MICROBIAL QUALITY OF ROOF HARVESTED RAINWATER GOOD ENOUGH TO DRINK?

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ABSTRACT

A research project is currently examining the potential for microbial pathogens to be present in roof harvested rainwater. A initial survey has determined that the faecal microbial indicators *E. coli* and enterococci were present in more than 50% of the rainwater tanks sampled. A preliminary survival experiment has indicated that locating rainwater tanks in the shade may increase the survival potential of microorganisms. The research outcomes to-date suggests that the microbial quality of roof harvested rainwater may not be as good as believed, however, further study will determine if a wider range of uses within households is still possible.

INTRODUCTION

Roof harvested rainwater (RHRW) is one of the major potential alternative water sources that can be used in South East Queensland. RHRW has the potential to replace significant volumes of grid water in and around domestic dwellings and industry if used for the most appropriate potable water substitutions. The use of RHRW for the flushing of toilets, as a cold water supply for washing machines and for outdoor irrigation is currently the most popular of the mandated options for all new establishments in south east Queensland. The potential areas for further replacement of current potable water uses are primarily focusing on use in the hot water system but there is consideration for even further possible expansion of uses up to, in some cases, even replacing potable uses (eg, cooking and drinking). There are currently restrictions, however, on further uses within the house where potable water is available from the grid due to uncertainty on the degree of health risks associated with these water sources. The uncertainty on health risks relates predominantly to the potential presence of microbial pathogens.

There is some limited information about the occurrence of microbial pathogens in rainwater tanks in the literature which suggests that RHRW can be contaminated by a number of zoonotic bacterial and protozoan pathogens (Ahmed et al. 2010, Simmons et al, 2001, Spinks et al. 2006). Pathogens have been reported to be detected in RHRW including the bacteria *Salmonella,* and *Campylobacter*¸ and the protozoa *Giardia* and *Cryptosporidium* spp. (Ahmed et al. 2008, Albrechtsen 2002, Crabtree et al. 1996, Simmons et al. 2001). It has also been suggested that animals and birds that have access to the roof could be the major potential source of these microbial pathogens (Koplan et al. 1978, Merritt et al. 1999).

This research project reports on the outcomes of the first stage of the research toward gaining detailed information on the type, prevalence and source of pathogens that can be present in rainwater tanks from a range of different locations within south east Queensland. In addition, the project is researching the survival and behaviour of different pathogens during storage in rainwater tanks. The resulting final information will be used to manage the optimal uses of RHRW for the maximum savings of potable water while maintaining appropriate health risk levels.

METHODOLOGY

Survey of Roof Harvested Rainwater in Rainwater Tanks

A series of rainwater tanks are being tested for the presence of microbial pathogens and indicators. A total of 83 rainwater tanks have been selected for an initial microbial survey. The survey involves the collection of 20 litres of RHRW from each tank, primarily directly from the off-take tap at the base of the tank. In addition, a number of dwellings have supplies from the rainwater tank to inside the house and in these instances; the internal tap was also sampled.

At the time of sample collection at each site, a range of additional information including tank location and age, the presence of trees overhanging the roof and the time since the last rain event was also recorded to determine if there were physical conditions that could be linked to the level of risk from microbial pathogens.

The collected RHRW was transported back to the laboratory and processed within six hours for the detection of specific microorganisms using the methods outlined in Table 1. On arrival in the laboratory, the collected water samples were stored in a cold room at 4ºC until being processed.

The common microbial indicators were analysed by detection on appropriate selective media. This was done by filtering triplicate 100 ml volumes of collected RHRW through 0.2 um nitrocellulose membrane filters which were then placed on the respective selective isolation media. Following incubation overnight at 37ºC, the plates were examined for colonies with the appropriate morphology that indicated the presence of the microbial indicators.

For the detection of specific microbial pathogens, the collected water samples were concentrated from the original 20 litres to a maximum of 100 mL using tangential-flow filtration. The resulting concentrates were then used for the detection of the microbial pathogens. The detection of the microbial pathogens listed in Table 1 was being undertaken using PCR. The DNA extraction and PCR methods used are those listed in Ahmed et al. (2008).

Table 1: Detection methodology for target microorganisms in RHRW.

Microorganisms	**Analysis Method**	**Detection**
E.coli	Culture	mTEC medium
Enterococci	Culture	mEI medium
Aeromonas hydrophila	PCR	*lip* gene
Campylobacter jejuni	PCR	*mapA* gene
Salmonella spp.	PCR	*invA* gene
Legionella spp.	PCR	*mip* gene
Cryptosporidium spp.	PCR	COWP gene
Giardia lamblia	PCR	*β*-giardin gene

Assessment of Potential Sources of Pathogens in Roof Harvested Rainwater

To determine the most likely potential sources of microbial pathogens in roof harvested rainwater, faecal samples have been collected from animals and birds that commonly have access to domestic roofs in south east Queensland. These animals and birds include possums and rats, fruit bats, and a range of birds common to the south east Queensland region. Each of the collected faecal samples is currently being processed to determine the presence of any of the microbial pathogens listed in Table 1.

In addition to the detection of the microbial pathogens, *E. coli* and enterococci strains have been isolated from each of the faecal samples and stored for comparison with *E. coli* and enterococci strains isolated from rainwater tanks using microbial source tracking methods outlined by Ahmed et al. (2009).

Survival of Microbial Pathogens in Rain Water Tanks

Even if microbial pathogens and indicators are being flushed into rainwater tanks with RHRW there is no information on the behaviour and survival potential of these microorganisms in the rainwater tank. An understanding of the persistence of different microbial pathogens in rainwater tanks will be important information for the development of improved management tools for rainwater tanks.

To gain a better understanding of the potential persistence of the different pathogens in rainwater tanks, a series of survival experiments are underway to assess the survival potential of different faecal microorganisms in a series of experimental tanks that are located either in full sunlight or in shaded conditions. The pathogens are being tested using diffusion cells as outlined in Toze et al. (2010).

RESULTS AND DISCUSSIONS

Preliminary Survey of the Quality of Roof Harvested Rainwater

The results obtained for the detection of the microbial indicators *E. coli* and enterococci in the collected rainwater tank samples are given in Table 2 and the range of number of *E. coli* cells per 100mL of tank water are presented in Figure 1. The results show that *E. coli* could be detected in more than 50% of the rainwater tanks sampled and that enterococci were present in greater than 90% of the tanks. This is higher than a survey of rain water tanks in Victoria by Spinks et al. (2006) who found that 33% of the tanks were positive for *E. coli* and 73% positive for enterococci, but less than what as been reported internationally with greater than 70% of Danish and Korean rainwater tanks sampled being positive for *E. coli* (Albrechtsen 2002, Lee et al. 2010).

Table 2: Number of rainwater tank samples positive for E. coli and enterococci.

Microorganisms	**% positive samples (n=49)**
E. coli	59
Enterococci	92

There is little current information on the potential correlation of faecal indicators and pathogens in rainwater tanks. The greater prevalence of enterococci in rainwater tanks compared to *E. coli* raises the question on the suitability of each of these microorganisms as an indicator for assessing the potential for faecal contamination of RHRW. A preliminary study on the potential presence of pathogens in south east Queensland rain water tanks indicated that there were potential discrepancies between faecal indicators and pathogens (Ahmed et al. 2010). This correlates with reports that neither *E. coli* nor enterococci correlated well with the presence of bacterial or protozoan pathogens in environmental waters (Harwood et al. 2005, Horman et al. 2004, Lemarchand and Lebzaron 2003). An assessment using microbial source tracking will need to be undertaken to determine the source of these microbial indicators to assess which, if either of these microorganisms is suitable for indicating a potential health risk from pathogens in RHRW.

Detected *E. coli* numbers ranged from 1 to 630 colony forming units (cfu) 100mL^{-1}. Detected enterococci numbers ranged from 1 to 693 cfu 100mL^{-1}. Similar to the need to determine if the presence of *E. coli* or enterococci cells has any correlation with microbial pathogens, it remains to be assessed if there is any link between the number of microbial indicators and the presence of pathogens in RHRW. It is anticipated that the present study will provide greater clarity on the efficacy of different microbial indicators for signifying increased risk from microbial pathogens.

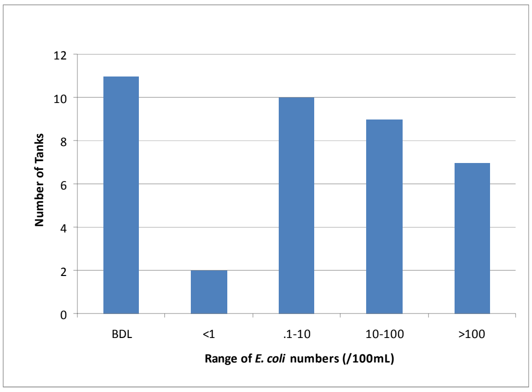

Figure 1: Range of E. coli cell numbers detected in individual rainwater tank samples (BDL = below detection limit).

The assessment of the quality of RHRW in rainwater tanks and in taps delivering into household for dwelling that had rainwater plumbed into the house has shown that *E. coli* and enterococci can be present both in the tank and in the water collected from the internal tap (Figure 2). Apart from three tank/tap samples, the results indicated that there was no significant different in the number of *E. coli* or enterococci cell numbers detected in the rainwater tank or in the RHRW sourced form the internal tap. Further analysis is planned to determine if the microbial pathogens can be detected in the RHRW samples collected from the internal tap or if the presence of the *E. coli* or enterococci for the tap indicates an increased health risk as it would for potable water supplies.

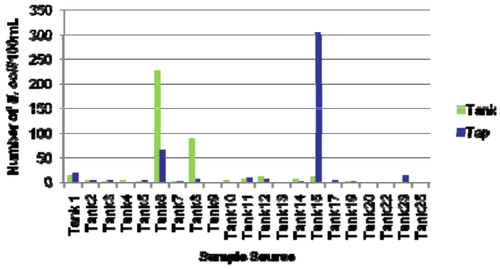

Figure 2: Comparison of E. coli numbers detected in rainwater tanks and the respective household tap in dwellings where rainwater is supplied to inside the house.

At the time of this paper the initial 49 collected rainwater tank samples had been tested for the presence of *Salmonella* and *Giardia*. These preliminary results have shown that *Salmonella* was detected in only one of the 49 rainwater tank samples. In contrast, 7 of the 49 rainwater tank samples tested gave a presumptive positive for *Giardia lamblia* (Table 3).

Table 3: Detection of Salmonella spp. and Giardia lamblia in rainwater tank samples

Microorganisms	**Rainwater** (n=49)	**Possum faeces** (n=25)	**Bird faeces** (n=11)
Salmonella spp.	1	0	1
Giardia lamblia	7	ND	ND

Further testing of the rainwater tanks samples is still needed. In a preliminary survey of rainwater tanks in southeast Queensland, Ahmed et al. (2008) demonstrated that there can be a range of microbial pathogens present in RHRW. Using PCR and pathogen specific primers they were able to detect the presence of DNA for *Salmonella*, *Campylobacter*, *Aeromonas*, *Legionella* and *Giardia.* They determined that as many as 41% of the tanks tested positive for microbial pathogen DNA. Once completed, this larger survey should enable the determination of the prevalence of pathogens in rainwater tanks and give an indication on the potential sources of the pathogens.

Based on the outcomes of the initial survey in the current reported study, it is intended to undertake a time series of sampling from a smaller number of selected tanks taken from the larger preliminary survey group. This smaller group of rainwater tanks will be selected by being classed either as a high or low risk of being contaminated with microbial pathogens. It is anticipated that the long term survey will provide valuable information relating to any changes in the microbial status of each tank and any resulting changes in the relative health risk from pathogens. It is expected that this will assist in the development of effective management plans for the use of rainwater tanks in south east Queensland.

Sources of Pathogen in Roof Harvested Rainwater

At the time of this paper, the faecal samples are still being processed to determine the potential presence of the microbial pathogens listed in Table 1. Of the results obtained to-date, *Salmonella* has been detected in one of the bird faecal samples not in an of the possum faecal samples tested. The possum and bird faecal samples are still to be tested for *Giardia.*

More than 300 *E. coli* and 200 enterococci samples have been collected from the range of animals sampled. These strains will be compared with the isolated from the rainwater tanks to determine if any of these animals and birds is a significant source of faecal microorganisms present in RHRW.

Survival Experiment

An initial survival experiment has demonstrated that *E. coli* and *Salmonella* have limited survival in RHRW and that the physical location of the tank in relation to exposure to sunlight and shade conditions may influence the decay rate (Figure 3). The results indicate that *E. coli* can have an increased survival in the rainwater tank if it is kept cooler by being located in shaded conditions. More research is currently underway to test a wider range of faecal microorganisms and zoonotic pathogens along with the impact of additional conditions in the rainwater tank such as seasonal impacts, the influence of sediment, regular or irregular flushing of the tank, and the role of non-pathogenic microorganisms.

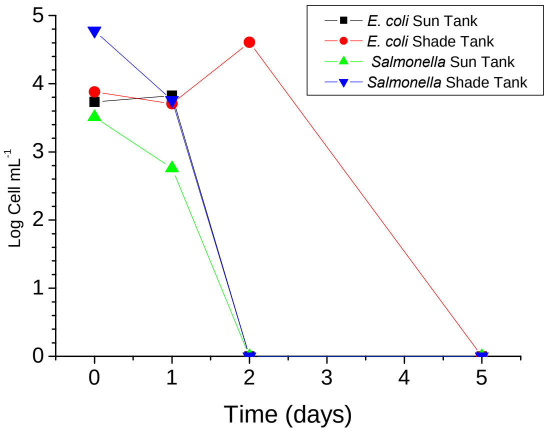

Figure 3: Survival of E. coli and Salmonella cells in rainwater tanks located in full sun or in shade

CONCLUSION

The research to-date is suggesting that the microbial quality of RHRW may not be as good as previously believed with faecal indicator microorganisms being detected in greater than 50% of the rainwater tanks surveyed. Further testing will demonstrate if there are also microbial pathogens in any of these collected samples and if there is any correlation between indicator microorganisms and pathogens. The outcomes of the preliminary survival experiment suggest that the placement of rainwater tanks in shaded locations may enable pathogens to survive longer than in tanks exposed to full sunlight.

The detailed information being obtained through tis research will allow a more detailed and accurate health risk assessment to be undertaken to determine of RHRW can be used for a wider range of uses within households than currently permitted or if the current restricted uses should remain.

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