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Inactivation of faecal indicator bacteria in a roof captured rainwater system under ambient meteorological conditions

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Abstract

Aims: In this study, faecal indicator bacteria (FIB) namely *Escherichia coli* and *Enterococcus* spp. were seeded into slurries of possum faeces and placed on the roof and in the gutter of a roof-captured rainwater (RCR) system. The persistence of FIB in these circumstances was determined under ambient climatic conditions. FIB persistence was also determined under *in-situ* conditions in tank water using diffusion chambers.

Methods and Results: The concentrations of surviving FIB at different time intervals were enumerated using culture-based methods. Both FIB were rapidly inactivated on the roof under sunlight conditions (T_{90} = 2 h) compared to shade conditions (T_{90} = 9-53 h). Significant differences were observed between sunlight and shade conditions on the roof for both T_{90} values of *E. coli* ($P < 0.001$) and *Enterococcus* spp. ($P < 0.001$). *E. coli* showed biphasic inactivation rates under both clean and unclean gutter conditions. *Enterococcus* spp., however, showed rapid inactivation (T_{90} = 2 h for the clean gutter and T_{90} = 6 h for the unclean gutter) compared to *E. coli* (T_{90} = 22 h for the clean gutter and T_{90} = 20 h for the unclean gutter). Significant differences were also observed between the T_{90} values of *E. coli* and *Enterococcus* spp. for both clean ($P < 0.001$) and unclean ($P < 0.001$) gutters. Both *E. coli* and *Enterococcus* spp. showed non-linear biphasic inactivation in tank water. Significant difference was observed between the T_{90} value of *E. coli* inactivation compared to *Enterococcus* spp. (P = 0.0003) in the tank water.

Conclusions: In this study, FIB were observed to survive longer (T_{90} = 9-53 h) on the roof under shade conditions compared to sunlight conditions (T_{90} = 2 h). If there is a rainfall event within a week after the deposition of faecal maters on the roof, it is highly likely that FIB would be transported to the tank water. When introduced into the tank, a relatively slow inactivation process may take place (T_{90} = 38-72 h).

Significance and Impact of the Study:

The presence of FIB in water indicates faecal pollution and potential presence of enteric pathogens. Therefore, the information on the resilience of FIB, as obtained in this study, can be used for indirect assessment of health risks associated with using roof-captured rainwater for potable and non-potable purposes.

Keywords: Faecal indicator bacteria; Roof-captured rainwater; Inactivation; Pathogens; Health risks

Introduction

Roof captured rainwater (RCR) is considered as an alternative water source in many countries because it has the potential to replace significant volumes of potable water in and around domestic dwellings as well as industries (Despins *et al.* 2009; Evans *et al.* 2006). The most significant issue in relation to RCR use, however, is the potential public health risks associated with the exposure to pathogens that could be present in the faeces of birds, insects, mammals and reptiles (Ahmed *et al.* 2012a; Chilvers *et al.* 1998; Marino *et al.* 1992). Faeces from animals and other organic debris deposited on the roof and in the gutter can be introduced into the tank via roof runoff during rain events. The presence of potential bacterial pathogens, such as *Aeromonas* spp.,

Campylobacter spp., *Salmonella* spp., *Legionella pneumophila*, and protozoa pathogens such as *Giardia* spp., and *Cryptosporidium* spp., along with the traditional faecal indicator bacteria (FIB) namely *Escherichia coli* and *Enterococcus* spp. have been frequently reported in rainwater tank samples (Ahmed *et al.* 2008; Crabtree *et al.* 1995; Savill *et al.* 2001; Simmons *et al.* 2008).

The presence of FIB in water indicates the potential for presence of pathogens of faecal origin. A recent study reported that the microbiological quality of rainwater tanks in an Ecovillage in Southeast Queensland (SEQ), Australia was highly variable. The presence of FIB, along with the occurrence of bacterial and protozoa pathogens in rainwater tank and related household tap water samples suggests that untreated RCR may not be suitable for drinking (Ahmed *et al.* 2012). Recent studies have also reported the presence of clinically significant virulence genes associated with FIB in rainwater tank samples in SEQ (Ahmed *et al.* 2011; Ahmed *et al.* 2012b). Questions have, therefore, arisen regarding the persistence of FIB and pathogens in rainwater tank samples as well as in faecal deposits on the roof and in the gutter.

A range of climatic and biological factors have been shown to influence the inactivation of FIB and pathogens. These factors include temperature, moisture content, solar radiation, relative humidity, pathogen type, presence of bio-degradable organic matter and interaction with other microorganisms (Hurst *et al.* 1980; Jiang *et al.* 2002; Sidhu *et al.* 2008; Wanjugi and Harwood 2013). The FIB and pathogens from bird and animal droppings deposited on the roofs and in the gutters are expected to inactivate rapidly due to harsh environmental conditions such as temperature, UV radiations and loss of moisture. However, certain conditions such as the shade portion of the roof, instant rain, availability of bio-degradable organic matter in the gutter may prolong the inactivation of FIB and pathogens. However, very little has been documented about the inactivation rates of FIB and pathogens on the roofs, in the gutters and in the tank water.

The primary aim of this study was to investigate the persistence of *E. coli* and *Enterococcus* spp. on the roof and in the gutter of a model RCR system. The specific aims of the study were to: (i) determine one $\log_{10}$ inactivation time (T_{90}) of *E. coli* and *Enterococcus* spp. on the corrugated iron roof and in the gutters; (ii) one $\log_{10}$ inactivation time (T_{90}) of FIB in the tank water. The experiments were undertaken to obtain information on the inactivation of FIB in the time periods between faeces being deposited on the roof and entering the tank.

Materials and methods

Study site and experimental RCR system construction

This study was conducted at the Ecosciences Precinct, Dutton Park, Qld, Australia (27º29' 45.62"S; 153º01' 46.41"E) from March to April 2012 (late summer to early autumn). A model RCR system (similar set up to RCR systems commonly in use in Australian domestic dwellings) comprising of a 5,000 L polyethylene tank, a roof (2 m^2) constructed with corrugated iron sheets and steel guttering and plastic downpipe leading water into the tank to simulate an urban RCR system. The tank was placed under direct sunlight (received minimum 5 h sunlight per day during the experiment).

Sources of faecal indicator bacteria (FIB) used for roof, gutter and tank water inactivation experiments

E. coli and *Enterococcus* spp. were isolated from fresh faeces of possums and wild birds (crow, pigeon, honey eaters and magpie) since these were the most likely sources of faecal deposition on the roof and in the gutters of dwellings in SEQ. Possum and bird faeces were pooled separately, serially diluted with phosphate buffer saline (PBS), and were streaked on Chromocult™ coliform agar (Merck, KGaA, Darmstadt, Germany) and Chromocult™ enterococci agar (Merck) plates. Agar plates were incubated overnight at 37°C. Ten *E. coli* colonies (five from possum and five from birds) and 10 *Enterococcus* spp. colonies (five from possum and five from birds) were isolated from the respective agar plates and streaked twice on the agar plates to obtain pure colonies. All these colonies were confirmed as *E. coli* and *Enterococcus* spp. by PCR amplifications of 23S rRNA genes as described elsewhere (Frahm and Obst 2003; Haugland *et al.* 2005). Mixed *E. coli* and *Enterococcus* spp. colonies (pure) were inoculated in flasks containing Nutrient Broth (BD, Sparks, MD, USA) and Brain Heart Infusion Broth (Merck), respectively. The flasks were kept in a shaking incubator at 100 rpm overnight at 37°C. Prior to seeding, the mixed bacterial cultures were washed twice in 20 ml sterile phosphate buffer saline (PBS). The cultures were centrifugation at 6,000 rpm for 3 min. followed by resuspension of the pellet in fresh PBS to remove culture media. The cultures were then acclimatised in PBS overnight at room temperature as described elsewhere (Gordon and Toze 2003). At the same time, the concentrations of FIB were enumerated from the culture suspension using spread plate method. In brief, serial dilutions were made for the culture suspension and streaked on Chromocult™ coliform agar (Merck) and Chromocult™ enterococci agar (Merck) plates for the isolation of *E. coli* and *Enterococcus* spp., respectively. Agar plates were incubated overnight at 37°C for 24 and 48 h. Plates with 20-200 colonies were enumerated.

FIB inactivation on the roof and in the gutter

The FIB inactivation study was designed to simulate the event of roof and gutter contamination with possum and bird faecal materials by using homogenized possum faecal slurry. Possum faecal pellets were chosen as seeding matrix as they generally contain more organic matter and as a result dry out more slowly which potentially provide more favourable condition for pathogen survival on the roof and in the gutter compared to small-sized bird or lizard faecal droppings which desiccate more rapidly. Briefly, possum faecal pellets (n = 8) were collected from the Currumbin Wildlife Sanctuary and Orphan Native Animal Rear and Release Association Inc., SEQ, and transported to the laboratory on ice, where they were stored at 4^{o}C. The collected pellets from several possums were pooled and homogenized into approximately 900 ml slurry using sterile PBS. The concentrations of *E. coli* and *Enterococcus* spp., in possum faecal slurry were determined within 24 h of collection using the spread plate method as described elsewhere (Sidhu *et al.* 2008). The concentrations were approximately 2×10^4 ml^{-1} of slurry for both FIB. Since the concentrations of FIB were low in possum faecal slurry, known concentrations of mixed *E. coli* and *Enterococcus* spp. were seeded into the possum faecal slurry to a final concentration of approximately 10^8 *E. coli* and 10^6 *Enterococcus* spp. ml^{-1} of slurry.

For the roof and gutter inactivation experiments, 5 mL of faecal slurry was poured into a series of 50 mm petri dishes (without lids), and placed on the corrugated iron roof and in the gutter of the experimental structure. The petri dishes were exposed to diurnal cycles of insolation. For the roof experiment, 30 petri dishes were kept directly under sunlight and another 30 were kept in the shade. The shade on the roof was artificially created by placing a tarpaulin over the petri dishes. Enough air space was provided between the petri dishes and the tarpaulin to ensure that sufficient airflow could still occur. For the gutter experiment, 30 petri dishes were placed in the clean segment of the gutter (free from faecal matters and organic debris), and another 30 petri dishes were placed in the unclean gutter. The gutter was made unclean by filling with moist sediment (similar to unclean urban household gutters) containing vegetation and organic debris. The petri dishes were kept under the vegetation and organic debris.

Roof and gutter experiments started in the early morning of the day and ended after 96 h and 48 h, respectively. Triplicate petri dishes containing FIB were randomly collected at 0, 1, 2, 3, 4, 6, 8, 24, 48, 72, and 96 h time intervals from the roof and respective gutter segments and the concentrations of surviving FIB were enumerated. The collected petri dishes on each sampling occasion were placed on ice, transported to the

laboratory and processed within 2-4 h. The volume of slurry was adjusted to 5 mL with PBS in petri dishes where desiccation was observed to correct for evaporation loss. Rehydrated materials were scraped carefully from the petri dishes and transferred to 15 mL tubes. Enumeration of *E. coli* and *Enterococcus* spp. was then performed in triplicate. Serial dilutions were made for each replicate, and the concentrations of FIB were enumerated using a spread plate method as described earlier.

FIB inactivation in tank water

The inactivation experiment in tank water was undertaken using diffusion chambers as previously described (Sidhu and Toze 2012; Toze *et al.* 2004). The diffusion chambers with internal volume of 7 ml were made of Teflon and 25 mm diameter mixed cellulose esters membranes with a pore size of 0.025 µm (Millipore, Tokyo, Japan). The chambers were placed inside the rainwater tank under the water surface to rule out the influence of external factors such as sunlight or evaporation. The membranes on either side of the chamber allow passage of water and nutrients through the diffusion chamber but prevent seeded microorganisms escaping from the chambers (Pavelic *et al.* 1998; Sidhu and Toze 2012; Toze *et al.* 2010).

Prior to setting up diffusion chambers, a rainwater sample from the tank was collected in a sterile 1 L glass bottle, and stored at 4ºC. The background concentrations of *E. coli* and *Enterococcus* spp. in the collected rainwater sample were determined by membrane filtration method (US EPA 1997). The concentrations were determined to be < 10 CFU 100 ml^{-1} for both FIB. The concentrations of mixed *E. coli* and *Enterococcus* spp. in the prepared PBS suspension were added to the rainwater sample matrix to a final concentration of approximately 3.6×10^6 *E. coli* and 1.4×10^7 *Enterococcus* spp. ml^{-1} of water. The seeded rainwater sample was distributed equally into 24 diffusion chambers. This provided three replicate samples for collection on each of the 8 sampling occasions (after time 0). The residual of seeded volume remaining after filling the chambers was retained for use as the time 0 sample. All of the assembled diffusion chambers were suspended in the tank at a depth of 1 meter below the water level with a steel wire. Three replicates diffusion chambers were collected at on each sampling event (24, 48, 96, 144, 240, 408, and 800 h) intervals from the tank water and the concentrations of surviving FIB were enumerated. The collected samples were placed on ice, transported to the laboratory, and processed within 2-4 h. The 7 ml seeded water sample was transferred from each diffusion chamber to sterile 15 ml polypropylene tubes using a sterile 10 ml syringe and 21 gauge needles. Sample serial dilutions were made and the surviving

concentrations of *E. coli* and *Enterococcus* spp. were enumerated using the spread plate method as described above.

Meteorological data

Ambient temperature, rainfall, evaporation, relative humidity, wind speed and solar exposure data were collected from the Australian Bureau of Meteorology (BOM) web site during each of the inactivation experiments. Temperature data loggers (HOBO devices; Onset Computer Corporation, Pocasset, Mass.) were placed on the roof and in the gutter adjacent to the petri dishes to record the temperature at 1 h intervals for the duration of the roof and gutter experiment. A HOBO device was also suspended into the tank water to record the temperature at 4 h intervals for the duration of the tank water experiment.

Data analysis

For each FIB, all determined concentrations in each replicate at each sampling occasion were converted to $\log_{10}$ values and plotted over time. One $\log_{10}$ reduction time (T_{90}) for each FIB was determined from each plot using the following equation as previously described (Gordon and Toze 2003)

$T_{90} = -t / (\log 10\ C_t/C_0)$

Where C_0 is the concentration (CFU ml^{-1}) at day 0, C_t is the final concentration (CFU ml^{-1}) at day t. A linear regression was fitted to each plot and the slope was taken as the inactivation rate. The inverse of these calculated inactivation rates were then used as the determination of the one $\log_{10}$ reduction time (T_{90}). The average T_{90} on each sampling occasion was determined from the replicates of each FIB. Where the inactivation of FIB in some experiments was biphasic, two T_{90} values were calculated, one for the initial inactivation (first phase) and the other for the second stage of the inactivation (second phase) (Gordon and Toze 2003). The number of data points used to calculate T_{90} values varied in the biphasic inactivation curves. Only data points above the assay limit of detection were used in calculating the inactivation times. An analysis of variance (ANOVA) was performed on the T_{90} values of FIB on the roof and in the gutter under different conditions. For statistical comparison, T_{90} values derived from the first phases of various experimental conditions were used. The critical P-value for the test was set at 0.05.

RESULTS

Climatic conditions

The average ambient minimum temperature during the experiments ranged from 15.5 ± 3.10 °C (sunlight roof) to 18.0 ± 2.90ºC (shade roof) (Table 1). The average maximum temperature ranged from 26.6 ± 2.02 ºC (shade roof) to 30.2 ± 2.20 ºC (tank water). The evaporation rates were high throughout the inactivation experiments with values higher during the roof experiments compared to the gutter. Solar exposure was higher (16.9 ± 3.12 MJ m^{-2}) during the sunlight roof experiment compared to shade roof (12.6 ± 1.14 MJ m^{-2}) and gutter (10.3 ± 1.12 MJ m^{-2}) experiments.

The tank water temperature (measured by data logger) ranged from 21.4 to 28.5ºC (average 24.1 ± 2.42 ºC) during the tank inactivation experiment. The temperature of the corrugated iron roof and gutter was also measured using the data loggers. For the roof sunlight experiment, the temperature ranged from 23.7 to 39.3ºC (average 28.7 ± 7.42 ºC) and for the roof shade experiment the temperature ranged from 19.3 to 26.0ºC (average 24.4 ± 2.32 ºC). Gutter temperature ranged from 24.5 to 38ºC (average 29.3 ± 5.35 ºC) for the duration of the gutter inactivation experiment.

FIB inactivation on the roof

The inactivation rates of FIB in possum faecal slurry placed on the roof were evaluated under sunlight and shade conditions (Fig. 1). Under direct sunlight, *E. coli* rapidly inactivated (T_{90} = 2 h) compared to shade where slow non-linear (biphasic) inactivation rate [T_{90} = 53 h (first phase) and 9 h (second phase)] was observed (Table 2).

Similar results were also obtained for *Enterococcus* spp. which was inactivated faster under direct sunlight (T_{90} = 2 h) compared to shade condition where slow biphasic inactivation [T_{90} = 9 h (first phase) and 18 h (second phase)] was also observed. A rapid inactivation (1 log) was observed in the first 8 h followed by a slow decline. No significant ($P > 0.05$) difference was observed in T_{90} value of *E. coli* inactivation compared to *Enterococcus* spp. for sunlight conditions. Significant ($P < 0.001$) difference, however, was observed in T_{90} value of *E. coli* compared to *Enterococcus* spp. for shade conditions. Significant differences were also observed between sunlight and shade conditions for both T_{90} values of *E. coli* ($P < 0.001$) and *Enterococcus* spp. ($P < 0.001$).

FIB inactivation in the gutter

The inactivation rates of FIB in possum faecal slurry were evaluated in the clean and unclean gutters under direct sunlight with the organic matter and vegetation in the unclean gutter shading the faecal slurry from sunlight (Fig. 2). *E. coli* showed biphasic inactivation rates under both clean and unclean gutter conditions [T_{90} = 22 h (first phase) and 3 h (second phase)] for the clean gutter conditions and [T_{90} = 20 h (first phase) and 6 h (second phase) for the unclean gutter conditions. *Enterococcus* spp., however, showed rapid inactivation (T_{90} = 2 h for the clean gutter and T_{90} = 6 h for the unclean gutter) compared to *E. coli*. No significant ($P > 0.05$) difference was observed for the T_{90} values of *E. coli* inactivation between clean and unclean gutter conditions. Significant ($P < 0.001$) difference, however, was observed in T_{90} value of *Enterococcus* spp. between clean and unclean gutter conditions. Significant differences were also observed between the T_{90} values of *E. coli* and *Enterococcus* spp. for both clean ($P < 0.001$) and unclean ($P < 0.001$) gutter conditions.

FIB inactivation in tank water

The inactivation rates of FIB were determined under *in-situ* conditions in the rainwater tank kept exposed to sunlight (Fig. 3). *E. coli* fell below detection limit after 576 h whereas *Enterococcus* spp. were detected up to 816 h. Both *E. coli* [T_{90} = 72 h (first phase) and 273 h (second phase)] and *Enterococcus* spp. [T_{90} =38 h (first phase) and 195 h (second phase)] showed non-linear biphasic inactivation. A Student's paired *t-test* was performed on the T_{90} values between *E. coli* and *Enterococcus* spp for tank water. The critical *P*-value for the test was set at 0.05. Significant difference was observed between the T_{90} value of *E. coli* inactivation compared to *Enterococcus* spp. (paired t-test, $P = 0.0003$) in the tank water.

Discussion

Large numbers of rainwater tank samples in SEQ were reported to have faecal indicators above the Australian drinking water guideline value (Ahmed *et al.* 2008; Ahmed *et al.* 2010). Furthermore, PCR analysis of clinically significant virulence genes associated with *E. coli* and *Enterococcus* spp. indicated the presence of a wide array of virulence genes in *E. coli* and *Enterococcus* spp. isolated from rainwater tank samples (Ahmed *et al.* 2011; Ahmed *et al.* 2012b). Certain *E. coli* strains from rainwater tank samples harboring virulence genes were identical to those found in possum and bird faeces (Ahmed *et al.* 2012c). Pathogens such as *Campylobacter* spp., *Salmonella* spp., *G. intestinalis*, and *Cryptosporidium* spp. have also been detected in rainwater tank samples (Ahmed *et al.* 2010). No information is available on the inactivation rates of *E. coli* and *Enterococcus* spp. on

the roof, in the gutter and in the tank water. This study, therefore, was undertaken to obtain information on the inactivation of FIB under different scenarios which could assist in the assessment of health risks.

The inactivation potential of *E. coli* and *Enterococcus* spp. was investigated in this study due to their wide prevalence in RCR systems in SEQ and the former is the FIB recommended for monitoring microbiological quality of RCR in SEQ, Australia (Ahmed *et al.* 2010). The results showed that *E. coli* and *Enterococcus* spp. became inactivated more rapidly on the roof under sunlight conditions compared to shade conditions. The average ambient daily minimum and maximum temperature over the study period for sunlight and shade conditions were similar, and, therefore, appeared not to have played any significant role in FIB inactivation. Faecal indicator bacteria are part of the normal gut-flora of warm-blooded animals and the optimum growth temperature is 35°C for most enteric bacteria, although growth can occur at higher and lower temperatures (Sinton *et al.* 2002). However, the rate of inactivation at temperatures < 35°C may have little impact on the inactivation (Klein *et al.* 2011; Sinton *et al.* 2007).

A biphasic inactivation was observed under shade conditions for both FIB with slow inactivation rates up to 48 h followed by rapid losses. An important factor leading to the rapid inactivation under sunlight could be high intensity of ultraviolet radiation associated with direct sunlight (16.9 ± 3.12 MJ m^{-2}) compared to shade (12.6 ± 1.14 MJ m^{-2}) where the faecal slurry was sheltered from the direct sunlight. This was in agreement with a previous study undertaken by Moriarty *et al.* (2011). In this study, the roof experiment was undertaken on the corrugated iron roof, which can have extremely high surface temperature under sunlight as previously observed by Bretz *et al.* (1998). The temperature logger kept on the roof under sunlight in this study recorded temperature as high as 39.3ºC during the first few hours of the experiment. The combination of direct sunlight and high roof temperatures may have lead to a rapid inactivation of FIB. It should be noted that inactivation rates of FIB could yield different results on other roof types such as fiberglass, concrete or tiled roof, which were not included in this study. Loss of moisture through rapid evaporation (5.4 ± 2.10 mm per day) may have been another factor leading to the rapid inactivation observed for sunlight conditions compared to shade conditions as moist conditions are essential for the viability of metabolically active bacteria (Sinton *et al.* 2002; Ward *et al.* 1981). Under direct sunlight, the complete dessication (dried as evident by lack of moisture) of the slurry was observed to occur within 2 h compared to the shade conditions where the complete desiccation occurred in 8 h. Moriarty and colleagues reported a significant rise in inactivation rates of FIB in cow pats when the moisture content of the pats decreased from 80% to 40% (Moriarty *et al.* 2011).

For the gutter experiment, it was hypothesized that FIB would survive longer in the unclean gutter compared to the clean gutter due to micro-climatic factors such as moisture, nutrients and protection from UV in the leaf debris. No significant differences were observed for the T_{90} values of *E. coli* inactivation between clean and unclean gutters. Significant difference, however, was observed in T_{90} value of *Enterococcus* spp. between clean and unclean gutters. *Enterococcus* spp. in both unclean and clean gutters survived a relatively shorter period of time than *E. coli*. This could be due to the fact that *E. coli* showed biphasic inactivation rates in both types of gutters. The T_{90} values of *E. coli* in the first phases for clean and unclean gutters were higher (T_{90} = 20-22 h) compared to the second phases where T_{90} values were much lower (T_{90} = 3-6 h). The T_{90} values of *E. coli* in the second phase for both gutters, however, were similar to the T_{90} values of *Enterococcus* spp. We acknowledge that clean and unclean gutter experiments were undertaken under sunlight conditions only. It is highly likely that FIB would inactivate more slowly in the unclean gutter under shade conditions. Other conditions such as seasonal impacts and higher moisture levels in the leaf litter could also influence the inactivation of microorganisms.

When comparing the inactivation of two FIB groups in the tank water, the results indicated that *Enterococcus* spp. had a faster T_{90} time than *E. coli*. This concurs with a previous study that showed that faecal coliforms had greater persistence in freshwater than *Enterococcus* spp. (Anderson *et al.* 2005; Sinton *et al.* 2002). Slower inactivation rates were observed for both indicators in the tank water compared to the roof and gutter experiments. This was not unexpected, considering the fact that FIB in tank water were not exposed to harsh meteorological conditions such as sunlight, desiccation which could attribute faster inactivation on the roof and in gutter experiments. We acknowledge that FIB inactivation in tank water was undertaken using diffusion chambers which have internal volume of 7 ml. The diffusion chambers were filled with water sample (collected from the experimental tank) seeded with FIB to allow predation by existing predators. The concentrations of predators in 7 mL of water sample may be low which may have inflated the T_{90} values of FIB under the experimental conditions.

It is possible that certain strains of FIB survived better than others (Anderson *et al.* 2005) in the tank water, on the roof and in the gutter since mixed faecal strains were used for spiking in this study. The biphasic inactivation rates of FIB for the roof, gutter and tank experiments suggesting that perhaps certain strains of FIB survived better than others (Hellweger *et al.* 2009). Studies have shown that *E. coli* persistence in the

environment can be strain dependent (Anderson *et al.* 2005; Topp *et al.* 2003; Whittam 1989). Several studies have also documented slow inactivation of FIB of faecal origin similar to the results reported in this study (Sinton *et al.* 2002; Noble *et al.* 2004; Sommer *et al.* 1997). It is also possible that other factors such as dessication of faecal slurry, moisture content and UV radiation may have contributed to the biphasic inactivation rates. It is noted that inactivation experiment was undertaken in the tank, which was kept under direct sunlight. Many urban rainwater tanks are located in the shade or underneath the house shielding them from high temperature, and in such conditions, FIB inactivation rates may differ from the results obtained in this study.

In conclusion, FIB especially *E. coli* can survive longer (T_{90} = 53 h) on the roof under shade conditions compared to sunlight conditions. This could have an impact on health risks associated with tank water use. If there is a rainfall event after the deposition of faecal matter on a shade roof , it is highly likely that FIB and other faecal pathogens could be transported to the tank water. When introduced to the tank, a slower inactivation process may take place (T_{90} = 38-72 h). Further research is required to understand the persistence of bacterial and protozoa pathogens on the roof and in tank water in relation to FIB because certain pathogens are known to be more persistent in the environment than FIB. Maintenance of good roof and gutter hygiene and elimination of overhanging tree branches and other mounted structures on the roof where possible to prevent the flocking of possums and birds should be considered to minimize chances of faecal contamination on the roof and in the gutter. The magnitude of faecal contamination in rainwater tank immediate after rain events can be minimized by installing first flush device as the first flush runoff may contain a large amount of the faecal contamination load. Approximately, 10% (ABS 2007) of Australian people use rainwater as a major source of their drinking water, and therefore, it is recommended that rainwater should be treated with effective treatment procedures such as filtration, ultraviolet disinfection or simply boiling the water prior drinking.

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Conflict of Interest

The authors declare no conflict of interest.

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Table 1 Average (± SD) ambient meteorological data during inactivation experiments

Meteorological data	Experiments			
	Roof		Gutter [c]	Tank water [d]
	Sunlight [a]	Shade [b]		
Temperature minimum (ºC)	15.5 ± 3.10	18.0 ± 2.90	16.2 ± 2.02	15.6 ± 1.30
Temperature maximum (ºC)	25.3 ± 4.10	26.6 ± 2.02	24.9 ± 3.05	30.2 ± 2.20
Rainfall (mm)	3.40	7.40	0.00	NA
Evaporation (mm)	5.40 ± 2.10	4.06 ± 1.60	3.32 ± 1.01	NA

Relative humidity (%)	74.5 ± 5.26	79.3 ± 4.98	77.2 ± 2.05	NA
Wind speed (km h^{-1})	34.5 ± 5.30	23.6 ± 2.40	21.6 ± 3.24	NA
Solar exposure (MJ m^{-2})	16.9 ± 3.12	12.6 ± 1.14	10.3 ± 1.12	NA

[a]12-04-2012
[b]17-04-2012 to 21-04-2012
[c]23-04-2012 to 25-04-2012
[d]19-02-2012 to 25-03-2012
SD: Standard deviation
NA: Not applicable

Table 2 T_{90} inactivation time of *Escherichia coli* and *Enterococcus* spp. on the roof, in the gutter and tank water

Faecal indicators	Experiments	Conditions	T_{90} (h) (R^2)	
			First phase	Second phase
E. coli	Roof	Sunlight	2 (0.72)	
		Shade	53 (0.85)[b]	9 (0.95)[a]
	Gutter	Clean	22 (0.66)[a]	3 (0.99)[a]
		Unclean	20 (0.73)[b]	6 (0.88)[a]
	Tank water		72 (0.86)[a]	273 (0.84)[c]
Enterococcus spp.	Roof	Sunlight	2 (0.95)	
		Shade	9 (0.92)[b]	18 (0.80)[c]
	Gutter	Clean	2 (0.80)	
		Unclean	6 (0.97)	
	Tank water		38 (0.99)[a]	195 (0.94)[d]

[a]: Three data points were used to calculate T_{90} inactivation times
[b]: Four data points were used to calculate T_{90} inactivation times
[c]: Five data points were used to calculate T_{90} inactivation times
[d]: Six data points were used to calculate T_{90} inactivation times
R^2: Coefficient of determination

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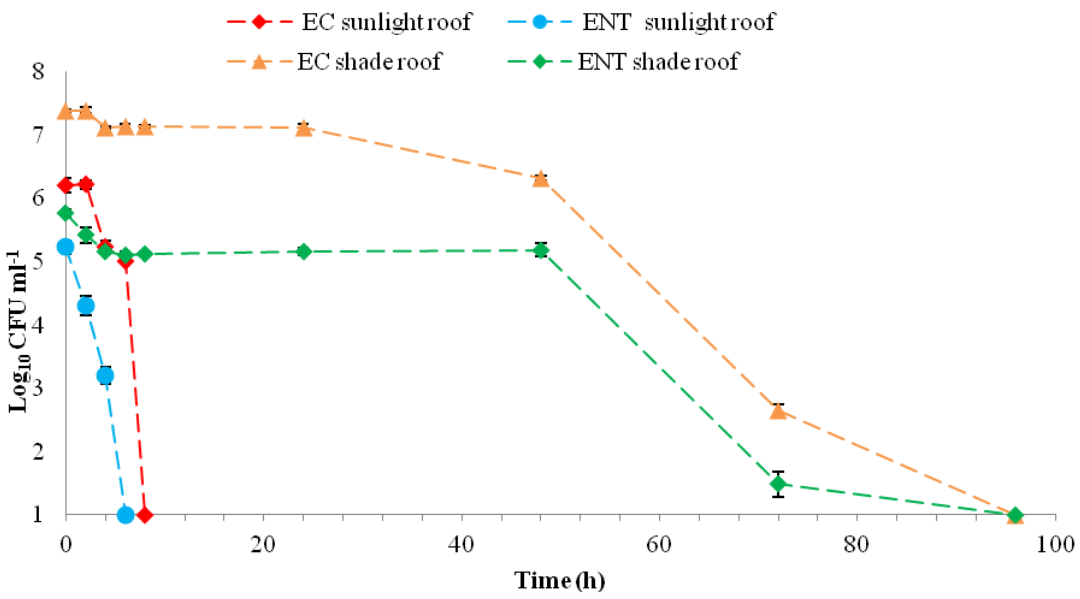

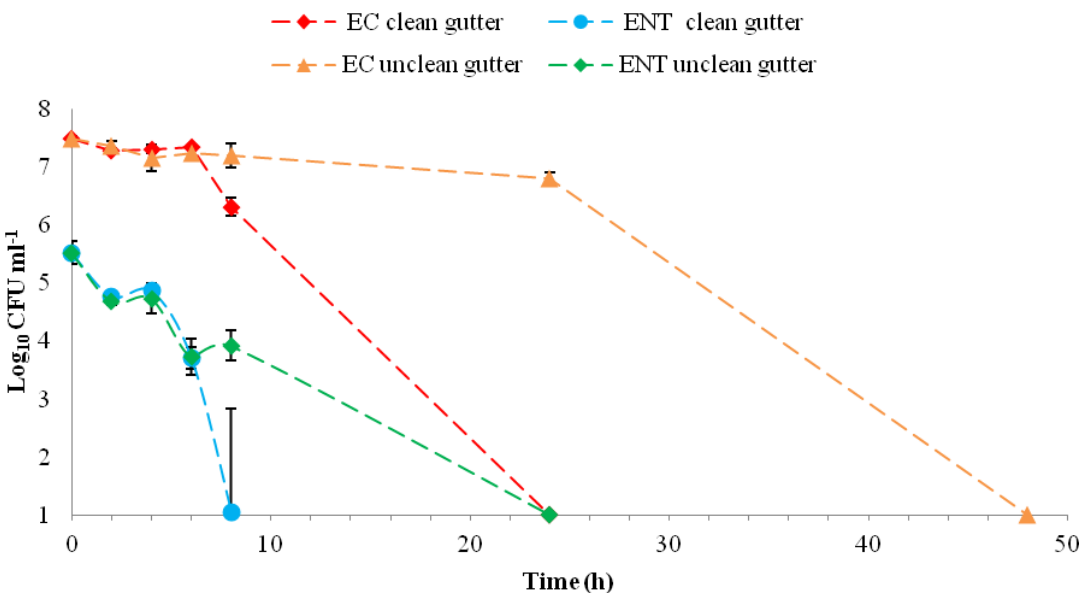

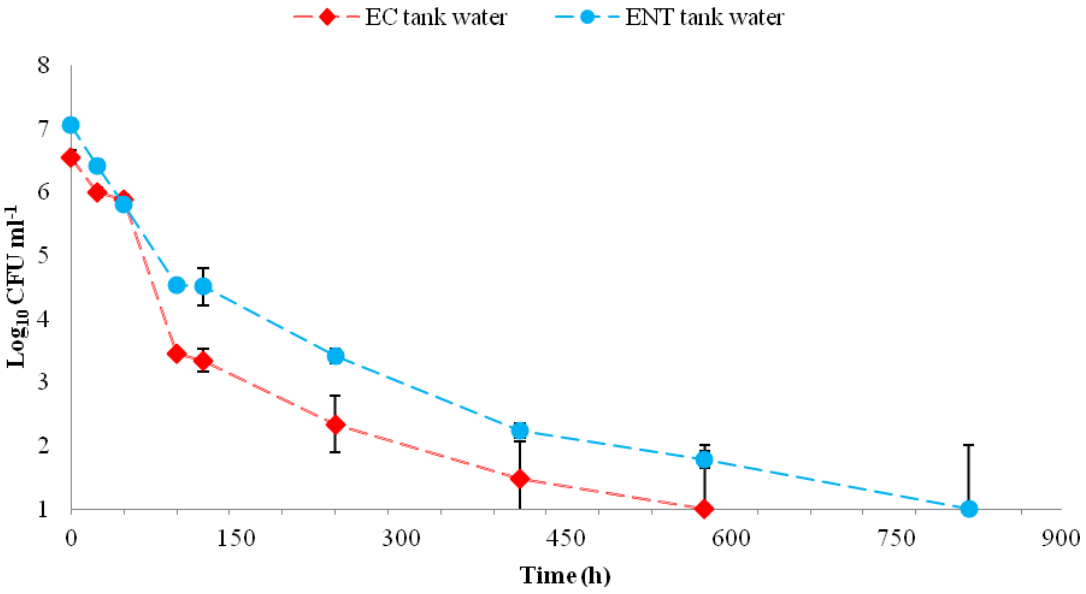

Figure 1 Log_{10} (mean ± standard deviation) colony forming units (CFU) of culturable *Escherichia coli* (EC) and *Enterococcus* spp. (ENT) during the course of inactivation on the roof under sunlight and shade conditions.

Figure 2 Log_{10} (mean ± standard deviation) colony forming units (CFU) of culturable *Escherichia coli* (EC) and *Enterococcus* spp. (ENT) during the course of inactivation in the clean and unclean gutters.

Figure 3 Log_{10} (mean ± standard deviation) colony forming units (CFU) of culturable *Escherichia coli* (EC) and *Enterococcus* spp. (ENT) during the course of inactivation in the tank water.