 1986, Dennis et al., 1984). Research findings generally indicated that *L. pneumophila* has little thermal resistance capacity above 60°C. As a result of this research, Australian standards (AS3500.4.2) have been developed that state that hot water systems should be maintained above 60°C in order to inhibit the growth of *L. pneumophila*. Lye (1991) found that three hotwater systems supplied by harvested rainwater maintained below 53°C were positive for *Legionella*-like isolates, as shown in Table 5. The two systems maintained above 60°C were negative for such isolates. When the operating temperatures of the three hotwater systems operating below 53°C were increased to above 60°C, *Legionella*-like isolates could no longer be isolated.

Martinelli et al. (2000) found that *L. pneumophila* were isolated much more frequently from hot water tanks (30%) as opposed to instantaneous hotwater systems (6.4%) in an area of Italy suffering an endemic outbreak of Legionellosis. The study concluded that temperature was critical, as the hotwater tanks in the study had been maintaining water at 50˚C +- 5˚C, while the tap water averaged 45˚C and dropped to 40˚C during flow. Instantaneous systems, however, always maintained temperatures above 60˚C (Martinelli et al., 2000). Ezzeddine et al. (1989) found that the main reservoir for *L. pneumophila* were mixing tanks where hotwater at 60-65˚C mixed with cold water to achieve 45˚C. Maintaining temperatures of 60˚C or accelerating flow rate were found to be the most effective methods of controlling *L. pneumophila* (Ezzeddine et al., 1989). Lee et al. (1988) discovered that water temperatures in electrically heated tanks were significantly lower than in gas-heated tanks. They concluded that the presence of *L. pneumophila* was associated with systems maintained below 48.8˚C. Wadowsky et al. (1985) detected *L. pneumophila* in water and sediment samples taken from hotwater systems maintained at 30-54˚C, but not from systems maintained at 71-77˚C.

The nature of the contact with water must be understood before an evaluation of risk can be made. The uses of hotwater are typically quite restricted within households, generally limited to showering, washing dishes, and cooking. Few people drink directly from the hotwater tap without prior additional heating such as boiling for coffee/tea or cooking. Ingestion of large amounts of water while showering is not common practice, and needs to be considered when assessing hotwater quality against ADWG which are based on the ingestion of 2L per day (enHealth, 2004). The threat of respiratory infection caused by inhalation of *L. pneumophila* may be of greater significance than that posed by direct ingestion. However, hotwater systems maintained at 60°C in accordance with Australian Standards should pose minimal health risks from either ingestion or inhalation. The immuno- and nutritional-status of a population also influences susceptibility to illness. Immuno-compromised individuals are at greater risk of infection from many sources including harvested rainwater and hotwater systems. It is recommended that immuno-compromised individuals exercise care when using harvested rainwater, such as boiling water intended for consumption.

Conclusions

Large reductions in *E. coli*, Total coliform, *Pseudomonas*, and HPC concentrations were consistently observed in harvested rainwater passing through domestic hotwater systems maintained between 50 – 70°C. Low numbers of viable total coliforms were present in hotwater samples, although further identification of isolates showed that these were predominantly *Bacillus spp*. This may have significant implications when assessing rainwater-fed hotwater systems as current ADWG operate on a presence/absence criteria for determining the acceptability of a water. Laboratory experiments showed that enriched bacterial populations cultured from harvested rainwater decreased rapidly with initial exposure to thermal stress, with reductions in activation rates during subsequent heating. Hotwater systems operating between 50-70°C all performed well achieving substantial reductions in bacterial loads, although due to the threat of *L. pneumophila* in hotwater systems, it is recommended that hotwater services be maintained above 60°C.

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