# Identifying the Major Influences on the Microbial Composition of Roof Harvested Rainwater and the Implications for Water Quality.

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Perceptions of the quality of roof harvested rainwater remain an impediment to widespread implementation of rainwater tanks on urban allotments. Previous literature reports on roof water quality have given little consideration to the relative significance of airborne environmental micro-organisms to roof catchment contamination and the issue of tank water quality. This paper outlines the findings of a recent study into the influence of weather on roof water contamination conducted at an urban housing development in Newcastle, on the east coast of Australia. Samples of direct roof run-off were collected during a number of separate rainfall events, and microbial counts were matched to climatic data corresponding to each of the monitored events. Roof run-off contamination was found to be under the strong influence of both wind speed and direction. The preliminary findings of an investigation currently underway into the microbial diversity of rainwater harvesting systems are also presented. The results indicate that the composition of organisms present may vary considerably from source to source and throughout the collection system. In all cases, evidence of faecal contamination was found to be negligible. The implications of these findings to the issues of tank water quality, health risk analysis and monitoring protocols are discussed.

*Keywords:* rainwater tanks; roof run-off; water quality; bacterial composition; airborne micro-organisms; climatic variables.

## INTRODUCTION

The implementation of rainwater storage tanks on domestic allotments offers a number of benefits as a potential solution to the ongoing water supply crises experienced by many of Australia's urban centres. Nonetheless, perceptions of water quality and health risk, based on limited process understanding, remain an impediment to their widespread application. Such perceptions arise from speculation that without disinfection and other processes to which mains water is subjected, urban roof water is likely to be of unsuitable quality for human consumption. While caution in relation to health risk is wise, this speculation remains largely unsubstantiated.

In a review of factors affecting water quality in rainwater harvesting systems, Spinks et al (2003) referred to the paucity of knowledge in relation to processes occurring within the tank. Such processes would be central to the concept of a naturally occurring 'treatment train', invoked by Coombes et al (2000) in response to observed improvements in microbial water quality with passage through the collection system. This along with more recent findings outlined in this paper, indicate that adequate resolution of the tank water quality issue may require a more complete understanding of the diversity and major influences on microbial contamination of the rainwater tank.

In general, microbial water quality assessment is focussed on faecal contamination of the water source. Monitoring involves common enteric organisms as indicators of such contamination and hence, the possible presence of other species pathogenic to humans. As such, concerns over the microbial quality of tank water have largely been focussed on the accessibility of the roof catchment surface to birds, insects, small mammals and reptiles, and subsequent introduction of pathogenic organisms to the storage system through faecal deposits, insect breeding and the decay of dead organisms and other organic debris.

Certainly, a number of studies, reviewed by Gould(1999) and Lye(2002), have identified various pathogens including *Salmonella*, *Shigella*, *Vibrio*, *Clostridium*, *Campylobacter, Cryptosporidium* and *Giardia* spp. in tank water samples. In contrast with these examples, others have reported roof-harvested and tank-stored rainwater to be of acceptable quality for drinking and cooking purposes (Dillaha and Zolan 1985), presenting no increased risk of gastro-intestinal illness on consumption when compared with chlorinated and filtered public mains water (Heyworth 2001). Thus a clear consensus on the quality and health risk associated with roof collected rainwater has not been reached.

In addressing this issue, it should be recognised that from a microbiological perspective, two separate modes of contamination of the roof catchment are likely: either via the direct activities of insects, birds and small mammals as outlined above, or by atmospheric deposition of environmental organisms. To date, reports on roof water quality have given little direct consideration to airborne micro-organisms, despite general acknowledgement of the likely impact of atmospheric pollutants on the chemical quality of harvested rainwater.

Numerous studies of the chemical composition of urban rainwater and roof run-off (Bridgman 1992;Bucheli et al. 1998;Forster 1998;Forster 1999;Garnaud et al. 1999;Loye-Pilot and Morelli 1988;Willey et al. 1988;Zhong et al. 2001), have demonstrated relationships between concentrations of chemical contaminants and proximity to contaminant sources (emissions), weather patterns, and atmospheric transport and deposition. Furthermore, aerobiological studies reviewed by Lighthart (2000) and Jones and Harrison (2004), have repeatedly demonstrated seasonal and meteorological influences on atmospheric concentrations of bacteria and fungal spores, which have been correlated for certain species, with the incidence of allergic and infectious outbreak (Brouqui et al. 2004;Corden and Millington 2001;Hawker et al. 1998;Tissot-Dupont et al. 2004). Nonetheless, the potential significance of similar processes to the bacterial composition of roof run-off has not been widely explored in the published literature.

The question arises then - do airborne environmental micro-organisms represent a significant proportion of the bacterial load of roof harvested rainwater? If so, of what relevance are they to tank water quality and associated issues? Such questions provided the impetus for a recent study (Evans et al., 2005) conducted at an urban housing development site in Newcastle, which represents a preliminary examination of the influence of weather patterns on, and the relative contribution of airborne organisms to, the microbial composition of roof harvested rainwater. The investigation was based on the rationale that variations in the bacterial composition from one rain event to the next would likely reflect the influence of weather, if atmospheric deposition is significant. Conversely, if the direct activity of animals is the major contributor, the influence of weather should be less apparent. Preliminary data from a separate investigation of the microbial diversity of rainwater harvesting systems, currently underway at the University of Newcastle, is also of relevance and will also be discussed. The findings of both studies and their implications are of interest to the future assessment and monitoring of tank water quality.

## METHODS

*The influence of weather on the microbial composition of roof water:*

Roof run-off was collected directly from the catchment system during 11 separate rainfall events and analysed for several microbial parameters and inorganic ions. Microbial counts and ionic concentrations were matched to climatic data corresponding to both the dry intervals antecedent to each event, and the storm events themselves. In terms of the microbial analyses, samples were examined for heterotrophic plate count (HPC) as a measure of total bacterial load, coliform counts as a measure of likely faecal contamination, and *Pseudomonas* spp counts. As a genus of widespread environmental organisms, *Pseudomonas* counts allowed fluctuations in a single group of organisms to be monitored in comparison to both climatic variables and total bacterial load. Analyses were conducted using standard filtration, plating and incubation protocols.

For each storm event, both wind velocity and direction were ascertained from meteorological records. For the dry period antecedent to each monitored rain event, the predominant wind direction, and the average wind speed were also determined.

*Microbial diversity of the rainwater harvesting system:*

Samples were collected from six different sources - an open concrete pond (OP), a swab from a section of the zinc sheeting of a roof catchment surface (RS), and the cold and hot water outlets of rainwater tanks at an urban (UT) and a rural (RT) location. The heterotrophic count was determined by spread plate onto nutrient agar and incubation at 35 degrees, while faecal coliform counts were determined by filtration onto preferential media and incubation at 37 degrees.

## RESULTS AND DISCUSSION

In the context of the issues raised, a number of key findings have emerged from the roof water study. Firstly, mean counts indicated that on average faecal coliforms represented a very minor portion (<0.1%) of the total bacterial count. Secondly, regression analysis revealed HPC and *Pseudomonas* counts to be correlated with one another, however no correlation was observed between coliforms and either HPC or *Pseudomonas*. These findings together indicate that faecal contamination of the roof catchment was minimal, and that throughout the sampling period, coliform numbers were under influences independent of those responsible for the bulk of the overall bacterial load.

The most striking outcomes were those revealing the influence of wind on microbial counts. When the dry intervals antecedent to each rain event were considered, it was found that events could be separated into those dominated by NW wind regimes and those dominated by SE regimes. Likewise, prevailing storm winds were separated broadly into those of either northerly or southerly origin. On this basis *Pseudomonas* counts were found to be heavily influenced by winds from the N-NW (Figure 1).

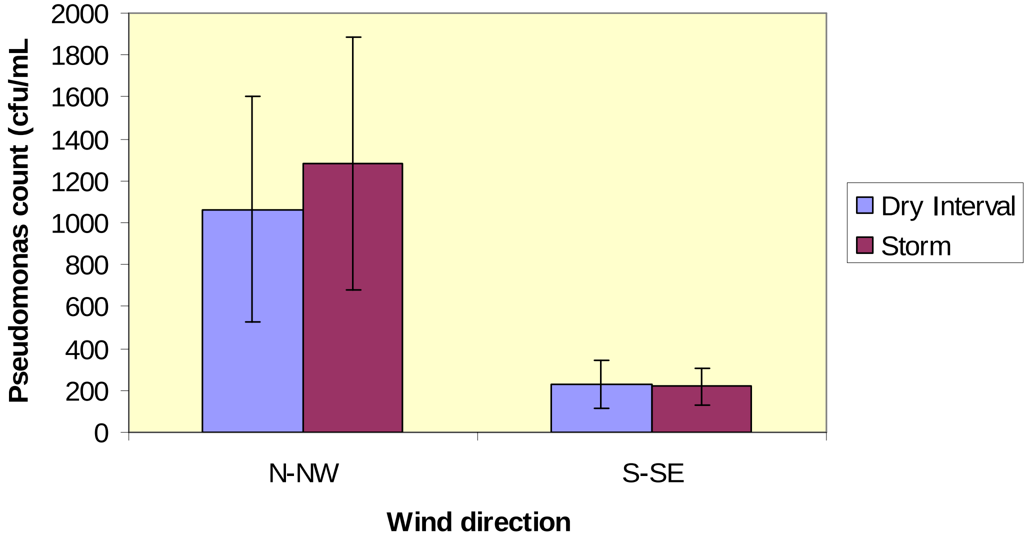

Figure 1: Caption

By comparison HPC appeared to be determined more by wind velocity. When events were grouped irrespective of direction into those of wind speed above or below overall mean wind speed, mean HPC for higher velocity events were found to be approximately 2.5 times greater than for low velocity events, whether considering storm winds or antecedent dry interval winds (Figure 2).

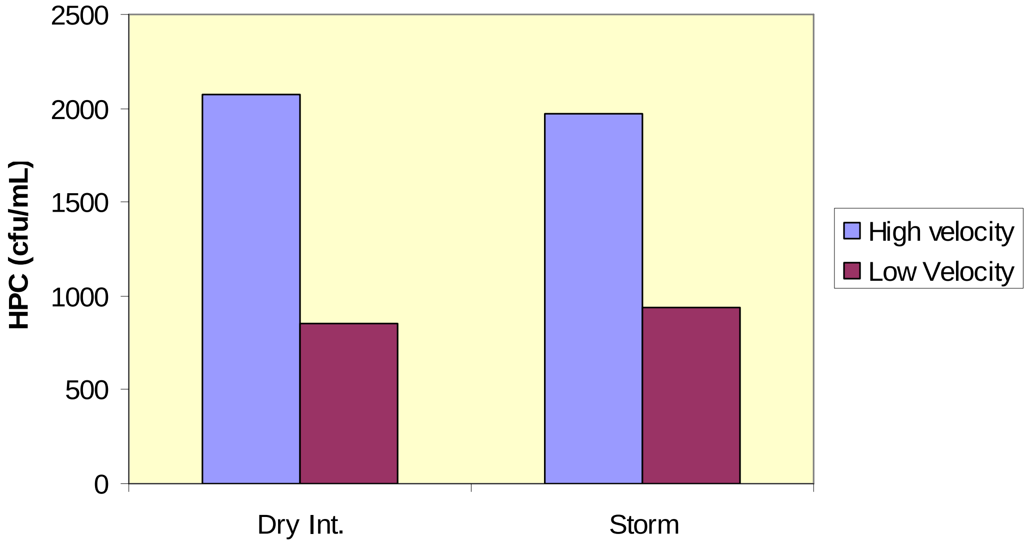

Figure 2: Caption

While faecal coliform counts did not bear any relationship to either wind speed or direction, total coliform counts were observed to be elevated for events where winds with a westerly component operated either prior to or during the rain event. It should be remembered that while faecal coliforms are potentially enteric organisms that are likely to be of faecal origin, the total coliform count may comprise environmental organisms not necessarily common to the digestive tracts of vertebrate animals (Prescott et al. 2002). Thus the westerly wind influence on the total coliform count may reflect transport of the Coliform group of bacteria present in soil from sources inland of the study site.

The broad picture emerging from these results is that the total bacterial load of the roof water, reflected by the HPC, is largely a function of wind velocity, probably due to increased uplift of organisms from source surfaces and more arrivals at the catchment surface per unit time, while the profile of that load is source dependant and therefore determined by wind direction. Essentially, winds of any direction will bring micro-organisms to the catchment surface, however the specific organisms present in the run-off and their relative abundances, are dependant upon the direction of prevailing winds in relation to the location of contaminant sources. Furthermore, a strong relationship was found between HPC and storm wind velocity (Figure 3). This may indicate that a substantial proportion of the bacterial load arrives with the storm front and possibly reflects the relative significance of wet deposition to microbial contamination of roof water.

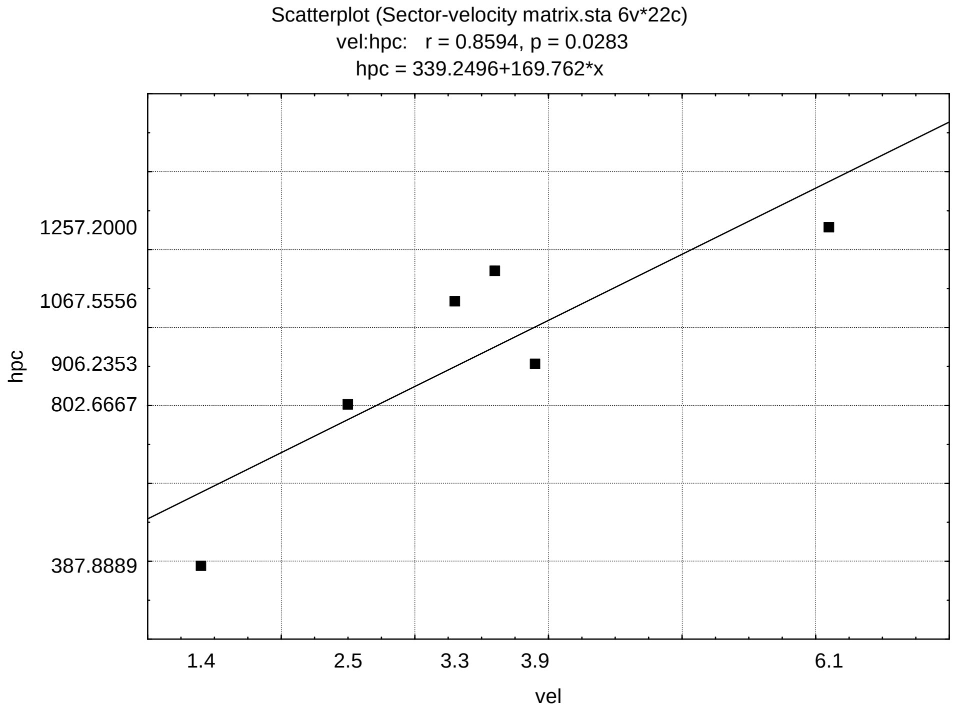

Figure 3: caption

The concept of wet and dry deposition of chemical pollutants is well established. According to Muller (1982), short range dry deposition is most significant for particulates > 2.0µM diameter, while wet deposition is more significant for particulates < 2.0µM. This is due to the propensity of fine particulate aerosols to accumulate in the atmosphere, extending residence time and allowing longer distance transport from point source emissions. The average bacterial cell size is such that they may be associated with aerosols in either category. Thus both scenarios are possible and dry deposition from nearby sources, as well as wet deposition of organisms transported from more distant sources, may both contribute to roof water contamination at any given site.

It is important in assessing these findings to recognize that the microbial counts reported are comparable with contaminant levels found previously in roof water studies (Thomas and Greene 1993;Uba and Aghogho 2000;Yaziz et al. 1989), and also consistent with those found in samples taken from roof fed rainwater tanks (Albrechtsen 2002;Dillaha and Zolan 1985;Simmons et al. 2001). Essentially, the study site does not represent an abnormal case in terms of run-off quality.

In addition, the results of parallel analyses conducted on chemical components of the run-off satisfy logical expectations. Due to the close proximity of the site to the eastern coastline it was expected that $Cl^-$ in the run-off would be derived mainly from sea salt, which appeared to be confirmed by the observed $Na^+$ to $Cl^-$ ratios in the samples and a two-fold greater concentration of $Cl^-$ in samples from events where the predominant winds were SE in origin. Furthermore, the ratios of sea salt to non-sea salt $SO_4^{2-}$ were also observed to be greatest for these same events. Hence, the chemical data appears to validate the reality of the observed relationships between microbial counts and weather patterns.

In dealing with the implications of these findings we might first consider the use of domestically harvested rainwater. Clearly, the greater the number of domestic applications for which rainwater is utilized, the greater the benefits derived in terms of water savings. Ideally, usage should extend beyond garden irrigation and car washing, to internal applications including hot water, laundry and toilet flushing. While quality guidelines, as they apply to mains water, are aimed at provision of drinking water, consumption for drinking and cooking purposes represents a relatively minor proportion of domestic water demand. Thus maximum benefit may still be realised if rainwater is excluded from use for this purpose.

In this case, assessing tank water quality based on traditional indicators of enteric pathogens (whose primary threat arises through ingestion), may be of little relevance. Furthermore, if the vast majority of the bacterial load is of non-faecal origin, then indicators of faecal contamination will reveal little of the likely composition of the microbial contamination. This composition may include environmental organisms of opportunistic pathogenicity, posing a potential health risk via pathways other than ingestion e.g. eye, ear and skin irritations, or through respiratory ailments arising from aerosols.

In this respect fungal species which have hitherto been given scant recognition in relation to rainwater quality, may be of significance. Certainly, atmospheric concentrations of some widely abundant genera have been linked directly to the incidence of respiratory conditions such as asthma (Corden and Millington 2001). It is also conceivable that accumulation of fungal spores on the catchment surface may result in a concentrating effect in the run-off and therefore within the tank. Respiratory infections resulting from introduction of pathogenic species to the internal environment via the water supply have been demonstrated as a result of aerosol formation, particularly in the bathroom environment (Anaissie et al. 2002). This is not to suggest that the likely health risk due to environmental organisms is necessarily substantial, or that the potential risk due to enteric pathogens should be ignored, simply that the full diversity of the bacterial contamination may need to be recognised for thorough assessment of tank water quality.

An interesting practical connotation of comparatively minor faecal contamination is that the bulk of the bacterial contamination is not due to random animal activity. In this sense, a contaminant load comprising atmospherically deposited environmental organisms, determined largely by weather patterns, implies an inherently greater potential for predicting bacterial water quality at a given site. Just as details of consumption, catchment surface area and rainfall patterns are incorporated into models for estimating tank capacity requirements for a site, knowledge of the site environment and regional weather patterns may be incorporated into health risk assessment, and determination of monitoring protocols and appropriate usage at any given site. Of course, practical viability of this approach would require a more detailed knowledge tank micro-diversity and possible use of additional or alternative indicator organisms.

The preliminary data from the microbial diversity study also appears to support the assertions of the preceding discussion, and prompts consideration of the rainwater tank as an ecologically discrete and potentially self regulating environment. Table 1 records the heterotrophic plate count for each of the samples, along with the % distribution of gram positive bacteria, gram negative bacteria, and fungal species within that count. Although determined separately, faecal coliform counts are also presented as a % of the corresponding heterotrophic count for comparative purposes. Table 2 records the number of different species of each category of organism based on examination of colony morphology, cell morphology and gram stain characteristics.

Table 1: Caption

| **Sample Source** | **Plate count** (cfu/mL) | **%of total plate count** Gram +ve | Gram -ve | Fungi | Faecal col. |
|---|---|---|---|---|---|
| OP | 164 | 28 | 65 | 7 | 0.03 |
| RS | 102 | 13 | 0 | 87 | 0 |
| UT(cold) | 294 | 39 | 41 | 20 | 0 |
| UT(hot) | 9 | 78 | 11 | 11 | 0 |
| RT(cold) | 825 | 5 | 95 | 0 | 0.18 |
| RT(hot) | 31 | 95 | 2.5 | 2.5 | 0 |

Table 2: Caption

| **Sample Source** | **Number of species** Gram +ve | Gram -ve | Fungi |
|---|---|---|---|
| OP | 7 | 8 | 5 |
| RS | 2 | 0 | 9 |
| UT(cold) | 12 | 15 | 16 |
| UT(hot) | 2 | 3 | 3 |
| RT(cold) | 3 | 13 | 0 |
| RT(hot) | 8 | 2 | 3 |

The results reveal gram negative bacteria to be abundant in cold water samples, while gram positive bacteria are more prominent in hot water samples. This is likely due to the predominance of spore forming bacilli in this group, which are by virtue of this characteristic, resilient to temperature extremes. Significantly, faecal coliforms, observed in only two samples, were found to represent a negligible portion of the overall bacterial load, reinforcing the findings of the aforementioned roof water study. Conversely, fungal species are comparatively well represented, particularly so in the case of the roof swab where they comprise the bulk of the organisms recovered. Again this is probably due to the capacity of spores to resist desiccation.

It should be noted that all organisms recovered are either strictly or facultatively aerobic, and that the results were obtained without any specific attempts to expand recovery of organisms using preferential media or incubation conditions. The data therefore represents the tip of the iceberg in terms of the true microbial diversity of the rainwater storage system. Nonetheless, this crude analysis does indicate that the balance of organisms may vary considerably from source to source, and across the catchment surface, and that the composition at the outlet will depend upon the conditions and interactions within the storage system.

In this respect the sparse evidence of faecal contamination is not surprising. The lower temperature, relatively nutrient poor and consequently highly competitive tank environment, would no doubt favour environmental organisms over enteric species adapted to the warmer, nutrient rich digestive tracts of animals. Thus, airborne environmental organisms, prominent in roof water, are likely to be important to processes occurring within the tank. While the exact nature of such processes is as yet unclear, they are likely to include biofilm formation and nutrient cycling, and may extend to sequestration of trace metals, breakdown of organic contaminants and competitive exclusion of pathogens. In this respect environmental micro-organisms may have beneficial rather than adverse impacts, and their potential role in regulating tank water quality is worthy of further exploration.

## CONCLUSIONS

1. Atmospherically deposited micro-organisms represent a significant proportion of the microbial composition of roof harvested rainwater.
2. The microbial load of roof water can be significantly influenced by weather patterns in relation to wind speed and direction.
3. Indicators of faecal contamination may have reduced relevance in assessing tank water quality with regard to the diversity of organisms present and the domestic applications of tank water.
4. Fungal species may be of significance to tank water quality and health risk assessment.
5. Environmental organisms may have an important role in regulating tank water quality with regard to both chemical transformations and exclusion of potential pathogens.

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