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# Thermal inactivation analysis of water-related pathogens in domestic hot water systems

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**This study aims to investigate whether hot water systems supplied with harvested rainwater present an increased risk to health over hot water systems supplied with potable mains water. It reviews previous studies investigating the health effects of utilising rainwater within domestic systems. The main risk to public health of mains-supplied hot water systems is the operation, maintenance, age, location and temperature of the system. Rainwater-harvesting systems contain an inherent water treatment train consisting of flocculation, settlement, sorption and bioreaction, and stored rainwater quality improves as metal and chemical contaminants settle to form sludge. Laboratory experiments were conducted using a variety of water-related bacteria to determine the time required to reduce a bacterial population by 90% at a given temperature. The results of this study show that after 5 min of exposure at 60 and 55°C, respectively, *Salmonella, Pseudomonas aeruginosa* and total viable count at 22 and 37°C concentrations were reduced to zero. Irish standards require hot water systems to be maintained at temperatures at or above 60°C. The conclusion from this pilot study is that hot water systems supplied with harvested rainwater do not present an increased risk to health over hot water systems fed with mains water.**

## Introduction

In Ireland, all public water supply is treated to potable standards, and harvested rainwater (RWH) is considered only for non-potable uses (toilet flushing). Hot water systems represent approximately 20% of household per capita consumption in Ireland and represent a logical extension for the use of RWH to reduce mains water demand (O'Hogain *et al*., 2007). This study sets out to investigate whether hot water systems supplied with RWH present an increased risk to health over hot water systems supplied with potable mains water. It also reviews previous studies investigating the health effects of utilising RWH within domestic systems (O'Hogain *et al*., 2011, 2012). The paper also presents the results from laboratory experiments conducted using a variety of water-related bacteria to determine the time required to reduce a bacterial population by 90% at a given temperature.

### Health concerns

The most common source of pathogens in drinking water supplies is recent contamination by human and/or animal excreta (Prescott *et al*., 1993). Pathogens of concern include bacteria, viruses and protozoa that can cause gastroenteritis, diarrhoea, dysentery, hepatitis, cholera or typhoid fever (NHMRC, 1996). While the majority of waterborne diseases are caused by pathogens that originate in the gastrointestinal tracts of humans or animals, there are microbes existing in the environment that can, in some cases, cause disease in humans (Prescott *et al*., 1993). *Escherichia coli* are a common intestinal bacteria found in large concentrations within warm-blooded animals. *E. coli* are commonly used as indicator organisms, indicating evidence of faecal material (WHO, 2003). *Salmonella* spp. are a group of human pathogens that can infect the gastrointestinal tract of humans, causing diarrhoea (Prescott, 1993). *Pseudomonas aeruginosa* is an opportunistic pathogen that may cause infection through skin lesions (WHO, 2003). *Enterococcus* spp. is an anaerobic bacterial genus that is a commensal inhabitant of the human intestine. It is reported to provide a higher correlation with many of the human pathogens found in wastewater than faecal coliforms (Jin *et al*., 2004).

### Persistence and growth in water

While typical waterborne pathogens are able to persist in drinking water, most do not grow or proliferate in water. Microorganisms such as *E. coli* can accumulate in sediments and are mobilised when water flow increases. After leaving the body of their host, most pathogens gradually lose viability and the ability to infect (Prescott *et al*., 1993). The rate of decay is usually exponential, and a pathogen will become undetectable after a certain period. Pathogens with low persistence must rapidly find new hosts. Persistence is affected by several factors, of which temperature is the most important. Decay is usually faster at higher temperatures, hence, the term 'thermal inactivation'. For pathogen-contaminated water to cause illness in humans, the pathogens must have an available route of infection and must overcome the defence barriers of the human body (stomach acidity, competition by natural gut flora and immunological responses, including acquired immunity). Routes of infection may include inhalation or ingestion. Successful infection by the pathogen is ultimately dependent on the pathogen dose (Prescott *et al*., 1993).

### Public health risks associated with rainwater harvesting

About three million Australians use roof-harvested rainwater from tanks for drinking in urban and rural regions (ABS, 1994). Fuller *et al*. (1981), Mobbs *et al*. (1998) and Cunliffe (1998) found that the quality of rainwater was often adequate for potable uses provided the rainwater tank and roof catchment were subject to

adequate maintenance (Coombes *et al*., 2002). Studies from both Ireland (O'Hogain *et al*., 2012) and New Zealand (Simmons *et al*., 2001) found that the RWH supplies sometimes exceeded drinking water guidelines for lead and microbial indicator organisms. Cunliffe (1998) stated that the probable source of indicator bacteria detected in rainwater tanks was excreta from small animals and birds. Of the epidemiological studies conducted on rainwater to date, two have compared the rates of gastroenteritis in young children. A study of cryptosporidiosis notifications in South Australia found a significantly reduced risk of cryptosporidiosis associated with tank rainwater compared to public mains water (Weinstein *et al*., 1993). A cohort study of 1016 4- to 6-year-olds, who were regular consumers of tank rainwater, concluded that they were at no greater risk of gastroenteritis than those who drank treated public mains water (Heyworth *et al*., 2006). The majority of rainwater tanks in this study were galvanised iron (59%) with 43% of tanks greater than 10 years old. Only 8% of the tanks had first-flush devices, and sludge was never removed in 42% of the tanks (Heyworth *et al*., 2006). A pooled epidemiological study of 13 case studies, which quantified the risk of gastrointestinal disease from rainwater consumption, concluded that there was no significant difference in risk comparing rainwater to improved water supplies (Dean and Hunter, 2012). A double-blinded randomised controlled trial among 300 households in Adelaide, South Australia, concluded that there were no appreciable differences in health outcomes from drinking untreated or treated rainwater (Rodrigo *et al*., 2011). Ahmed *et al*. (2011) carried out a review of available research reporting the microbial quality of RWH. This review suggested that the quality of RWH is strongly influenced by the season and the number of preceding dry days (Kus *et al*., 2010; Lye, 2009). Several of the case studies reviewed suggested links between gastroenteritis and consumption of untreated rainwater (Brodribb *et al*., 1995; Franklin *et al*., 2008; Murrell and Stewart, 1983). These reported outbreaks tended to involve small numbers of individuals, and the reported illnesses were often related to communal RHW systems.

Coombes (2015) hypothesised that RWH contains an inherent water treatment process ('treatment train') consisting of flocculation, settlement, sorption and bioreaction and that stored rainwater quality improves as metal and chemical contaminants settle to form sludge. This study highlighted the importance of first-flush devices to remove 11–94% of dissolved solids and 62–97% of suspended solids from the first 0·25 mm of roof runoff. Martin *et al*. (2010) assessed the microbial properties of RWH at two study sites at Newcastle on the east coast of Australia. They concluded that rainfall events contributed to the bacterial load in rainwater storage systems, but that processes within the rainwater storage ensured that these incoming loads were not sustained. Spinks *et al*. (2005) and Spinks (2007) concluded that biofilms that formed on RWH tank walls and at the base of the sludge layer act to improve water quality. Spinks (2007) concludes that the settlement of particulate matter to the bottom of rainwater tanks is probably the single most beneficial process within rainwater storage. The quality of rainwater supplies was not compromised by the accumulation of sludge in tanks. This confirms the observation by Coombes (2002) that it is preferable to avoid disturbing rainwater storages.

Further studies (Morrow *et al*., 2007, 2010) concluded that the majority of the rainwater-harvesting systems in national investigations were compliant with the chemical and metal values in Australian Drinking Water Guidelines. Given the variability of RWH quality throughout the system, the most reliable sampling location is point the of use for a given end use. Evans *et al*. (2009) used pioneering medical science techniques including polymerase chain reaction (PCR) methods (not experimental real-time analysis) to extract deoxyribonucleic acid (ribonucleic acid) of microbes in over 40 rainwater harvesting systems over a 3-year period. Each sample was also subjected to a comprehensive range of microbial, medical and biochemical tests to confirm the results of the PCR analysis. The research found that bacteria of faecal origin were rare and not abundant or persistent in rainwater harvesting systems. This research discovered that rainwater storages act as balanced ecosystems in a similar fashion to environmental systems that improve water quality (Evans *et al*., 2009).

## Hot water systems

Arguably, the most significant health risk in hot water systems comes from the respiratory pathogen *Legionella pneumophila*. *L. pneumophila* is the agent of Legionnaires disease, an acute form of pneumonia, which most commonly infects the respiratory tract of immunocompromised individuals. While *L. pneumophila* can be due to aspiration, the most likely route of infection of the respiratory tract occurs when the bacteria is entrapped in aerosols and inhaled. The ingestion of *L. pneumophila* is harmless as they are unable to cope with the stresses of the gastrointestinal tract. There may be a potential health risk from showering in hot water if the water supply contains *L. pneumophila* and the hot water is maintained below 60°C, as contaminated aerosols may be produced. However, this risk is equally applicable to mains water users. Thermal inactivation can be used as an effective method to inactivate *Legionella* bacteria. The degree of inactivation is dependent on the temperature and the length of time the bacteria are exposed to that temperature. The thermal inactivation of *Legionella* bacteria starts around 50°C but is more rapid at higher temperatures. At 60°C, 90% of *L. pneumophila* will be inactive after 3·2 min of exposure (average value) (Makin, 2014). Where the water contains 100 000 CFU/l *Legionella*, the bacteria need to be held at 60°C for approximately 10 min to reduce numbers to below UK's Health and Safety Executive (HSE) action level of 100 CFU/l. Hot water storage cylinders that maintain a temperature of 60°C throughout the whole storage vessel for a period of 1 h daily should achieve satisfactory control of *Legionella* bacteria, in line with the recommendations in UK's HSE code of practice (HSE, 2013). A study by Borella *et al*. (2004) investigated *Legionella* spp. and *Pseudomonas* spp. contamination of hot water systems in Italy. *Legionella* spp. and *Pseudomonas* spp. were detected in 22·6 and 38·4% of samples, respectively. The study found that system and building

characteristics were the main predictors for *Legionella*. *Legionella* contamination was associated with a centralised heater, distance from the heating point of >10 m and a water plant that is >10 years old. *Legionella* presence was not affected by the origin of water (Borella, 2004). A study by Kruse (2015) in Germany analysed water samples from 718 buildings for *Legionella* spp. The study concluded that the most important risk factor for contamination with *Legionella* spp. was the temperature of the circulated hot water.

### Heat inactivation rates for waterborne disease

While extensive research has been undertaken in the food industry to determine the heat inactivation rates for pathogens, studies for thermal inactivation in a freshwater medium are more recent and more specialised (Spinks *et al*., 2005). Spinks *et al*. (2006) carried out thermal inactivation analyses on eight species of non-spore-forming bacteria in a water medium at temperatures of 55–65°C, and susceptibilities to heat stress were compared using *D*-values. The *D*-value for this study was defined as the time required to reduce a bacterial population by 90% or 1 log reduction. The results suggested that the temperature range from 55–65°C was critical for the effective elimination of enteric/pathogenic bacterial components and supported the thesis that hot water systems should operate at a minimum of 60°C. The study also recommended that future rainwater harvesting investigations should focus on the microbial ecology of rainwater treatment trains and stored water to determine the types of organisms likely to exist in these systems. Ahmed *et al*. (2011) also concluded that any microbial assessment should involve the analysis of RWH for actual pathogenic species, not just the common faecal indicator bacteria. Coombes *et al*. (2006) and Evans *et al*. (2006) further highlighted that *E. coli* has a large number of non-faecal environmental strains that are prevalent in natural waters (such as rainwater). Later research by Luo *et al*. (2011) confirmed this key issue – that the isolation of *E. coli* in rainwater may not indicate faecal contamination.

## Methodology

This study sets out to determine the time required to reduce a bacterial population by 90% at a given temperature. Analysis was carried out to assess the thermal destruction rates of sterile water samples spiked with known quantities of bacteria and to show the suitability of rainwater as an alternative hot water supply within the domestic house. The temperatures chosen were 55 and 60°C as they mimic the conditions typically found in a domestic hot water system. Aliquots of the spiked water samples were taken at times 0, 5, 10, 15 and 20 min and processed for a concentration of bacteria. The bacteria chosen for the experiments included *E. coli*, *Enterococcus fecalis*, *P. aeruginosa* and *Salmonella* spp. They are all indicators of water quality, in particular, *E. coli* and faecal coliforms which are indicators of faecal pollution. *E. coli* were measured using the method employed by the Irish National Accreditation Board (Inab)-accredited laboratory using the Colilert 18 method, a most probable number (MPN) technique that is suitable for the examination of drinking waters, including samples from all stages of treatment and distribution, and those source waters of moderate to high turbidity (Idexx, 2012a). This method was chosen as it is the only US Environmental Protection Agency-approved 18 h test and is included in the *Standard Methods for Examination of Water and Wastewater* (Idexx, 2012a). Faecal coliforms were measured using a method based on those recommended in the 21st edition of *Standard Methods for the Examination of Water and Wastewater* (American Public Health Association *et al*., 2005). *Pseudomonas* spp. were measured using a method based on the *Microbiology of Drinking Water – Part 8* (Gov.uk, 2010), and the medium used was Pseudomonas Isolation Agar. Filtered samples were incubated at 37 ± 1°C for 48 h. The colony count of heterotrophic bacteria or total viable count ($T_{VC}$) was enumerated by the Inab-accredited testing laboratory using the pour plate method with yeast extract agar (Idexx, 2012b). *Salmonella* spp. were chosen as an extra parameter for these experiments as it is a human pathogen that can infect the gastrointestinal tract of humans causing diarrhoea (Prescott, 1993). The method employed by the laboratory for the detection of *Salmonella* spp. was that of the Tecra Unique Salmonella Kit.

The bacteria used for spiking the water samples were obtained from the Health Protection Agency in the UK. They were supplied in lenticule discs and were then reconstituted using sterile phosphate buffer solution. Lenticule is the registered trademark name for the reference materials supplied by Public Health England. These are mainly used as controls for food, water and environmental microbiology examination.

The water samples were spiked by transferring the shaken reconstituted Lenticule of known bacteria into sterile water. This was then analysed for colifoms, *E. coli*, faecal coliforms, *Salmonella*, *P. aeruginosa* and $T_{VC}$ at 22 and 37°C. Once the first aliquot was taken, the spiked water sample was placed in a heated water bath at 55°C. A calibrated temperature probe was placed in a control sample of water placed alongside the samples in the water bath in order to record the temperatures at given intervals of 5, 10, 15 and 20 min. Once the desired temperature of 55°C was reached, a timer was set for each of the 5 min intervals. Sample aliquots were then taken and analysed for the methods specified earlier. For the heat treatment experiments at 60°C, the same procedure as outlined earlier was repeated with the spiked water sample placed in a heated water bath at 60°C. All water samples were incubated at 60°C for 5, 10, 15 and 20 min, respectively. Samples were analysed immediately following treatment.

## Results

### Thermal inactivation experiments conducted at 55°C

Table 1 shows the microbiological results for coliforms, *E. coli*, faecal coliforms, *Salmonella*, *P. aeruginosa* and $T_{VC}$ (at 22 and 37°C). All samples were incubated at 55°C for 5, 10, 15 and 20 min, respectively. The sample was taken prior to heat treatment at 55°C. Both the coliform and *E. coli* results were 248·3 MPN/100 ml. After 5 min at 55°C, the values showed a marked decrease to 2·1 MPN/

Table 1. Overall microbiological results for heat treatment experiments at 55°C

| Parameter | Unit | Time: min | | | | |
|---|---|---|---|---|---|---|
| | | 0 | 5 | 10 | 15 | 20 |
| Coliforms | MPN/100 ml | 248·3 | 2·1 | 0 | 0 | 0 |
| *E. coli* | MPN/100 ml | 248·3 | 2·1 | 0 | 0 | 0 |
| $T_{VC}$ at 22°C | CFU/ml | 88 | 0 | 0 | 0 | 0 |
| $T_{VC}$ at 37°C | CFU/ml | 101 | 0 | 0 | 0 | 0 |
| *P. aeruginosa* | CFU/ml | 12 | 0 | 0 | 0 | 0 |
| Faecal coliforms | CFU/ml | 36 | 0 | 0 | 0 | 0 |

100 ml. At all other times, 10, 15 and 20 min, respectively, no coliforms or *E. coli* were detected in the samples. $T_{VC}$ at 22 and 37°C was also highest at time 0, 88 CFU/ml at 22°C and 101 CFU/ml at 37°C. After 5 min, no $T_{VC}$ at 22 or 37°C was detected.

### Coliforms and *E. coli*

The coliforms and *E. coli* samples taken prior to heat treatment at 55°C gave a concentration of 248·3 MPN/100 ml, as shown in Figure 1. After 5 min at 55°C, results showed a dramatic decrease to 2·1 MPN/100 ml. At times 10, 15 and 20 min, no coliforms or *E. coli* were detected in the samples.

### Faecal coliforms

The thermal inactivation rates for faecal colifoms at 55°C are shown in Figure 2. Faecal coliforms at time 0 prior to heat treatment at 55°C was 36 CFU/ml. After 5 min at 55°C, results showed a dramatic decrease to 0 CFU/ml. At times 10, 15 and 20 min, no faecal coliforms were detected in the samples.

### $T_{VC}$

The thermal inactivation rates for $T_{VC}$ (22 and 37°C) at 55°C are shown in Figure 3. $T_{VC}$ at 22 and 37°C prior to heat treatment at time 0 was 88 CFU/ml at 22°C and 101 CFU/ml at 37°C. After 5 min, no $T_{VC}$ at either 22 or 37°C was detected and none thereafter at times 10, 15 or 20 min.

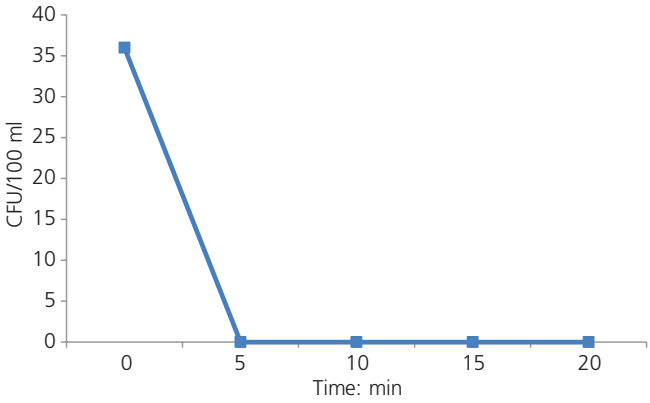


Figure 2. Thermal inactivation rates for fecal colifoms at 55°C

### *Pseudomonas aeruginosa*

The thermal inactivation rates for *P. aeruginosa* at 55°C are shown in Figure 4. *P. aeruginosa* prior to heat treatment at time 0

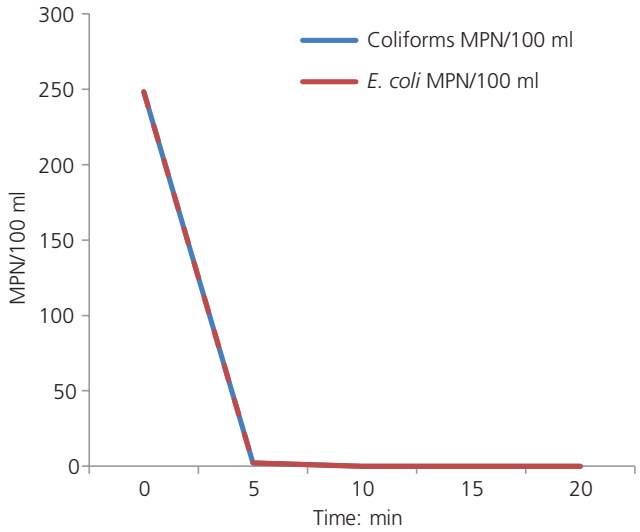


Figure 1. Thermal inactivation rates for coliforms and *E. coli* at 55°C

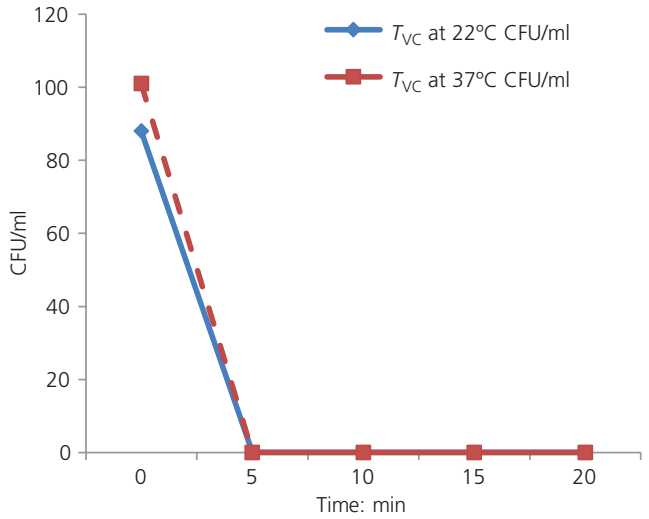


Figure 3. Thermal inactivation rates for $T_{VC}$ (22 and 37°C) at 55°C

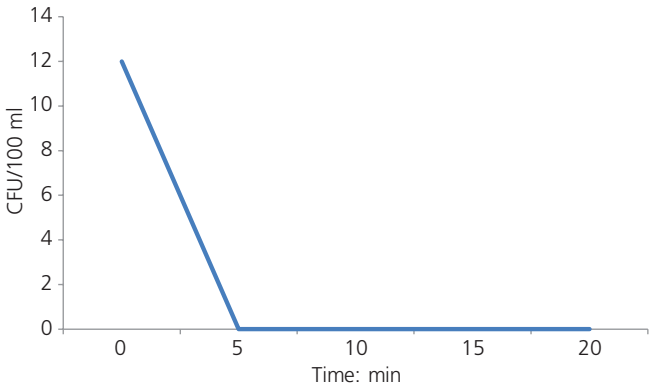


Figure 4. Thermal inactivation rates for *P. aeruginosa* at 55°C

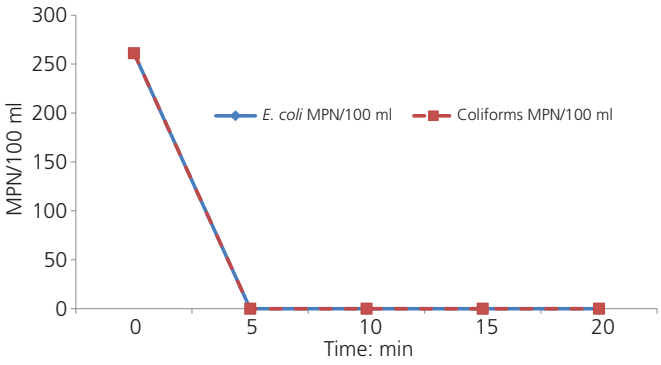


Figure 5. Thermal inactivation rates for coliform and *E. coli* at 60°C

was 12 CFU/100 ml. *P. aeruginosa* was not detected at 5, 10, 15 or 20 min.

### Thermal inactivation experiments conducted at 60°C

Table 2 shows the microbiological results for coliforms, *E. coli*, faecal coliforms, *Salmonella*, *P. aeruginosa* and $T_{VC}$ at 22 and 37°C.

#### Coliforms and *E. coli*

The thermal inactivation rates for coliform and *E. coli* at 60°C are shown in Figure 5. The coliforms and *E. coli* samples taken prior to heat treatment at 60°C gave a concentration of 261·3 MPN/100 ml. After 5 min, and thereafter at times 10, 15 and 20 min, no coliforms or *E. coli* were detected in the samples.

#### Faecal coliforms

The thermal inactivation rates for faecal coliform at 60°C are shown in Figure 6. Faecal coliform sample taken prior to heat treatment at 60°C gave a concentration of 34 CFU/ml. After 5 min at 60°C, the results showed a dramatic decrease to 0 CFU/ml. At times 10, 15 and 20 min, no faecal coliforms were detected in the samples.

#### $T_{VC}$ at 22 and 37°C

The thermal inactivation rates for $T_{VC}$ (22 and 37°C) at 60°C are shown in Figure 7. $T_{VC}$ at 22 and 37°C prior to heat treatment at time 0 was 90 CFU/ml at 22°C and 129 CFU/ml at 37°C. After 5 min, no $T_{VC}$ at 22 or 37°C were detected and none thereafter at times 10, 15 or 20 min.

#### *Pseudomonas aeruginosa*

The thermal inactivation rates for *P. aeruginosa* at 60°C *are* shown in Figure 8. *P. aeruginosa* prior to heat treatment at time 0 was 15 CFU/100 ml. No *P. aeruginosa* was detected at 5, 10, 15 or 20 min.

## Discussion of results

### Simulating heat shock

The results of this study indicate that after 5 min of exposure at 60 and 55°C respectively, *E. coli*, faecal coliforms, *Salmonella*, *P. aeruginosa* and $T_{VC}$ at 22 and 37°C concentrations will have been reduced to zero. The mechanism of cell inactivation would appear to be the breakage of bonds due to excessive energy. Previous studies have shown that bacterial cells cultured at higher temperatures display more resistance to heat than bacteria cultures at lower temperature (Pflug and Holcomb, 1991). Thus, the results from this study may be conservative when compared to the typical lower temperature of water stored in rainwater harvesting tanks.

### Comparison with other similar studies

These results are comparable with the results from international studies reported on similar experiments (Spinks *et al.*, 2003). In this study, laboratory experiments investigated the time required to reduce a bacterial population by 90% or 1 log reduction (*D*-value) at 65, 60 and 55°C. The results reported *D*-values at 65 and 60°C for *E. coli* of 3 and 62 s, respectively, while at 55°C, *E. coli* displayed an initial *D*-value of 21 min followed by 4 min.

Table 2. Overall microbiological results for heat treatment experiments at 60°C

| Parameter | Unit | Time: min | | | | |
|---|---|---|---|---|---|---|
| | | 0 | 5 | 10 | 15 | 20 |
| Coliforms | MPN/100 ml | 261·3 | 0 | 0 | 0 | 0 |
| *E. coli* | MPN/100 ml | 261·3 | 0 | 0 | 0 | 0 |
| $T_{VC}$ at 22°C | CFU/ml | 90 | 0 | 0 | 0 | 0 |
| $T_{VC}$ at 37°C | CFU/ml | 129 | 0 | 0 | 0 | 0 |
| *Pseudomonas* | CFU/ml | 15 | 0 | 0 | 0 | 0 |
| Faecal coliforms | CFU/ml | 34 | 0 | 0 | 0 | 0 |

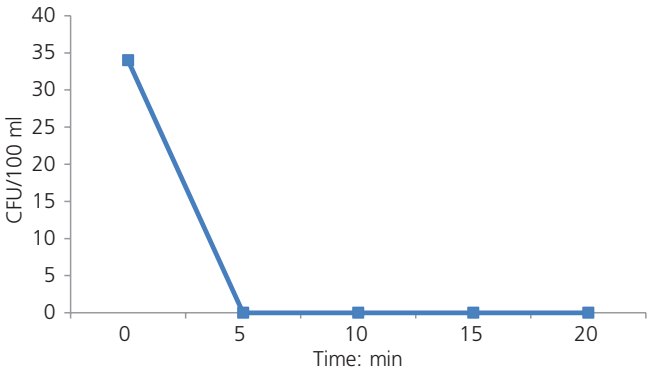


Figure 6. Thermal inactivation rates for faecal coliform at 60°C

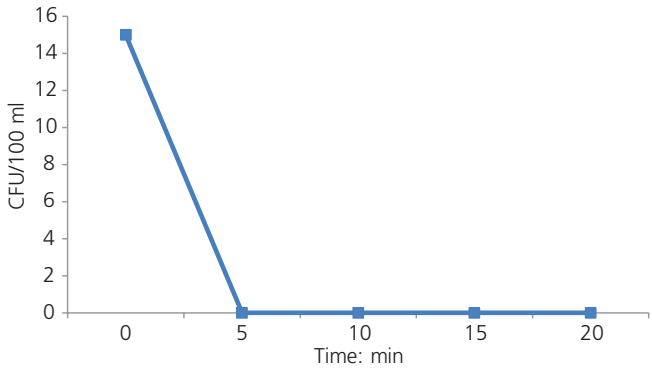


Figure 8. Thermal inactivation rates for *P. aeruginosa* at 60°C

For *P. aeruginosa*, the *D*-value at 65, 60 and 55°C were 5 and 49 s and 5 min; for *S. typhimurium*, <2, 4 and 77 s; and for *Klebsiella pneumoniae*, <2, <2, and 35 s, respectively. The results indicate that after 12 min at 60°C, *E. coli* concentrations will have been reduced by 15 log reductions, while the other pathogens experienced similar or even greater reductions.

Human pathogens are restricted to temperature ranges of around 37°C. Enterohemorrhagic *E. coli* has a growth range between 8 and 48°C, and *Aeromonas* spp., between 4 and 45°C (Szewzyk *et al*., 2000). At temperatures exceeding maximum growth limits, the thermal death rates are high, such as for *Campylobacter* sp. that survive for only a few hours at temperatures exceeding their optimal range of 37°C (Szewzyk *et al*., 2000). As well as studying *Legionella* species, Stout *et al*. (1986) also investigated the *D*-values for species from other genera. At 60, 70 and 80°C, *P. aeruginosa* had *D*-values of 2·6, 1·3 and 0·7, respectively (Stout *et al*., 1986).

## Conclusions

The results of this study show that after 5 min of exposure at 60 and 55°C, respectively *Salmonella*, *P. aeruginosa* and $T_{VC}$ at 22 and 37°C concentrations were reduced to zero. *E. coli* and faecal coliforms required 10 min of exposure at 55°C to reduce the population to zero and 5 min at 60°C. This is in agreement with other international studies on domestic rainwater tanks and hot water systems (Coombes *et al*., 2003; Spinks, 2003). Thermal inactivation in RWH-fed hot water systems at a minimum of 60°C, combined with the treatment train inherent within RWH systems, is likely to deliver water quality comparable to mains hot water. From the literature, the main risk to public health from hot water systems is not the water source but rather the operation, maintenance, age, location and temperature of the hot water system. Irish Standards require hot water systems to be maintained at temperatures at or above 60°C. Hot water is stored at a minimum of 60°C and distributed so it reaches a minimum temperature of 50°C (55°C in healthcare premises) within 1 min at outlets (HSE, 2013). The conclusion from this pilot study is that hot water systems supplied with RWH do not present an increased risk to health over hot water systems fed with mains water. Further studies in Ireland should be commissioned to characterise the RWH treatment train and the maintenance of RWH systems to establish appropriate indicator organisms and sampling points, and determine the efficiency of first flush devices.

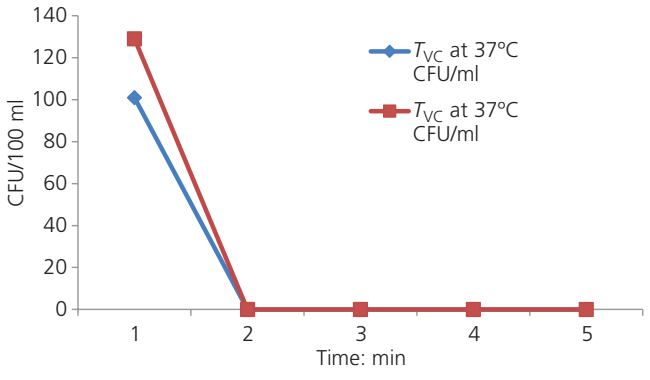


Figure 7. Thermal inactivation rates for $T_{VC}$ (22 and 37°C) at 60°C

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