Coliforms, biofilms, microbial diversity and the quality of roof-harvested rainwater

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Abstract

Previous studies of the bacterial quality of roof-harvested rainwater have been confined to either detection of specific pathogens and faecal indicator organisms, or epidemiological studies of health effects associated with consumption of stored rainwater. As yet however, a general consensus on tank-water quality has not been reached. In this paper it is proposed that achieving reliable microbial quality assessment will require a more detailed understanding of the true microbial diversity of rainwater harvesting systems and microbial processes at work within the tank environment. The paper details the preliminary findings of an investigation into the microbial diversity of rainwater harvesting systems currently underway in eastern Australia. The study includes rainwater tanks at both urban and rural locations, from which a wide variety of micro-organisms have been cultured and identified. The results have indicated considerable variation in the level of contamination and bacterial composition between sites, and over time. In all cases free living environmental species were predominant, and evidence of faecal contamination was minimal by comparison. The key outcomes of a parallel pilot study into the formation of biofilms in rainwater tanks, and their potential impact on water quality, is also presented, revealing a possible relationship between the extent of biofilm development and the quality of water delivered. Further support for the bio-remedial capacity of biofilms was provided by in-vitro experiments on biofilms which confirm the capacity of an established biofilm to remove lead from the main water body. It was concluded that a greater understanding of the microbial ecology, and the factors influencing microbial composition of the rainwater tank, will be critical to the optimum utilization of domestic rainwater harvesting as part of an integrated solution to water supply crises faced by many of Australia's major urban centres.

Introduction

The increasing demand of growing populations and ongoing drought conditions in catchment areas has left many of Australia's major urban centres facing water supply shortages, with no foreseeable improvements into the future. The implementation of rainwater harvesting on domestic allotments has emerged as a viable solution to these supply crises, with potential to decrease demand on municipal supplies, mitigate stormwater discharge and reduce infrastructure costs for new housing developments. Nonetheless, concerns exist over the quality of rainwater harvested in the urban setting.

Current knowledge of the microbial characteristics of rainwater harvesting systems is limited to that provided by studies involving attempts to detect specific pathogens in rainwater storages, or monitoring for the presence of faecal indicator organisms (coliforms). The results of these studies, reviewed by Gould (1999) and Lye (2002) have indicated considerable variation in tank water quality. In several cases rainwater storages have been linked to isolated disease outbreaks including *Campylobacter* enteritis (Merrit et al. 1999;Palmer et al. 1983)

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and Salmonellosis (Taylor et al. 2000), while in other studies not associated with any outbreak of illness, pathogens have been detected (Lye 1992;Simmons et al. 2001;Tuffley and Holbeche 1980), fuelling perceptions that rainwater cisterns generally present a significant potential health risk. In contrast to this, larger scale epidemiological investigations have revealed that consumption of rainwater presented no increased risk of gastro-intestinal illness by comparison with chlorinated and filtered mains water (Heyworth et al. 1998;Heyworth 2001), and no correlation between indicator presence and adverse health affects (Strauss et al. 2001). In essence, a clear consensus on tank water quality has not been reached. Nor has our understanding of the underlying reasons for variations in quality between sites been greatly advanced, or how to predict such variations and accommodate them within the framework of an effective rainwater usage strategy.

No doubt, microbial presence is central to the water quality issue, however the impact may not be exclusively adversarial. A recent investigation of the microbial composition of roof run-off (Evans et al. 2006) concluded that the vast majority of the bacterial load entering the tank comprised environmental organisms of non-faecal origin. Coombes et al (2000) had previously observed improvements in both the microbial and chemical quality of rainwater with passage through the collection system, which were attributed to naturally occurring processes within the tank described collectively as an 'incidental treatment train'. In a review of factors affecting water quality Spinks et al (2003) emphasized the lack of knowledge of such processes, and of process understanding in general with regard to rainwater harvesting systems.

In the context of these findings, the presence and interactions of non-pathogenic environmental species may be of significance to the quality of water delivered by rainwater tanks. Of particular interest is the formation and activity of biofilm communities and their potential to remediate the tank by removal of heavy metals and other undesirable contaminants.

This report focuses on the findings of a preliminary investigation into the microbial diversity of rainwater harvesting systems, with an assessment of bacterial counts and species profiles of cold and hot water samples from an urban and a rural rainwater tank in eastern Australia. In addition, the key outcomes of two separate studies are also presented, one a pilot study into biofilm presence and character in two rainwater tanks in the Newcastle region of eastern Australia, and the other an in-vitro study of the potential bio-remedial capacity of biofilms.

The paper therefore represents the initial phase of an effort to understand more fully the microbial complexity of rainwater harvesting systems, and the importance of the diversity present to processes within the tank that impact directly on the quality of water delivered.

Experimental Detail

Microbial Diversity of 2 Rainwater Tanks

Tank and site description: The study involved one tank located in Newcastle city and another at rural Gulgong, approximately 250km inland of Newcastle on the east coast of Australia. The Newcastle site has duel 2.2kL galvanized iron tanks in series, fitted with a mains water trickle top-up system. The Gulgong tank is a 10kL aquaplate tank without any mains water connection. Both tanks are fed by a galvanized iron roof and gutter catchment, and at both sites water is pumped to the house and delivered as either cold water, or hot via a water heating system. The hot water systems of the urban and rural sites were set at 60°C and 50°C

respectively. At the Newcastle site trees overhang part of the roof catchment, and at Gulgong a large tree is found next to the house but does not directly overhang the roof catchment. No routine maintenance of the tank or roof/gutter catchment is undertaken at either site.

Sample collection and analysis: Samples were collected from the tap outlets in the kitchen at both sites. A total of 4 cold and 4 hot water samples were collected at Newcastle, and 5 cold and 4 hot samples from Gulgong, over a 12 month period.

E. coli and total coliform counts were determined via filtration and incubation on Millipore 'm-coli blue' media as per the manufacturer's instructions. All other bacterial determinations were made by direct plating onto nutrient agar, while fungal species were recovered by plating onto Sabouraud's dextrose agar. Initial differentiation of the species recovered was made by microscopic examination of the morphological characteristics of separate colony isolates, with further distinctions made via a combination of simple biochemical assays, gram staining and microscopic examination of cell morphology. DNA extraction of pure colony isolates and subsequent PCR and sequence analysis were applied for species identification.

Biofilm Development and Characterization in 2 Rainwater Tanks

Location and tank descriptions: This study involved rainwater tanks located on properties in Newcastle on the east coast of Australia. One was located in an outer suburb (tank A), and the other at an inner city suburb (tank B). Both tanks were situated above ground. Tank A was a 14 year old galvanized iron tank with no history of maintenance, and Tank B a 3 year old plastic tank.

Sample collection and analysis: For the determination of tank water bacterial counts, samples were collected both from within the tank, by submersing a sterile container 30cm below the water surface, as well as from the tap outlet connected directly to the tank. Determination of the *E. coli* and heterotrophic plate counts were made by filtration and incubation on Millipore 'm-coli blue' and 'heterotrophic plate count' media respectively, as per the manufacturer's instructions.

For the characterization of biofilm species, the inner wall of each tank was scraped with a sterile cotton swab approximately 30cm below the water line, with 3 replicates of each sample taken. The swab tips were placed in sterile milli-Q water and vortexed to release the biofilm from the tip. The suspension was serially diluted and plated onto nutrient agar media. Following incubation, colony isolates were differentiated in the manner described above (refer microbial diversity study) with each colony type subsequently identified by DNA sequence analysis.

Lead Uptake by an In-vitro Cultivated Biofilm

E. coli biofilms were cultivated in-vitro by inoculating the wells of perspex tissue culture micro-plates with a stock bacterial culture. The micro-plate wells were filled with a nutrient broth and incubated for 72 hours. Following incubation the broth was replaced with a solution of sterile milli-Q water spiked with lead (Pb^{2+}) to a concentration of 100ppb. Using high resolution ICP-MS, the Pb^{2+} concentration of the solution in each experimental and each control (no *E. coli* culture added) well, was determined at time intervals of 0, 5, 10, and 20 hours following addition of the solution.

Results

Microbial Diversity of 2 Rainwater Tanks

This investigation aimed to focus on the extent of microbial diversity present in rainwater tanks, differences between tanks with regard to the profile of bacterial species present, the relative contribution of faecal coliform organisms to the overall contaminant load, and the impact of hot water systems on the contaminant load.

Microbial analyses of samples taken from the Newcastle and Gulgong tanks revealed the presence of a wide array of species, with a total of 104 different bacterial species identified among the colony isolates recovered from the combined samples of the two tanks. The average level of contamination was found to be more than 6-fold greater in the cold water samples of the Gulgong tank compared with the Newcastle tank, with substantial variance evident in both cases (table 1). Despite the significantly greater contaminant load, microbial diversity in the Gulgong cold water was found to be 25-30% less than that of the Newcastle cold water, in terms of both the total number of species identified, and the mean number of species per sample (table 1).

Table 1: *Mean bacterial plate counts and species diversity derived from 4 cold and hot water samples from Newcastle, and 5 cold and 4 hot water samples from the Gulgong tank.*

Tank	**Newcastle**		**Gulgong**	
Sample type	Cold	Hot	Cold	Hot
Total no. of species identified	64	20	49	39
mean no. species/sample	23±3.4	8±1.2	16±2.3	14±4.3
Mean plate count (cfu/mL)	366±153	17±2.6	2263±1070	94±31

A more specific comparison of the microbial profile of the two tanks is provided in figure 1, in which the average count per cold water sample of the most abundant bacterial genera recovered from each tank is recorded. Only three of the nine most abundant genera (*Pseudomonas* spp., *Serratia* spp., and *Bacillus* spp.) were prominent at both sites. The relative contributions of these organisms to the overall bacterial load varied considerably between the two tanks, with *Pseudomonas* spp. and *Serratia* spp. clearly the dominant genera in the Gulgong tank, but only moderately abundant in the Newcastle tank. Strikingly, the two most abundant genera in the Newcastle samples (*Herbaspirillum* spp., *Sphingomonas* spp.) were not recorded at all in the Gulgong samples. While fungi were not characterized to individual genera level, the collective fungal counts of the two tanks have been included in figure 1, revealing a10-fold greater average contribution of fungi to the contaminant load of the Newcastle tank than to the Gulgong tank. It has been confirmed through DNA sequence analysis that the as yet unidentified bacteria observed to be prominent at the two sites (figure 1) are not the same organism.

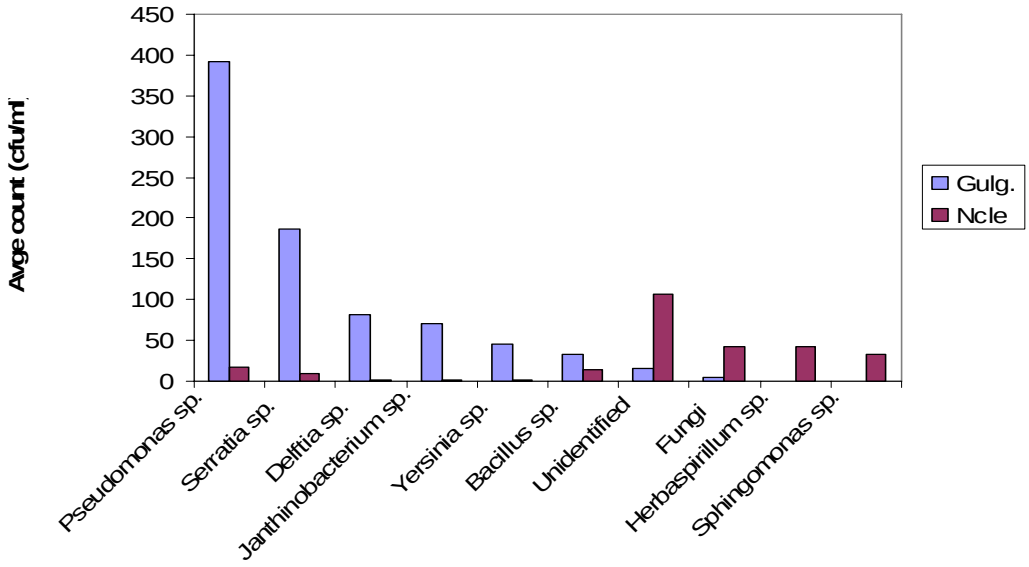

Figure 1: *Profile of the Newcastle and Gulgong tanks based on average counts per sample of the most abundant genera recovered from each.*

As faecal contamination, due to the activities of birds and/or other animals on the catchment surface, has generally been considered the major source of bacterial contamination of tank water, it is of some significance that none of the recognized faecal coliform species are among the most abundant found in these storage systems. A comparison of the presence of standard quality indicator organisms, and their abundance in the context of overall contaminant levels, for both cold and hot water samples is detailed in table 2.

Coliform organisms were generally found to be present in cold water samples from these sites with total coliform detected in all samples, faecal coliform in 77% and *E. coli* present in 50% of samples (table 2). *Enterococci* were the least frequently encountered enteric group, recorded in only 23% of cold water samples. Despite their general presence the coliform groups were found to comprise a very minor proportion of the total bacterial load of the cold water samples with total coliform representing < 4% on average, faecal coliform < 1% and *E. coli* and *Enterococci* < 0.1%.

By comparison with the cold water these organisms were less frequently encountered in the hot water samples with total coliform and faecal coliform recorded in 60% fewer samples and *E. coli* not recorded in any hot water samples (table 2). While *Enterococci* appear to have been found in a greater proportion of hot water samples (28% compared with 23% of cold samples), they were in fact found in the same number of hot and cold samples and the higher percentage frequency is simply a function of there being fewer hot water samples overall.

Table 2: *The percentage of samples positive for 4 groups of indicator organisms, and the average proportion of total plate count represented by each, in the combined cold and hot water samples from the Newcastle and Gulgong tanks.*

Sample type	Cold		Hot	
	Presence (% of samples)	Avge % of plate count	Presence (%of samples)	Avge % of plate count
E.coli	50	0.06±0.03	0	0
Enterococci	23	0.05±0.03	28	0.62±0.41
Faecal col.	77	0.52±0.27	28	0.62±0.41
Total col.	100	3.77±2.08	43	3.36±2.21

The impact of hot water systems is evident in table 1 where contaminant levels are shown to be reduced on average by approximately 95% in hot water samples by comparison with the cold water sample average at both sites. As table 2 indicates the proportion of total plate count comprised by total and faecal coliforms remained essentially constant between the cold and hot samples, thus they too were reduced in number by approximately 95%. While *E. coli* appears to have been completely eliminated by passage through the hot water systems, *Enterococci* comprised a slightly higher proportion of the hot water samples than they did in the cold water (table 2). However, balanced against the overall reduction in counts, this still represents a 50% reduction in *Enterococcus* counts. It should be noted that both *Enterococci* and faecal coliform were only found in the hot water samples from the Gulgong site where the hot water temperature was set at a moderate 50°C, and not in the Newcastle hot water set at 60°C.

The apparently greater resilience of *Enterococci* to the elevated temperature of the hot water systems, by comparison with the other coliform groups, is consistent with the overall trend observed in the comparative composition of gram positive and gram negative bacteria in the cold and hot water samples. Figure 2 reveals that gram negative bacteria were predominant in cold water samples from both sites, representing 73% of the contaminant load at Newcastle and 89% of organisms recovered from the Gulgong tank. However, passage through the hot water system was seen to have a dramatic impact on the gram negative population in both cases, with the relative abundance of this group reduced to 16% of the total load at Newcastle and just 7% of organisms present in the Gulgong hot water. Indeed the 4 most abundant genera in the Gulgong cold water (*Pseudomonas, Serratia, Delftia* and *Janthinobacterium*), and the 2 most abundant at Newcastle (*Herbaspirillum* and *Sphingomonas*), are all gram negative bacteria of which none have been recorded in the hot water samples at either site. While *Enterococci* and other gram positive cocci have prevailed in the 50°C Gulgong hot water, the 60°C Newcastle hot water samples were dominated by spore forming bacilli. Overall the hot water systems have been shown to produce a log reduction in total cell counts, delivering water that is virtually free of coliforms and associated gram negative bacteria.

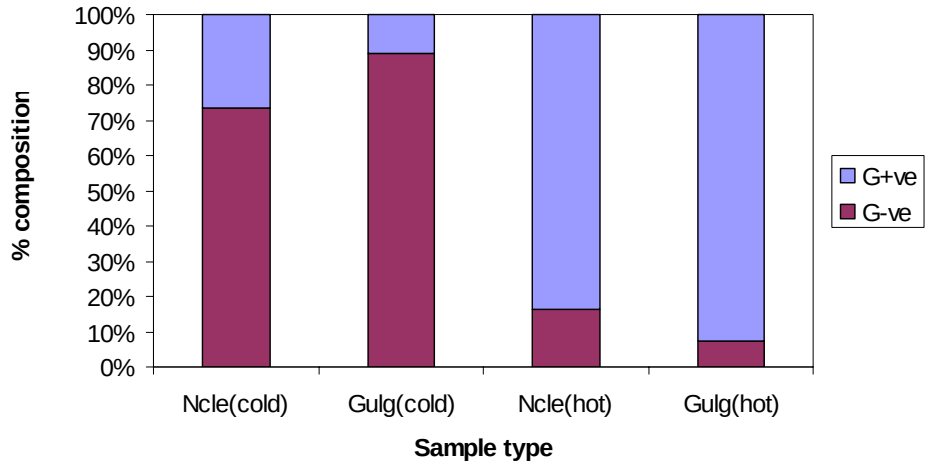

Figure 2: *Comparative composition of gram negative and gram positive bacteria in cold and hot water samples from the Newcastle and Gulgong tanks.*

Biofilm development and characterization in 2 rainwater tanks

The rainwater tank biofilm study represented a pilot study into the presence, character and potential water quality impact of biofilms in rainwater harvesting systems, and involved two tanks at separate locations in Newcastle as described previously (refer experimental details).

A qualitative assessment of the extent of biofilm formation in the experimental tanks was made by visual inspection of the interior walls. The most advanced biofilm growth was observed in tank A where a heavy slime layer exhibiting extensive clumping was readily apparent, while tank B revealed a moderate and comparatively uniform slime film around the entire interior.

An assessment of the general water quality of tanks A and B was made via water samples taken from both the main water body inside the tank and from the tap outlet. These samples were analysed for both *E. coli* and heterotrophic plate count (HPC) (table 4). While HPC's were observed to be higher in tank A, the highest *E. coli* counts were recorded for the tank B samples. For both parameters, the greatest improvements to water quality in terms of reduction in bacterial counts at the outlet, compared with those in the tank water column, were observed for tank A, the tank with the most extensive biofilm formation.

Table 4: *Comparison of the* E. coli *and heterotrophic plate counts from tanks A and B, for samples taken from just below the water surface within the tank, and from the tap outlet. The % improvement refers to the reduction in counts from the tank interior to the tap outlet.*

Tank	Sample type	Bacterial count	
		E. coli (cfu/100ml)	**HPC** (cfu/mL)
A	Tank water column	48	610
	Tap outlet	29	390
	Improvement	**34%**	**36%**
B	Tank water column	70	200
	Tap outlet	60	160
	Improvement	**14%**	**20%**

For both tanks, samples of the tank wall biofilm were analysed to determine bacterial species composition. A total of 13 different species of bacteria were recovered and identified from the biofilm samples, with 3 of these common to both tanks (figure 3). All 3 were *Bacillus* sp. suggesting that members of this genus are both widely distributed and primary biofilm colonizers. While *E. coli* was evidently incorporated into the biofilm of tank A, it is unclear whether this is in any way related to the lower counts observed in the water samples for this tank compared with those of tank B.

PCR amplification and subsequent DNA sequencing was not carried out for the water column samples. However, the colony and cell morphology characteristics of the biofilm species isolates were examined microscopically and compared to colony isolates recovered from the tank water samples of these tanks, allowing the composition of species in the biofilm to be compared with that of the main water body. This analysis revealed that the bacterial species found in the biofilm represented only a fraction of the total bacteria present as planktonic cells in the main water column. In tank A biofilm species were found to comprise <1% of the bacterial load in the main water body, while none of the biofilm species were recovered from the water samples of tank B.

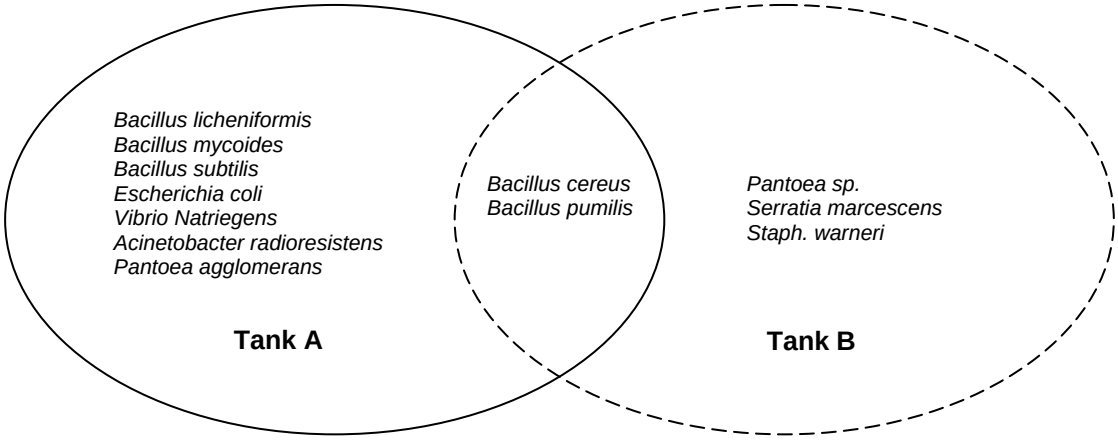

Figure 3: *Comparison of the biofilm composition of tanks A and B.*

Lead uptake by in-vitro cultivated biofilm

Finally we consider the uptake of lead (Pb^{2+}) from the adjacent water body by an in-vitro cultivated *E .coli* biofilm (refer experimental details). Figure 4 traces changes in the Pb^{2+} concentration over a 20 hour period in spiked (100ppb lead) solutions placed in perspex wells containing a 72 hour *E. coli* biofilm, compared with changes in control wells with no biofilm. While reductions in the Pb^{2+} concentration were observed in both the biofilm and control solutions over the first 5 hours, no further change was observed for the control samples at subsequent time points. In contrast, Pb^{2+} removal was observed to continue in the biofilm wells throughout the entire experimental period. The initial reduction in Pb^{2+} in the control wells was most likely due to adsorption to the perspex of the wells, which appears to have reached equilibrium or saturation during the first 5 hours. Continuing removal of Pb^{2+} from the solution beyond this time in the biofilm wells must therefore be attributed to the presence of the biofilm. Ultimately, Pb^{2+} concentration was reduced by 40% on average in the biofilm wells over 20 hours, compared with less than 20% reduction in the control wells, all of which occurred in the initial 5-hour period.

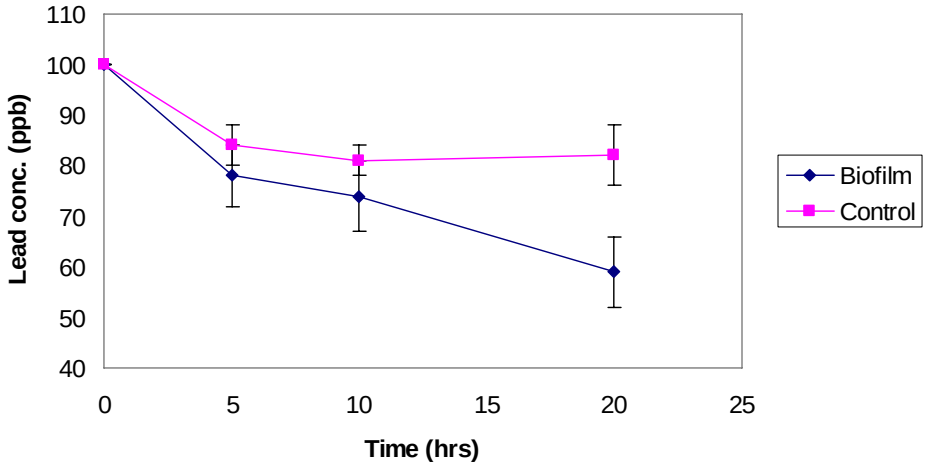

Figure 4: *Changes in the lead concentration of solutions in perspex wells containing a 72 hr* E.coli *biofilm, compared with changes in wells containing no biofilm (control). Initial lead concentration was 100ppb in both cases.*

Discussion

Numerous features of the data presented are of potential significance to our perception and understanding of the quality of water delivered by rainwater harvesting systems, and therefore provide several points of discussion. These include the scope of diversity present; evidence of minimal faecal contamination; absence of known waterborne pathogens; temporal variance in the overall level of contamination; regional variation in the profile of the contaminant load; the impact of hot water systems; and the potential importance of biofilm communities.

The wide array of species encountered in this study highlights the potential complexity of the rainwater tank environment as a microbial habitat. The presence of such diversity also serves as a reminder of the superficial level of our current understanding of potential microbial interactions within the tank and the manner in which they might impact on the quality of water delivered. Limiting our microbial analysis of tank water to simple monitoring of coliform indicators or screening for specific pathogens, is adequate for identifying quality problems associated with faecal contamination as they arise. However, such measures provide little insight into either the underlying reasons for the conflicting reports on tank water quality found in the literature, or the true nature of the bulk of the contaminant load which appears increasingly to be non-faecal in origin.

Indeed the apparently minor contribution of faecal contamination to the overall bacterial load observed in these tanks is consistent with the roof run-off quality findings of Evans et al (2006). In that investigation faecal coliform counts were similarly low by comparison, and variations over time did not correlate with the overall bacterial load which appeared to be strongly influenced by wind velocity and direction, leading to the conclusion that airborne environmental micro-organisms provided the major contribution to roof water contamination.

Nonetheless, the mere presence of *E. coli* and other faecal coliform species in more than 50% of the samples analysed would render the tanks in this study non-compliant with Australian drinking water guidelines (NHMRC 2004). However, the residents at these sites have reported no adverse health affects from consumption of this water, supporting conjecture that the strict guidelines applied to town water supplies may be inappropriate for domestic rainwater storages (Fujioka et al. 1991;Krishna 1993;Ruskin et al. 1990). Furthermore, assessment of the bacteriological quality of water from these tanks would vary depending upon the particular indicator chosen for monitoring purposes. Superimposed on this is the knowledge that pathogen presence does not necessarily correlate with indicator presence (Savill et al. 2001;Schetes et al. 2005), raising the question of just how accurately indicators represent health risk.

Of potentially greater interest and significance are the distinct differences observed in the microbial composition of the urban and rural tanks, and several features of the data bear consideration. Firstly, the general disparity in the extent of contamination observed between these tanks appears indicative of differences in either the surrounding site environment, or of conditions within the tank environment. Secondly, it is apparent that the diversity of species present does not vary proportionately with overall bacterial counts and is therefore not necessarily congruous with the level of contamination. A more evenly distributed population may be indicative of a more competitive and stable resident microbial community in the Newcastle tank, with the abundance of all organisms kept in check, preventing the proliferation of transient contaminants.

It is also apparent, based on the degree of variance around the mean values observed for plate counts of cold water samples (table 1), that considerable fluctuations in bacterial counts occur over time. Such variance may reflect the influence of several factors including, seasonal fluctuations in the atmospheric concentrations of micro-organisms deposited on the catchment surface; wind patterns and temperature conditions during the intervening period between rainfall events; or bacterial 'die-off' as a function of the time interval between sampling and the most recent rainfall event.

Certainly, the varying survivability of species within the tank environment would impact on the relative abundance of species present over time. So differences in the time interval between sampling and the most recent rainfall event may result in variations between microbial profiles of two separate tanks, as a function of the differential decay rates of the species involved. However it is unlikely that highly abundant species at one site would not be encountered at all at another site if contaminant sources and inputs were similar. Thus the differences in the profiles of the urban and rural tanks observed in figure 1, more likely reflect variations in contaminant inputs on a regional scale.

The impact of hot water systems on the bacterial composition of water delivered from these tanks is of significance with regard to the potential scope of domestic use of rainwater. Certainly, maximising the use of rainwater to include use for showering/bathing etc is necessary in order to derive maximum benefits in terms of water savings (Coombes et al. 2003). The log reduction in the overall contaminant load following passage through the hot water system resulted in water of quality suitable for bathing purposes, consistent with the findings of Spinks et al (2006), even for the moderate temperature setting (50°C) of the rural hot water system.

Although it is pertinent to consider the potential opportunistic pathogenicity of the environmental bacteria that comprise the majority of the contaminant load, it should be noted that while some of the more prominent genera found in these tanks (eg. *Pseudomonas* and *Yersinia*) contain species recognized as water-borne pathogens, none of the pathogenic species were found to be present in this study. In fact the potentially beneficial impacts of these environmental organisms on tank water quality are likely to be of far greater significance in the long term. Clearly environmental species, better adapted to lower temperature relatively nutrient poor conditions than enteric pathogens adapted to the warm, nutrient rich digestive tracts of animals, are likely to survive the competitive tank environment at the expense of these pathogenic species. More important is their potential role in processes contributing to the 'incidental treatment train', as described by Coombes et al (2000). While the exact nature of such processes remains unclear, they have been linked with the formation and activity of biofilm communities in the tank environment.

With respect to this the results of the biofilm pilot study presented here are significant. The reduction in bacterial counts at the tap outlet by comparison with those near the surface of the tank water column, are consistent with the treatment train effect. In addition, the observation that improvement to quality was greatest in the tank where biofilm development was most advanced, supports the implication of the biofilm in this phenomenon. Furthermore, characterization of the biofilm composition and comparison with the planktonic species in the main water body has revealed the tank to be a somewhat ordered environment, rather than a random microbial soup, with selective partitioning of species between the tank wall biofilm and the water column.

Finally, the in-vitro biofilm model although simplistic, involving a single species biofilm and a single metal contaminant, does confirm the capacity of an active biofilm to remove lead from the surrounding medium. This result has important implications in the context of water quality and health risk associated with rainwater harvesting in the urban environment, especially with regard to concern over lead and other pollutants common to motor vehicle and industrial emissions. It is therefore of more than cursory interest that a number of environmental bacteria have demonstrated a capacity to facilitate removal of such pollutants from solution in other scenarios (Choi et al. 2003;Remoudaki et al. 2003;Salehizadeh and Shojaosadati 2003), and that among them are several *Bacillus* species, found to be prominent biofilm colonizers in the tank biofilm investigation presented in this paper.

The implications of the issues outlined above can be summarized as follows. Evidence of regional variation in the contaminant profile indicates a requirement for greater knowledge of the factors determining microbial composition of run-off. Indeed, recent evidence of the influence of wind on microbial composition suggests the possibility of incorporating knowledge of the site environment, and regional weather patterns, into health risk assessment for rainwater use at specific locations. The continued evidence that faecal contamination of rainwater tanks is generally minimal, and an understanding of the nature of temporal variations in the contaminant load, are both likely to be important factors in the development of appropriate protocols for monitoring and assessing rainwater quality. Importantly, the delivery of harvested rainwater from hot water systems, of microbial quality suitable for bathing purposes, substantially increases the potential for water savings. Finally, with regard to concerns over the chemical quality of rainwater harvested in the urban environment, the presence and potential remedial capacity of biofilms may influence future design strategies and maintenance practices applied to rainwater tanks.

Conclusions

The results presented in this report indicated that rainwater tanks harbour a wide diversity of microbial species, and that the profile or species composition may vary considerably, both spatially (from site to site) and temporally (at the same site). Faecal contamination appeared to be minimal in these tanks in the context of the overall contaminant load, while hot water systems dramatically reduced the level of contamination, delivering water of bacterial quality apparently suitable for bathing. Clearly, distribution of micro-organisms within the tank environment is not random, with distinct partitioning of species between the biofilm and the main water body. Furthermore, the potential of biofilms to chemically remediate tank water by facilitating removal of heavy metals from the water column has been demonstrated.

It seems that a greater understanding of the true diversity of organisms present in rainwater tanks, the factors influencing both regional and temporal variations in composition, and the interactions of both biofilm communities and planktonic species with chemical components of the rainwater, may be critical to the optimization of rainwater harvesting systems and effective utilization of rainwater in the urban environment.

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