Water Quality, Incidental Treatment Train Mechanisms and Health Risks associated with Urban Rainwater Harvesting Systems in Australia

By

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I hereby certify that the work embodied in this thesis is the result of original research and has not been submitted for a higher degree to any other University or Institution

Signed ____________________

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Abstract

The increasing variability of rainfall in many Australian cities has recently highlighted major inefficiencies in the traditional centralised approach to urban water-cycle management. Rainwater tanks are fast becoming accepted as a best practice device in urban water-cycle management due to their ability to reduce mains water demand and reduce stormwater runoff. Unlike in rural areas, rainwater tanks in the urban environment can be interconnected with the municipal water supply via a trickle top-up system to allow the tanks to be plumbed in as a permanent supply for designated household end-uses. The greater the utilisation of tank waters within households the greater their contribution to increasing the efficiency of the urban water-cycle. However, what can be considered as appropriate utilisation of harvested rainwater is dependent upon the hygienic quality of the water and currently, very little is understood about the phenomena related to tank water quality.

This thesis investigates three areas relating to water quality in urban rainwater harvesting (RWH) systems that were considered poorly understood. In Section I (Chapter 3) of this thesis, water quality was monitored across a range of RWH systems to allow tank water quality to be compared to official water quality guidelines and to municipal water supplies, as well as to assess design variables by comparing *inter*-system water quality variations. The water quality results of two field trials showed that harvested rainwaters generally did not comply with drinking water guidelines primarily due to the presence of coliform bacteria and *E. coli,* though did consistently comply with bathing water guidelines. Several design and environmental parameters were assessed for correlations with water quality parameters. Strong correlations were found between tank size and microbial parameters, with increasing tank size being associated with increasing concentrations. This was thought to be related to the greater frequency of top-up with chlorinated municipal supplies in smaller tanks as a result of more rapid emptying. Water quality was also found to be poorest immediately after rain events with improvements as storage time increased.

Section II (Chapters 4 – 8) of this thesis investigated a series of mechanisms within RWH systems that influenced *intra*-system water quality variations, termed here 'the incidental treatment train'. The mechanisms investigated were defined as *incidental* in that they were not deliberately installed or established for the purpose of improving water quality and were potentially common to all RWH systems. Investigations into the incidental treatment train confirmed that a number of speculated processes were indeed occurring. Within the water column of several rainwater tanks, both spatial and

temporal variations in water quality were observed. Spatial water quality variations were seen through the stratification of microbial concentrations, with higher concentrations observed in the upper layers of the water columns. Temporal variations in water quality were observed through repeated sampling of the water column in specific tanks, providing evidence of the existence of incidental treatment mechanisms within the tanks. Heterogeneous microbial populations were also observed in the water columns with several species having been previously identified as containing some form of bioremediation potential.

The hypothesis that biofilms would develop on the internal walls of tanks and would demonstrate the potential to improve water quality was also shown to be true. Microbial communities were observed to develop on galvanised iron, polyethylene and glass slides at varying depths within a rainwater tank. Viable cell counts in these biofilms were found to be three orders of magnitude greater than concentrations in the water column, while culturable cell counts were found to be over four orders of magnitude greater within the biofilms. Greater concentrations of cells were generally observed in the biofilms grown in the lower layers of the water column while minimal variations in concentrations were observed between slide materials. A mixture of bacteria was detected within the biofilms and a core group of 5 species were found on each material. Plastic and metal-slide biofilms contained species exclusive to those materials, indicating that slide material did have a bearing on the species present. Biofilms were found to have a substantial capacity for removing heavy metals from the water column. This phenomenon was observed for all of the heavy metals that were analysed. The most significant accumulation rates were observed with lead, for which concentrations within biofilms were consistently 2,000 – 10,000 times greater than concentrations in the water column.

The process of sedimentation and the accumulation of sludge were observed in many rainwater tanks. It was found that sediment was not distributed evenly across the base of rainwater tanks and greatest accumulation occurred directly under the inlets. Extremely high concentrations of heavy metals were found in sediments from all tested tanks. Lead concentrations in sludge ranged up to 6.6g/L, equating to magnifications of up to 340,000 times the concentrations found in water columns. This highlights the importance of the role of passive sedimentation in rainwater tanks and provides impetus for tank design to optimise the effectiveness of this process. The ability of two types of sludges to act as flocculants when re-suspended was also tested but found to be of minimal practical benefit. A number of factors were thought to influence the risk and extent of sludge re-suspension relating to design and environmental variables.

The potential of domestic hotwater systems (HWS) to produce waters of sufficient hygienic quality for showering was of great interest in this research project. From the major field studies, massive reductions in bacterial concentrations were achieved when harvested rainwaters were passed through a range of domestic HWS. A minimum of three log-reductions or 100% inactivation was achieved for *E. coli* in HWS operating at 55°C or above. HWS operating at 60°C produced comparable quality to municipal water supplies in both regions. Laboratory-based thermal destruction analyses were conducted on a range of common indicator and pathogenic organisms to ensure susceptibility of these organisms to heat inactivation. Rapid inactivation of all pathogens was achieved at temperatures relevant to domestic HWS (55°C – 65°C). *E. coli* was found to possess the greatest heat resistance capacity of the tested organisms, which was shown in the field studies to be inadequate for surviving domestic HWS. *Bacillus sp.* were found to be the most common survivors in both laboratory analyses and field tested HWS. This was expected due to the spore-forming ability of *Bacillus* though was not considered alarming due to negligible potential for these organisms to cause disease transmission through water systems. The use of rainwater in HWS was therefore concluded to pose minimal human health risks, though HWS should be maintained at a minimum of 60°C in accordance with current Australian Standards to ensure human safety.

The third and final section (chapter 9) evaluates the health risks of utilising harvested rainwater for non-drinking purposes and critiques the inadequacies of the current guideline framework for regulating urban RWH systems. A review of the literature relating to pathogen transmission pathways and water-borne outbreaks of disease from rainwater tank and non-rainwater tank supplies revealed that the risk of serious illness from drinking untreated harvested rainwaters is relatively low due to the exclusion of the major disease vectors (cattle and human faeces) from roof catchments. Previously reported epidemiological studies and an analysis of outbreak frequency supported this conclusion. A review of the current framework of water quality guidelines revealed a deficiency in their relevance to non-potable utilisation of harvested rainwaters. Specific guidelines were therefore developed and proposed for three categories of secondary-use and recommendations were made for developing robust drinking rainwater guidelines. In essence, this thesis has argued that a series of incidentally occurring treatment mechanisms contribute significantly to producing a high quality freshwater resource and that with an appropriate guideline framework, the fuller and more appropriate utilisation of this sustainable water resource for secondary uses including showering and bathing, toilet flushing, laundry and washing, and outdoor irrigation applications, should be achieved.

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Glossary

AAS	Atomic Absorption Spectroscopy
ABS	Australian Bureau of Statistics
ADWG	Australian Drinking Water Guidelines
ANOVA	Analysis of Variance
AS/NZS	Australian Standards / New Zealand Standards
BCC	Brisbane City Council
BOM	(Australian) Bureau of Meteorology
CBD	Central Business District
CFU	Colony Forming Units
DALY	Disability Adjusted Life Year
EC	Electrical Conductivity
EPS	Extracellular Polymeric Substance
FC:FS	Faecal Coliform : Faecal Streptococci
GDWQ	Guidelines for Drinking Water Quality
GRWQA	Guidelines for Recreational Water Quality and Aesthetics
HCGI	Highly Credible Gastrointestinal Illness
HPC	Heterotrophic Plate Counts
HSD	Honest Significant Difference test
HWS	Hotwater System
ICP-MS	Inductively Coupled Plasma – Mass Spectrometer
NCC	Newcastle City Council
NSW	New South Wales
RCS	Rainwater Catchment System
RPZD	Reduced Pressure Zone Device
RWH	Rainwater Harvesting
RWT	Rainwater Tank
SAR	Sludge Accumulation Rate
SAS	Scientific Analytical Services
TDA	Thermal Destruction Analysis
TDS	Total Dissolved Solids
TTC	Thermotolerant Coliforms
WHO	World Health Organisation
WSUD	Water Sensitive Urban Design

CHAPTER 1

Introduction

1.1 Urban Rainwater Harvesting

The sustainability of the urban water cycle in many of the world's cities has come under increasing pressure over the last century (Beecham & Khiadani, 1997). Freshwater supplies, including surface and ground waters, face increasing pressures from growing urban populations and increasing rates of water consumption (Appan, 1999; Wei, 1991). Water demand in most cities today is met by importing large quantities of water from neighbouring catchments via extensive pipeline distribution systems. Although in Australia only about 1% of water demand is for drinking purposes, all municipal waters are treated to a drinking water standard. In parallel to this, rainfall, which quickly becomes stormwater, is removed through large hydraulically efficient pipes and channels and discharged into downstream water bodies. Environmental degradation of receiving waterways such as creeks and lakes caused by increasing volumes of poor quality stormwater runoff have resulted from this, accentuated by the expansion of impervious surface areas prevalent in city landscapes (Beecham & Khiadani, 1997).

However, the practice of rainwater harvesting (RWH) within the urban environment has the potential for substantial mitigation of these pressures (Coombes *et al.*, 2002; 2003b; Teck, 1989). The practice of RWH is distinct from that of stormwater detention. The focus of this thesis will be on harvested rainwater, which is rainfall captured from elevated roof surfaces and stored in rainwater tanks. Stormwater detention involves the storage of surface runoff waters which are significantly more contaminated as a result of interaction with ground surfaces. Urban RWH is one of the central components of Water Sensitive Urban Design (WSUD), an environment-based design concept for urban water management proposed by Whelans *et al.* (1994). Along with rainwater harvesting, WSUD includes the utilisation of underground stormwater detention basins, water sensitive landscaping, and the re-use of greywater and wastewater at the allotment scale. WSUD is a major driving force behind the current decentralisation of the traditional 'pipe paradigm' urban water management approach. Urban RWH and WSUD are starting to be recognised as part of a set of best practice management options for urban development and are being adopted by many water planners and local councils in Australia and many other countries.

1.1.1 History: From Jars of Clay to WSUD

Various forms of RWH have evolved independently throughout the world for thousands of years. Gould and Nissen-Peterson (1999) and Wahlin (1997) provided reviews of the history of rainwater harvesting and suggested that the first rainwater harvesting systems probably predate recorded history. Many ancient civilisations, particularly in arid and semi-arid regions, applied water harvesting methods to divert, collect, and store rainwater for drinking and irrigation purposes. Basic rainwater harvesting techniques were believed to have been developed in Ancient Iraq 4000 to 6000 years ago (Helms, 1981) and the oldest known house–cisterns were found in Palestine belonging to the Chalcolithics, thought to be in existence before 3000 B.C. (Evanari *et al.*, 1982). In the central Chinese province of Gansu, clay pots were being produced over 6000 years ago, thought likely to have been used for collecting rainwater runoff from the roofs of ancient shelters and other structures when needed (Gould & Nissen-Peterson, 1999).

Within Australia's modern history, rainwater tanks have also played a prominent role in supplying potable water needs of earlier residents. Prior to the late nineteenth century and the emergence of centralised water and wastewater systems, rainwater harvesting was a widespread practice in Australia (Lloyd *et al.*, 1992). The construction of centralised water supply systems was motivated by demand for increased drought security and improved fire-fighting capacity and was not related to public health concerns. Public health concerns were primarily associated with inadequate sewage disposal systems. Poorly constructed underground rainwater tanks were also a source of illness though aboveground tanks were often favoured over municipal water supplies by NSW residents in the late nineteenth century (Coombes, 2002).

In many parts of the world today harvested rainwater is still the primary source of potable water. This includes areas in many developed countries where centralised water systems do not reach, including 11% of the Australian population (ABS, 2004). However, we are currently on the verge of a completely new approach to urban water cycle management. The view that only a single water source can be the predominant supply is being overturned in favour of an integrated water management approach (Geiger, 1995). Rainwater tanks are now being used to complement mains water for non-drinking uses, reducing demand pressures on mains waters while reducing dependence on the need for constant rainfall. Hence, modern urban RWH encompasses two separate water supplies for two different categories of use, harmonising in interdependence through the mutual benefit bestowed on each one by the other.

1.1.2 Modern Urban Rainwater Harvesting Systems

Rainwater harvesting in the modern urban environment is taking a form never contemplated before. Today, the use of rainwater harvesting systems is emerging as a desirable mainstream practice (Sun *et al.*, 1995; Wei, 1991). Consequently, the design and manufacturing processes employed have resulted in systems of greatly improved quality than those popularly used in the nineteenth and twentieth centuries. Significant developments have been made in construction materials as well as in design and manufacturing processes, and rainwater supplies now complement and embrace municipal supplies rather than compete with them.

1.1.2.1 Construction Materials

Developments in our understanding of health and disease have enabled us to identify elements, compounds and infective agents that may be toxic or antigenic to the human body. This knowledge, coupled with improvements in the sensitivity of analytical laboratory equipment for detecting and measuring harmful agents, has allowed significant design improvements in the building and water supply sectors. Rainwater tanks now come in a variety of durable yet inert materials that overcome rusting problems and reduce chemical contamination from tank and catchment materials. The most commonly accepted rainwater tank materials now include concrete, galvanised iron, Aquaplate® and plastic (food-grade polyethylene).

1.1.2.2 Modern Designs

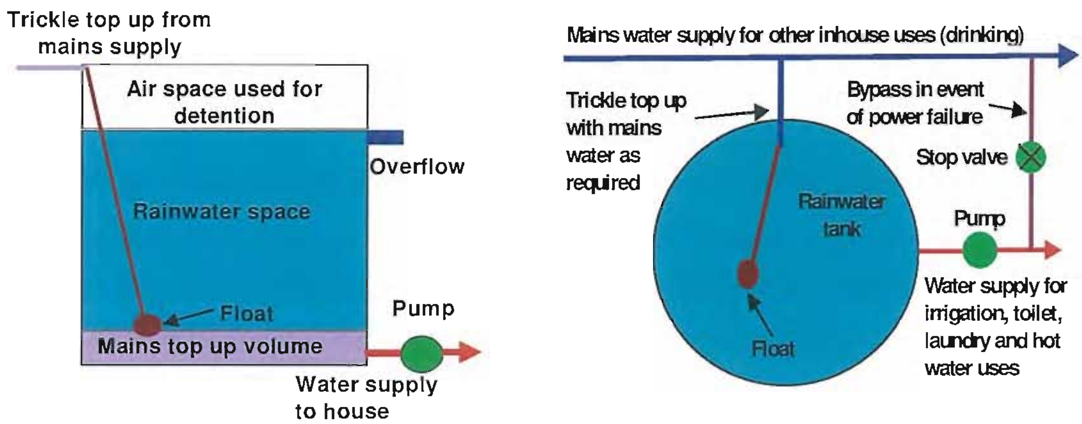
Innovative modern RWH system designs have also contributed to enhancing water quality in tanks along with providing a range of aesthetically and physically convenient tank shapes and sizes. Modern rainwater tanks are designed to be fully enclosed in order to restrict the entry of potentially disease transmitting animals (vectors) such as mosquitoes, amphibians, small reptiles and mammals. Along with this, a number of design modifications and additions are now standard options, such as the widespread use of first flush devices to separate and discard the initial dose of rainwater which typically contains elevated levels of contaminants. Filters, UV and other disinfection systems are also employed in some systems when the water is intended for drinking purposes.

Furthermore, modern tank designs have focused on aesthetically pleasing options, available in a range of colours, shapes and sizes to maximise their physical convenience. Innovative shapes have been designed to suit urban and city residences with little spare room for tradition circular tanks. Tanks are now manufactured in the shapes of fences and can be designed to 'squeeze' into gaps behind garden sheds or under houses. A number of large companies in Australia are involved in the continual development and supply of modern rainwater tanks, including BlueScope Water and Bushman's Tanks, which has resulted in improved designs.

1.1.2.3 Integrating Tank & Mains Water Supplies

The key innovation of modern urban rainwater harvesting systems is the ability to complement municipal water supplies without the need for deliberate householder intervention. There are two main mechanisms available to achieve this. The first is the trickle top-up design which uses a float in the tank to trigger the slow input of mains water to maintain a designated minimal water level, as shown in Figure 1.1 (Coombes & Kuczera, 2001). In this design, the rainwater tank is connected to the household plumbing in a way that allows water from the tank to constantly supply the designated uses. During dry periods the tank will contain a mixture of rainwater and mains water and will therefore always be in use. One of the major advantages of this design is that tanks can be topped-up with mains water during off-peak times and used during peak times, effectively reducing the peak demands for municipal water suppliers.

The second design used to integrate tank supplies with municipal supplies is a bypass valve that switches from rainwater supplies to mains water supplies when rainwater is not available through the use of a pressure-triggered solenoid valve (Coombes *et al.*, 1998). One of the most commonly installed products of this type is the Rainbank®, manufactured by Davey. In this design, mains water does not enter the tank but is diverted past the tank when needed. The disadvantage of this design is that it does not slowly accumulate storages of mains water in the tank and hence does not contribute to reducing municipal peak demands. However, it saves energy and may extend the life of the pump by avoiding the use of the pump to pressurise water when the tank is empty.



Adapted from Coombes & Kuczera (2001)

Figure 1-1: Trickle top-up system in an urban rainwater harvesting system shows the minimum water level maintained in the tank, as publicised by Coombes and Kuzcera (2001). Mains water enters the tank through an inlet at the top of the tank allowing for a 300mm air gap. Top-up is triggered by a float valve when water levels fall below a pre-determined level.

The plumbing configuration that achieves the most optimal utilisation of both tank and mains water has been the subject of relatively recent research. Coombes *et al* (2003) found that when rainwater tanks in the urban environment are utilised exclusively for outdoor garden irrigation then only limited water savings are achieved. This is primarily due to the seasonal mismatch between outdoor water demand and supply, whereby the garden water demand comes after periods of no rainfall enabling a maximum utilisation of only one tank volume of rainwater per rain event (Coombes *et al.*, 2003). The most optimal utilisation of urban rainwater is achieved when draw down rates in the tank are maximised. Therefore, supplying indoor demand can maximise harvestable yield by continually creating available storage capacity during rain events. Using harvested rainwater to supply toilets as the only indoor demand has been found to marginally improve optimisation. However, connection to laundry and hotwater systems has been found to result in significant improvements to urban water cycle efficiency (Coombes *et al.*, 2003).

1.2 The Benefits of Urban Rainwater Harvesting

Rainwater harvesting within the urban environment produces both environmental and economic benefits. The four major benefits include increased drought security for city water supplies, greater environmental flows, reduced stormwater runoff, and cost savings on municipal water and stormwater infrastructure.

1.2.1 Improved drought security

The need for greater drought security in many of the world's urban regions has become increasingly obvious in recent years (e.g. Heggen, 1999; Appan, 1999; Gumbo, 1998). Increased frequencies of droughts along with the realisation of the limits of traditional centralised water supply systems to protect cities against water shortages have encouraged the use of a multitude of water savings devices along with rainwater harvesting and greywater reuse schemes. The use of harvested rainwater to supplement existing municipal water supplies for domestic non-drinking applications has been shown to significantly reduce demand on municipal waters, easing pressures on reservoirs during dry periods and allowing them to fill more quickly and to a fuller capacity during wet periods (Coombes *et al.*, 2002). The logic of urban rainwater harvesting is further highlighted by the fact that for many major cities of the world, including Sydney, the quantity of rainfall in the city region itself is regularly greater than in the city's inland water supply catchment (e.g. ABM, 2006).

1.2.2 Allowance for environmental flows

The decreased reliance of municipal suppliers on abstracting water from natural water ways results in lower levels of environmental stress imparted on these natural aquatic systems and associated ecological habitat (DEH, 2006b). Natural water ways, in particular rivers, often contain delicate aquatic ecosystems that are sensitive to both flow volumes and seasonal patterns of flow (Brownlow *et al.*, 1994). During dry periods, over-abstraction of such systems may reduce flow levels to below threshold values required for the maintenance of ecosystem integrity. During these times, water chemistry becomes increasingly unstable and may lead to irreversible changes within the biological habitat. A number of water quality problems may be encountered when flow volumes are significantly reduced, including increased levels of salinity, turbidity, nutrients and an increased risk of algal blooms and eutrophication (EPA SA, 2003; Davies & Kalish, 1994). The maintenance of seasonal flow trends, including seasons of high flow or flooding, are also important for plant and animal species in ecosystems surrounding rivers and in receiving wetlands (Frazier & Page, 2006; Richardson *et al.*, 2004). However, the utilisation of rainwater during wet periods reduces the duration and the extent of dependence on natural water ways during times of drought allowing environmental flows to more closely mimic natural patterns (DEH, 2006a).

1.2.3 Reduced stormwater runoff

Furthermore, when rainwater is not harvested in the urban environment it quickly becomes a source and cause of environmental pollution to downstream water bodies (Beecham & Khiadani, 1997). This is so for two reasons. Firstly, flowing water is one of the most efficient transporters of particulate matter, with many substances readily dissolving in it. A wide range of contaminants accumulate on roads, gutters, roofs and gardens from car and industrial emissions, which are readily scavenged and mobilised in stormwater runoff. Secondly, the consequence of the traditional stormwater disposal approach involving the channelling of stormwater runoff into single contour lines of drainage for rapid removal creates rapidly flowing waters capable of causing erosion. The problem is accentuated by the typically large proportion of impervious surface areas in city landscapes which effectively produce greater volumes of stormwater runoff with higher flow velocities. Smaller tributary streams and creeks are particularly susceptible to increased erosion while the receiving water bodies are degraded by the deposition of the mobilised contaminants. The implementation of urban rainwater harvesting along with other components of WSUD has the ability to reduce stormwater runoff volumes significantly (Coombes *et al.*, 2002; Phillips *et al.*, 1997).

1.2.4 Water-Infrastructure Cost Savings

A range of significant savings can be made on municipal infrastructure cost when rainwater harvesting is used to complement municipal water supplies. Firstly, the reduction in demand on mains water results in the deferment and potential elimination of the need for construction or upgrading of additional head-works infrastructure required to cater for growing demand. Such headwork infrastructure includes dams, water treatment plants, pumps and desalination plants which require large capital investments. Secondly, reductions in peak demand on municipal water systems allow the use of smaller diameter distribution pipes, smaller pumps and lower capacity treatment plants which are sized according to peak demand. Considering the distribution infrastructure accounts for the majority of infrastructure costs, reductions in pipe diameters can lead to savings of hundreds of millions of dollars for municipal water suppliers (Coombes *et al.*, 2002).

Reductions in peak demand on mains water are achieved due to the fact that rainwater is captured locally and does not therefore contribute to the load on distribution infrastructure, as well as through the use of the trickle top-up strategy. As explained above, the trickle top-up strategy enables tanks to be interconnected with household plumbing in such a way that tank waters constantly supply water to their designated applications. When tank water levels fall below a predetermined level, the minimum water level in the tank is maintained through the gradual input of mains water. This allows tanks to partially refill during non-peak times and supply water during peak hours even when there is an absence of rainfall, hence flattening out the demand curve (Coombes *et al.*, 1999). Finally, reductions in the erosion potential and volumes of stormwater runoff mean that traditional stormwater drainage designs can be re-evaluated and re-designed to be more landscape-based and less financially expensive.

1.2.5 Water Awareness

The presence of a visually obvious symbol of water conservation acts to continuously remind householders of the finiteness of freshwater supplies and consequently generates respect for the resource. Rainwater tanks are in many ways a classical 'Aussie Icon' and many people feel they connect in some way to their symbolic status. Rainwater tanks, therefore, generally serve as a positive enforcement symbol of water conservation. In urban areas where water conservation has historically been seen to be the responsibility of governments and water suppliers, the rainwater tank helps to re-awaken people's awareness of their own role in water conservation.

1.3 The Barriers to Implementation

The emerging popularity and re-introduction of urban rainwater harvesting by local councils and water demand analysts has not been without objection. Both real and perceived problems have been raised relating to the cost effectiveness of RWH, the modified role and profit potential of municipal water suppliers, and water quality and public health risk issues.

1.3.1 Cost effectiveness

Rainwater tanks do provide low cost water to householders once the systems have been set up and are operating well (Coombes *et al.*, 2004). However, despite rainwater being a free resource, the capital investment and maintenance costs associated with pumps and accessories results in payback periods varying substantially. Estimates of the length of time until 'break even' range from 2 to greater than 20 years depending on the tank material and design, levels of harvested rainwater utilisation, local rainfall, and local municipal water costs (Coombes *et al.*, 2003c; Sun *et al.*, 1995). In situations where pumps or other operational accessories are problematic, tank water may prove to be more expensive per kilolitre than mains water (Berghout, 2005). Therefore, without significant subsidies from local governments or water municipal water suppliers, householders considering the installation of a RWH system for purely economic reasons will generally not be motivated to install such a system.

This analysis assumes that the costs for mains water suppliers are fixed and independent of the influence that widespread installation of RWH systems would make. However, this analysis ignores the substantial economic benefits RWH can confer to municipal water suppliers, as discussed in subsection 1.2.4. The net effect of widespread urban RWH can be a significant system cost savings arising from the deferral of major capital investment in centralised supplies to cope with growth in demand. The barrier to the widespread implementation of urban RWH is then not the high costs to the householder, but the absence of a systematic mechanism that facilitates the redistribution of cost savings from municipal or government providers back to householders. In a holistic economic analysis, an equitable distribution of cost savings derived from the widespread installation of RWH systems can provide economic benefits to all stakeholders (Coombes & Kuczera, 2003).

1.3.2 Authoritarian Objections

Institutional resistance to urban RWH has occurred in various forms over recent years. These objections appear to be a result of a mixture of valid and perceived concerns relating to legal liability resulting from cross contamination, responsibility for maintenance and revenue loss.

1.3.2.1 Responsibility, Liability & Revenue

Responsibility (maintenance), liability (dual supply) and revenue (profit in the water market) are three main areas of contention expressed by centralised water suppliers. In Australia, municipal water suppliers typically bear a legal responsibility to meet Australian Drinking Water Guidelines (ADWG) standards through the incorporation of these standards into statutory requirements of operating licences. Currently in Australia, for the majority of water suppliers, the scope of responsibility for maintaining water quality extends to waters in the distribution pipes and ends at the water meter of residential properties (e.g. Sydney Water, 2005). Rainwater tanks plumbed into household plumbing effectively provide a mixed water source with the water supplier responsible for municipal water quality and the householder responsible for tank water quality.

Understandably, water authorities do not want the responsibility of maintaining a large number of small decentralised systems, nor are they generally willing to risk mains water contamination through backflow as this would compromise the quality of water in distribution systems. In NSW, the general response to these issues has been to push the onus of responsibility for managing each RWH system back onto the householder and permitting cross connections between mains and tank water plumbing on the condition an air-gap or reduced pressure zone device (RPZD) was employed which effectively eliminates the potential for backflow. This requirement has recently been relaxed and at present an above-ground rainwater tank with mains water top-up requires only a non-testable (dual check valve) backflow prevention device while a below ground tank requires a testable double check valve (AS/NZ 3500.1.2).

However, this still leaves local health departments with a responsibility to recommend appropriate uses for harvested rainwater. To date, most health departments make conservative recommendations for uses of tank water including for gardening and toilet flushing. Householders are typically recommended not to use harvested rainwater for drinking, and at the beginning of this research project the utilisation of rainwater in hotwater systems was rarely discussed (such as NSW Health, 2002). These recommendations have been based on perceived health risk and not on epidemiological results (such as Heyworth *et al.*, 2006) and have been shown to produce suboptimal benefits to urban water cycle management (Coombes *et al.*, 2003b). As discussed in subsection 1.1.2, the most significant reductions in mains water demand and stormwater runoff occur when rainwater is supplied to indoor uses with significant demand, namely hotwater systems, with the supplying of non-hotwater uses providing only marginal benefits.

The final concern for state governments and municipal water providers is loss of revenue from the sale of water. In Australia, water authorities are becoming increasingly corporatised and held to return profitable dividends to state government owners. In other parts of the world, privatised water providers are a common occurrence, and the loss of market share is not consistent with 'bottom line' objectives for these companies. However, as outlined in section 1.2.4, the significant cost savings from deferment of capital works can far outweigh reductions in revenue incurred by urban rainwater harvesting (Coombes & Kuczera, 2003). The loss of revenue for water utilities is offset by gains in household income, which results in a net benefit to the whole community. The problem becomes complicated when the initial capital investment costs are heavily subsidised or met by government authorities rather than water providers, making the involvement of all stakeholders and authorities extremely important in the decision making process.

1.3.3 Lack of Understanding of Water Quality

Arguably the most contentious barrier to the re-introduction of rainwater tanks into urban regions has been the fear of poor tankwater quality and the perception of significant public health risk from their use. The basis of these fears has generally been a mixture of hypothetical speculation and limited field data. Field investigations into the water quality of RWH systems have generally been limited either by the dimensions of their sampling regime (i.e. either the number of systems sampled or the level of sampling repetition of each system), the range of water quality parameters measure, or by limited geographical representation.

Various studies in New Zealand (Simmons *et al.*, 2001), Australia (Plazinska, 2001; Tuffley & Hollbeche, 1980), Micronesia (Dillaha & Zolan, 1985), and Palestine (Abu-Sharekh & Subuh, 1995), have investigated a substantial number of systems (>100) during a brief period of time (no. samples typically = 1), effectively providing a 'snapshot' of water quality in the systems. These studies have generally been designed to compare the influence of design parameters on water quality between systems. Other studies have used greater repetition of individual systems though have been restricted to fewer systems in the sample group (e.g. Fujioka *et al.*, 1995; Coombes *et al.*, 2003c; Rijal & Fujioka, 1995; Michaelides, 1989). These studies have given more focus to seasonal variations in water quality.

Many studies have also been limited in scope by the range of water quality parameters measured. Methodological issues, including cost, equipment and expertise, can impede on desired sampling strategies, reducing the focus of investigations to a limited range of parameters. The range of microbial parameters investigated in many studies has been restricted to indicator organisms (e.g. Plazinska, 2001; Coombes *et al.*, 2003c; Pinfold *et al.*, 1993; Dillaha & Zolan, 1985). This is a justifiable approach given that most water quality guidelines (including previous World Health Organisation guidelines) prescribe standards based on the presence of indicator organisms. However, this approach does little to determine the relevance of these organisms for health risk assessment and neither furthers our understanding of the microbial ecology in these systems (Lye, 2002).

Furthermore, the majority of previous investigations into the water quality of RWH systems have taken place in tropical, semi-tropical and rural areas of developing countries where harvested rainwater is used as the primary drinking water supply (e.g. Vasudevan *et al.*, 2001; Uba & Aghogho, 2000; Ariyananda, 1999; Hussein *et al.*, 1997; Yang *et al.*, 1995; Fujioka et al., 1991; Wirojanagud, 1991). The context of this project and many other emerging RWH projects, however, is the complimentary use of harvested rainwater with mains water supplies in urban areas of developed countries for which relatively few investigations have been conducted (e.g. Albrechtsen, 2002; Lye, 2002; Simmons *et al.*, 2001; Coombes *et al.*, 1999). A range of practical differences exist between the regions that were the focus of past RWH research and the regions that are the focus of substantial future RWH development. These include differences in system design, climate, meteorological conditions, and prevalence of contaminants in the local environments resulting from industrial, agricultural, and sanitary practices. The validity of extrapolating microbial and chemical water quality data from widely differing geographical areas to estimate water quality in urban areas of developed countries is therefore scientifically unsound.

1.3.4 Lack of Understanding of Water Quality Dynamics

Water quality is further complicated by the dynamic properties of water and its constituents in RWH systems. Water in RWH systems interacts with numerous components of the system, including catchment and collection components, the storage tank and the distribution system including a pump, pipes and possibly hotwater systems. Evidence has been emerging that each of these components influences water quality to some degree, though investigations have not differentiated between the influence of the components themselves and the influence of the microbial ecologies associated with the components (Coombes *et al.*, 2000; Scott & Waller, 1987; Fujioka & Chinn, 1987). In the majority of these interactions, water quality reportedly improves as it progresses through the systems. Hence, the water quality of a sample taken from one part of the system provides only an arbitrary representation of the water quality in the entire system. Extrapolating water quality 'downstream' or 'upstream' of the given sample will provide inaccurate results if the system has not previously been experimentally interrogated.

Similarly, sampling at one point in time may not provide an accurate indication of water quality in the previous or following time period. A limited number of previous studies have found that water quality generally improves with storage (Kitamura *et al.*, 1997; Yang *et al.*, 1995; Wirojanagud *et al.*, 1991). While these spatial and temporal variations in water quality provide indirect evidence for some form of incidental water treatment, only Wirojanagud *et al.* (1991) and Coombes *et al.* (2000) have provided brief speculation as to the possible underlying phenomena involved in contributing to these water quality improvements. Water and wastewater treatment plants operate by exploiting a series of naturally occurring treatment processes which have been well investigated (Lester & Birkett, 1999). It would not be surprising that a comparative treatment train may operate within rainwater harvesting systems. However, as centralised structures have traditionally been seen as of higher importance than decentralised ones, research into the dynamics of water quality in small scale systems has been largely neglected.

1.3.5 Lack of Appropriate Health Risk Assessment

Within the scope of WSUD, the defined purpose of RWH systems in the urban environment is not as a drinking water source but as a complement to mains water by supplying non-drinking applications. Despite this, authors on the subject and health departments typically take a conservative health risk approach and apply stringent drinking water guidelines due to the absence of specific secondary-use water quality guidelines (Uba & Aghogho, 2000; Yaziz *et al.*, 1989; NSW Health, 2004). Water quality in RWH systems often does not comply with guideline requirements of drinking water standards and appears to pose a high level of public risk due to the detection of faecal indicator organisms and opportunistic pathogens in several published studies (e.g. Simmons *et al.*, 2001; Wirojanagud *et al.*, 1991; Fujioka, 1991). However, the relevance of using faecal indicators to assess tank water quality and the public health significance of random detections of opportunistic pathogens in tank waters may be limited (Pinfold *et al.*, 1993). In the only significant epidemiology study conducted on harvested rainwaters, Heyworth *et al.* (2006) found that tank waters did not pose a significant risk of enteric illness. In this study, the rates of highly credible gastrointestinal illness (HCGI) in the control group of children drinking only chlorinated mains water was in fact marginally higher than rates of HCGI in the experimental group (>900 children) consuming only harvested rainwater.

However, the global disease burden attributable to transmission of waterborne pathogens cannot be taken lightly, warranting tank waters to be the subject of their own specific microbial and chemical health risk assessment. This must be conducted using methodology, data and assumptions specific to the realities of urban RWH systems (Simmons & Heyworth, 1999). However, informed and objective health risk assessments have not always been applied to rainwater harvesting systems. As has been experienced in Australia and the United Kingdom in the past, the categorisation of tank waters as Category 5 Hazardous Fluid Materials by water supply authorities (equating it with nuclear waste, effluent from hospitals, paint factories and morgues and stating it to be more hazardous than raw sewage) appears not to have been based on available scientific data (Coombes, 2002; Hassell, 2005). Despite the emerging popularity of RWH in the urban environment and the potential benefits of such a practice, no health risk assessments have been conducted which have incorporated disease transmission and exposure data relevant to harvested rainwaters intended for non-drinking applications.

1.4 Research Aims & Thesis Structure

To comprehensively investigate water quality in urban RWH systems, a number of major aims must be considered. For each of these to be clearly defined and investigated, this thesis has been structured into three sections, with each section containing their own specific research goals and hypotheses.

1.4.1 SECTION I – Water Quality

The first section of the thesis, comprised of chapter 3, examines the endpoint water quality in a range of RWH systems and evaluates the results against various water quality guidelines. The evaluation of tank water quality using multiple water quality guidelines, including Australian and World Health Organisation (WHO) drinking and bathing guidelines, was conducted to provide a balanced assessment which avoids the bias resulting from the exclusive use of inappropriately conservative drinking water guidelines.

This section assumes water quality is static and that a single sample is representative of the entire systems' water quality, ignoring the premise of section II that water quality within systems is dynamic. This is necessary when evaluating water quality against established water quality guidelines, and allows for a simpler analysis when making inter-system comparisons. The discussion of water quality in chapter 3 is based on the results of two rainwater harvesting projects conducted in Brisbane and Newcastle, the first being conducted by Brisbane City Council (BCC) and the latter being conducted by the University of Newcastle. These complementary projects also provided the basis for inter-system water quality analysis, enabling the influence of design variables on water quality to be assessed.

The research aims of Section I included:

- Evaluating water quality in a range of urban RWH systems against drinking and non-drinking water quality guidelines, and

- Comparing and evaluating the effects of a variety of design components and environmental factors on tank water quality through inter-system comparisons.

1.4.2 SECTION II – Treatment Train Mechanisms

In Section II, water quality is assumed to be dynamic, changing over time and space. In this section, intra-system water quality comparisons are made in an attempt to understand the nature of tank water quality dynamics and elucidate the mechanisms comprising the incidental treatment trains inherent within RWH systems. Chapters 4 to 8 examine the various components of the treatment train in detail.

The research aims of Section II include:

- Determining whether water quality significantly improves as it passes through the RWH system, and hence confirm the presence of an 'incidental treatment train'
- Elucidate the mechanisms operating within this treatment train, including specifically to evaluate the functions of:
    - The roof catchment (chapter 4)
    - Water storage (chapter 5)
    - Microbial Biofilms (chapter 6)
    - Sedimentation processes (chapter 7)
    - Hotwater systems (chapter 8)

1.4.3 SECTION III – Health Risks

The final section, chapter 9, examines a variety of issues inherent in the risk assessment process. The focus of the health risk assessment is novel in that it focuses on secondary uses of tank water rather than drinking uses. To do this, a number of issues require clear expounding. Firstly, the different routes of disease transmission are discussed, including which vectors are common within urban environments. Further, previous outbreaks of illness arising from the use of harvested rainwater are examined, along with the type of human/water contact that various secondary uses create. A critique of the current water quality guideline framework is then made, highlighting relevant components and inadequacies. Finally, a new risk assessment procedure for urban rainwater harvesting is developed and novel secondary use water quality guidelines are proposed.

The research aims of Section III include:

- Evaluating tank water quality in the light of possible disease transmission pathways and assessing consequent health risk for secondary domestic uses
- Evaluating the suitability of current potentially relevant water quality guidelines for application to assessing harvesting rainwater for secondary uses
- Proposing secondary use water quality guidelines for rainwater harvesting systems.

CHAPTER 2

Literature Review of Harvested Rainwater Quality and Treatment Train Mechanism

2.1 Introduction

Recently, interest has been given to rainwater harvesting systems as a means of improving water cycle management in urban and metropolitan areas. The re-introduction and retrofitting of rainwater tanks into cities, which have long been serviced with treated municipal supplies for all water uses, has consequently raised a number of questions relating to the quality of this alternative supply. These water quality issues have included the ability of harvested rainwaters to meet chemical and microbial guideline standards, the suitability of this water for various end uses, and the potential health risks associated with its use. The novel application of rainwater tanks as a supplement to mains water supplies has created a number of disparities in the available data and limited the ability of currently published literature to address these issues.

In the past, the promotion and development of rainwater harvesting has occurred primarily in developing countries or in rural areas of developed countries as a means of securing an improved water supply. The majority of literature investigating water quality in rainwater harvesting systems has therefore focussed on water quality issues within this context. Consequently, literature relating to water quality of harvested rainwater and health risks from the non-drinking utilisation of tank waters in urban areas of developed countries is relatively sparse.

Water quality in rainwater harvesting systems has been found to vary significantly between systems (Albrechtsen, 2002; Ariyanada, 1999; Fujioka *et al.*, 1995). Significant variations in water quality have also been found within individual systems over time and at spatially discrete points throughout the collection, storage tank, and distribution network (Coombes *et al.*, 2000; Kitamura *et al.*, 1997; Kita & Kitamura, 1995; Fujioka & Chinn, 1987). A series of physical, chemical and microbiological processes intrinsic to the normal functioning of rainwater harvesting systems have been speculated as the cause of these temporal and spatial water quality variations within systems. While some of the more obvious mechanisms have been identified in the literature, such as sedimentation and pasteurisation (Coombes *et al.*, 2000; Scott & Waller, 1987), other more subtle mechanisms have not been identified and none have been thoroughly investigated.

This thesis has been structured into three parts which have been mirrored in the three sections of this literature review. The first two sections of the literature review relate to water quality and incidental treatment mechanisms and are presented in this chapter. The aims of this chapter were to review the published literature on water quality in RWH systems and to identify possible components of the incidental treatment train from the literature. The review of water quality encompasses microbial (indicator organisms and pathogens) and chemical quality in RWH systems in both urban and rural areas. The second section introduces the concept of the treatment train and reviews evidence from the literature of an incidental treatment train in domestic rainwater harvesting systems while highlighting the deficit of knowledge relating to the individual mechanisms within the treatment train.

The third section of the literature review relates to health risk assessment and non-drinking water quality guidelines and is presented in chapter 9. The third section combines a summation of published cases of illness and epidemiological data relating to the use of harvested rainwater, critiques the inadequacies of current water quality guidelines for regulating harvested rainwater quality, and presents a health risk assessment and recommendations for secondary use guidelines for the use of harvested rainwater.

2.2 Water Quality

Harvested rainwater quality has been investigated in a number of sporadic studies throughout the world over recent decades. The quality of harvested rainwater, as with any water body, relates not to the water molecule (i.e. H_2O) itself but to the types and concentrations of additional constituents present in the solution. Given the efficient solvency characteristics of water and its capacity to mobilise and transport material, the additional constituents in a water body may include an extensive range of microbial, inorganic and organic molecules (Lester & Birkett, 1999). The concentrations of these additional constituents will fluctuate as physical, chemical and biological processes continuously occur. Water quality is therefore ultimately a dynamic process rather than a static condition.

However, in order to compare water quality in different systems or evaluate water quality against guideline standards, single numerical values must be used to represent water quality. Therefore, the discussion of water quality in this section (2.2) is based on literature that generally assumes that harvested rainwater has a static value, and ignores the premise of the next section (2.3) that water quality in rainwater harvesting systems is dynamic. In order to do this with maximum scientific integrity, the water quality data reviewed in this section are based on water samples reported to be taken from the tap or from the nearest point to the end of the RWH systems as described by each study. The water quality issues discussed in this section include both microbial (indicator and pathogenic) and chemical water quality.

2.2.1 Index & Indicator Organisms in RWH Systems

Currently, it is not feasible for water quality monitoring regimes to routinely test for the full range of pathogenic (disease causing) or opportunistically pathogenic microorganisms. A number of limitations relating to culturing or identification methodology, time delay, equipment, expertise and cost, contribute to the difficulty in routinely monitoring for numerous specific pathogens. Therefore, at present, the contamination of water supplies by pathogenic organisms is typically measured as a potential, rather than an observed, presence through the use of index and indicator organisms. The World Health Organisation's most recent water quality guidelines (WHO, 2006) define these as follows:

> " – an *index organism* is one that points to the presence of pathogenic organisms – for example, as an index of faecal pathogens; and
>
> – an *indicator organism* is one that is used to measure the effectiveness of a process – for example, a process indicator or a disinfection indicator."

Most investigations into the microbial quality of harvested rainwater use a combination of index and indicator organisms. The most commonly used of these are Heterotrophic Plate Counts (HPC), Total Coliform counts, Thermotolerant Coliform counts (or specifically *E. coli*), and *Pseudomonas* counts. These can be preliminarily identified and quantified by culturing on selective media and counting the colony forming units (CFU). The single major surrogate measure for pathogenic organisms has been thermotolerant coliform bacteria, and specifically *E. coli*. However, it has been increasingly found that bacterial faecal index organisms do not accurately indicate the presence of protozoan or viral pathogens and often not even bacterial pathogens (Camper *et al*, 1991). Consequently new index organisms have been proposed such as the spore-forming *Clostridium perfringens* for protozoan pathogens and bacteriophages for viral contaminants (Ashbolt *et al*, 2001). However, many drinking water guidelines, such as the ADWG, still operate on a pass/fail system according to the absence/presence of *E. coli* or thermotolerant coliform bacteria. This standard is a simple and convenient test, however, there may be significant variations in the degree of correlation between the presence of thermotolerant coliforms and the presence of pathogenic microorganims for different water supplies.

Another major class of faecal indicator organism are Enterococci (also known as Faecal Streptococci). Previously, it was observed that animal faeces contain higher concentrations of Enterococci than coliform bacteria, whereas human faeces contain less than half the concentration. Using these general ratio rules for faecal coliforms to faecal streptococci (FC:FS) in fresh faeces, attempts were made to define the source of faecal contamination in water bodies (Geldreich & Kenner, 1969). However, due to the greater survival and resilience capacities of Enterococci, it is now realised that the FC:FS ratio can change significantly over time and is therefore not generally considered an accurate method for determining the source of faecal contamination (Ashbolt *et al.*, 2001). However, due to their greater survival characteristics, Enterococci are still commonly used as an independent faecal marker organism.

2.2.1.1 Limitations of Indicator Organisms

The limitations of the faecal indicator organism method must be understood in order for a more accurate interpretation of water quality data in relation to risk assessment to be made. Two assumptions are inherent with the use of indicator organisms. The first assumption is that a strong correlation exists between the presence of faecal indicator organisms and the presence of faecal contamination. The existence of a number of environmental coliform organisms (non-faecal origin), as well as the presence of pathogen exposure pathways not involving the faecal route, means that a degree of uncertainty must be acknowledged during data interpretation. While some strains of *E. coli* and thermotolerant coliform are known to be environmental, they are much more specific to faecal contamination than the wider total coliform group (Ashbolt *et al.*, 2001).

The second assumption is that the presence of faecal contamination equates to the presence of pathogenic organisms. This probability varies significantly between sources of faecal contamination, with human sewage almost certainly containing human pathogens, while reptile, small mammal and avian faecal contamination may often not contain human pathogens. Confidence in this assumption has been diminishing with recent findings revealing a number of cases where false positive (presence of index organisms and absence of pathogens) and false negative (absence of index organisms and presence of pathogens) results have been obtained (Smith & Rose, 1998; Ashbolt *et al*, 1997; Hollander *et al.* 1996). Furthermore, differing water sources as such coastal, surface, ground, and harvested rainwater supplies, contain unique and differing probabilities of being contaminated by differing sources of faecal contamination (e.g. Simmons *et al.*, 2001; Jones & Obiri-Danso, 1999; Ferguson *et al.*, 1996; McFeters *et al.*, 1974). However, testing for all pathogenic microorganisms is not currently feasible for frequent long-term monitoring programs, making indicator and index organisms the most appropriate and cost effective alternative currently available.

2.2.1.2 Presence in Rainwater Tanks

A number of published literature reviews have presented the occurrences of identified micro-organisms in harvested rainwaters in rural areas, such as those by Lye (2002) and Gould and Niessen-Peterson (1999). Studies from around the world have generally focussed on the detection of index and faecal indicator organisms, which are of limited relevance in rainwater harvesting systems (discussed in Chapter 9). In the past it has been common for studies to draw conclusions about water quality and health risks of harvested rainwaters by superficially comparing results of indicator organisms against prevailing drinking water guidelines. Few studies into harvested rainwater quality have critically evaluated the specific relevance of the assumptions inherent in the indicator-organism methodology.

The quality of water in RWH systems in urban areas is not well understood for a number of reasons. These include the fact that previous research into RWH systems has focused on rural areas, has focused on rainwater as a drinking water supply rather than non-drinking supply, has not examined the influence of a dual water supply (chlorinated mains water mixing with rainwater), and that significant variability has been observed in water quality between previous studies.

Compared to rural areas, very few relevant studies into microbial water quality exist for rainwater tanks in the urban environment. In the urban industrial city of Newcastle, Australia, tank water quality monitored in a Water-Sensitive-Urban-Design retrofit project at Figtree Place was found to contain thermotolerant coliforms in three of 13 samples with a maximum concentration of 8 CFU/100mL (Coombes *et al.*, 2000). Total coliforms were detected in nine samples ranging up to 1,000 CFU/100mL, while *Pseudomonas* were detected in all but one sample and ranged up to 250,000 CFU/100mL with HPC ranging between 10 and 30,000 CFU/mL (Coombes *et al.*, 2000). Coombes *et al.* (2003c) did further monitoring of a rainwater tank in the inner city suburb of Maryville, Newcastle. The authors found thermotolerant coliforms in only one of 12 samples (10 CFU/100mL), while average total coliform, *Pseudomonas* and HPC concentrations were 18 CFU/100mL, 1673 CFU/100mL and 784 CFU/mL, respectively.

In Denmark, the quality of urban harvested rainwater (via collection of roof and pavement runoff) was also assessed for its suitability to supply toilets (Albrechtsen, 2002). While *E. coli* was detected in concentrations of 4 to 990 CFU/100mL in 11 of 14 samples from seven rainwater tanks, concentrations taken from the toilet water supplied by rainwater were actually lower than the toilet flushing water supplied by the municipal supply. This was a similar trend seen for HPC (37°C), yeast and microfungi. *Pseudomonas aeruginosa* were only detected in one (20 CFU/100mL) of 14 samples from the rainwater tanks while HPC ranged from 13 to 11,000 CFU/mL. These studies provide a relatively limited view of tank water quality in urban areas intended for non-drinking applications and show significant variations in bacterial concentrations.

Numerous more studies into harvested rainwater quality have been conducted in rural areas in both developed and developing countries than in urban areas. Investigations into rural rainwater harvesting systems in developed nations have been conducted in Australia, New Zealand and USA. Over one hundred rainwater tanks were sampled in remote aboriginal towns in central Australia (Plazinska, 2001). None of the tanks contained any mechanical cleaning devices such as first flush devices, and no maintenance of the roof catchments was ever undertaken. Approximately 17% of tanks were estimated to contain HPC concentrations in the range of 100 to 3,500 CFU/mL, while 55% were in the range of 5,000 to 50,000 CFU/mL, and 28% of tanks contained high levels of HPCs in the range 30,000 to 300,000 CFU/mL. Total coliforms were also a common contaminant found in 84% of tanks, while thermotolerant coliforms were found in 59% of tanks, 41% of which contained less than 100 CFU/mL while 18% contained greater than 100 CFU/100mL.

Simmons *et al.* (2001) reported a similar degree of contamination in rainwater tanks in rural New Zealand with 56% of 125 tanks found to contain thermotolerant coliforms. Thermotolerant coliform concentrations ranged from <1 to 840 CFU/100mL, while HPC (CFU/mL), Total coliform and Enterococci concentrations ranged between 1 – 130 000, <1 – 19 000 and 2 – 19 000 CFU/100mL, respectively. In rural northern Kentucky, Lye (1987) reported only low levels of microbial contamination in rainwater tanks. Thermotolerant coliform contamination was found in only one of 30 systems (20 CFU/100mL) though HPC levels varied more significantly (20 – 2×10^9 CFU/mL).

A number of Islands belonging to the Americas have undergone rainwater tank monitoring projects including the Caribbean, the U.S. Virgin Islands and Hawaii. In the Eastern Caribbean, 20 of 57 samples were found positive for thermotolerant coliforms in 8 private and 3 public tanks that had been monitored for 4 months (Haebler & Waller, 1987). In the U.S. Virgin Islands, Ruskin and Callender (1988) found 197 of 271 samples (73%) from private cisterns contained total coliform bacteria and 191 of 271 samples (70%) contained *P. aeruginosa.* From public tanks in this same project, 33 of 75 samples were positive for total coliforms while 39 samples (52%) were positive for *P. aeruginosa* (Ruskin and Callender, 1988 cited in Krishna, 1989)

In Hawaii, nine cisterns sampled five times each over a one-month period contained 122 to 896,000 CFU/mL of HPC bacteria (Fujioka *et al.*, 1995). *E. coli* concentrations also varied between systems, with two systems never containing *E. coli* while three systems were never negative for *E. coli.* The overall average *E. coli* concentration was 138.2 CFU/100mL, ranging between 0 to 1000 CFU/100mL. The concentrations of Enterococci were generally lower than those of *E. coli* although the pattern of distribution was very similar, with the overall average being 35 CFU/100mL, ranging between 0 to 172 CFU/100mL. RNA phage was not detected in any water samples and the rare isolation of *C. perfringens* allowed the authors to conclude the faecal deposition was animal. H_2S producing bacteria were also monitored and found to follow a similar distribution to *E. coli* and Enterococci, averaging 113 CFU/100mL and ranging between 0 to 923 CFU/100mL, with two systems never containing H_2S producing bacteria and three systems always containing H_2S producing bacteria (Fujioka *et al.*, 1995). In another Hawaiian study involving five cisterns total coliform concentrations were found between 0 to 285 CFU/100mL (averaging 63.5 CFU/100mL), *E. coli* concentrations between 0 to 92 (average 13.64) CFU/100mL, thermotolerant coliform concentrations between 0 to 114 (average 22.32) CFU/100mL, faecal streptococci between 0 to 221 (average 30.72) CFU/100mL, and HPC between 45 to 1663 CFU/mL (average 517) (Rijal and Fujioka, 1995).

A number of rainwater harvesting projects have been monitored within Asia and South-East Asia. Wirojanagud *et al.* (1991) found *E. coli* contamination in 12% of outdoor cement rainwater tanks in Thailand. The study also found *E. coli* in 33% of in-house containers, suggesting human secondary contamination. Using the FC:FS ratio the authors suggested the faecal contamination in the outdoor tanks was primarily from an animal source (ratio <1) whereas the indoor containers was probably human (>4) indicating secondary contamination (Wirojanagud *et al.*, 1991). Although this method is now considered unreliable, the interpretation given in the study provided a logical explanation for the observed results.

In China's northwest, seasonal and geographical influences were found to affect concentrations of indicator organisms in harvested rainwaters (Yang *et al.*, 1995). In Yuzhong, the total coliform concentrations (16,560 CFU/100mL) and total bacteria concentrations (2,609 CFU/mL) were higher during the colder January sample (4°C) than in September (12°C) when total coliform (2,609 CFU/100mL) and total bacteria (780 CFU/mL) were lower. In Tongwei the reverse trend was seen with higher total coliform concentrations (16,600 CFU/100mL) observed during September (16°C) and lower concentrations (800 CFU/100mL) observed during January (6°C) (Yang *et al.*, 1995), with both locations reporting harvested rainwater quality to be higher than that of the local spring and river water.

Ariyananda (1999) reviewed the water quality in aboveground ferrocement tanks and belowground burnt brick tanks in Sri Lanka. Despite the belowground tanks often being contaminated with lizards, frogs and ants, none of 39 samples of these tanks was positive for *E. coli* and total coliform concentrations ranged from zero to 170 CFU/100mL (Mansur, 1999 cited in Ariyananda, 1999).

In the Middle East, 200 cistern systems collecting rainwater from flat concrete roofs were sampled on the West Bank of Palestine. 54 of the systems (27%) contained coliform bacteria, 3% contained greater than 50 CFU/100mL (Abu-Sharekh & Subuh, 1995). On the island of Mauritius, off the east coast of Madagascar, a single rainwater harvesting system was tested weekly for one year. Nine of 55 samples tested positive for thermotolerant coliforms, with the average concentration 1.1 CFU/100mL (Michaelides, 1989). The results of these studies have been summarised in Table 2.1 to demonstrate the significant variability in detection rates and concentrations of thermotolerant coliform bacteria between systems in both the urban and rural context.

The levels of contamination in harvested rainwater by faecal indicator organisms must be viewed within the context of the levels of contamination in other water sources. Harvested rainwater is usually judged on the basis of it being a finished drinking water. When compared to drinking water guidelines, which specify zero faecal indicator organisms, harvested rainwater is usually found to require some form of treatment/disinfection, although when untreated has still generally been found safe to drink (Gould, 1999). When harvested rainwater is assessed as a source water rather than a finished (treated drinkable) water source, the conclusions may be far different, with rainwater being a relatively appealing choice of water supply.

The bacteriological quality in most modern rainwater tanks is often higher than that of the surrounding surface waters and unprotected traditional source waters (Gould, 1999; Yang *et al.*, 1995; Lye, 1992). Even protected surface water catchments often contain higher levels of index organisms, such as *E. coli*, than some of the maximum levels in rainwater tanks, as seen from comparison of Tables 2.1 and 2.2. Maximum levels of thermotolerant coliforms in rainwater tanks vary between tens to hundreds of organisms, with occasional concentrations in thousands of CFU/100mL (Table 2.1).

Often a significant proportion of samples test negative for *E. coli*. Conversely, average levels of *E. coli* in surface water catchment tributaries were found to be up to tens of thousands of CFU/100mL, depending on rain events and the extent of catchment protection from agricultural practices (Table 2.2).

Table 2-1: Summary of Thermotolerant Coliform (TTC) Contamination in Rural and Urban Domestic Rainwater Harvesting Systems

Location	No. Samples (Systems)	TTC Positive	TTC Range (CFU/100mL)
Urban			
Australia[A]	12 (1)	8 %	0 – 10
Australia[B]	13 (4)	23 %	0 – 8
Denmark[C]	14 (7)	79 %	0 – 990[§]
Rural			
Sri Lanka[D]	39 (NS)	0 %	0 – 0*
USA[E]	30 (NS)	3 %	0 – 20
Mauritius[F]	55 (1)	16 %	0 – NS
Micronesia[G]	203 (203)	29 %	0 – 100
Thailand[H]	100 (3)	33 %	0 – <10*
Caribbean[I]	57 (11)	35 %	0 – 42
Thailand[J]	136 (76)	40 %	0 – 100
U.S. Virgin Islands[K]	38 (10)	50 %	0 – 770
New Zealand[L]	125 (125)	56 %	0 – 840
Australia[M]	>100 (>100)	59 %	NS
Hawaii[N]	45 (9)	60 %	0 – 1000*
Hawaii[O]	25 (5)	68 %	0 – 92*
Hawaii[P]	9 (9)	78 %	0 – 4700*

NS Not Stated *E. coli

[§]Included rainwater samples collected from a mixture of roof and pavement runoff.

[A]Coombes *et al.*, 2003
[B]Coombes *et al.*, 1999
[C]Albrechtsen, 2002
[D]Ariyanada, 1999
[E]Lye, 1987
[F]Michaelides, 1989
[G]Dillaha & Zolan, 1985
[H]Wirojanagud, 1991
[I]Haebler & Waller, 1987
[J]Pinfold *et al.*, 1993
[K]Crabtree *et al.*, 1996
[L]Simmons *et al.*, 2001
[M]Plazinska, 2001
[N]Fujioka *et al.*, 1995
[O]Rijal & Fujioka, 1995
[P]Fujioka *et al.*, 1991

Table 2-2: Average *E. coli* concentrations in Sydney Catchment Tributaries

System Type	*E. coli* CFU/100mL	
	Dry	Rain Event
Fully Protected A	26	400
Fully Protected B	30	1190
Part Impacted A	31	6250
Part Impacted B	130	6690
Urbanised	450	10400
Intensive Agriculture	210	17700

(CRC for WQT, 2004)

The current body of literature relating to indicator organisms in urban rainwater harvesting systems contains a number of large data gaps. This is mainly due to past research focussing primarily on rural areas and developing countries where harvested rainwater is a source of drinking water. Within the literature, significant variability has also been presented on the temporal distributions and concentrations of indicator organisms in rainwater tanks, making it difficult to generalise or extrapolate results. Furthermore, the strategic use of rainwater in the urban environment is a relatively recent concept. The influence of a dual water supply on the presence of indicator bacteria in rainwater tanks has therefore not been well investigated, nor has their role in representing health risk been established.

2.2.2 Detection of Pathogens in RWH Systems

A number of factors currently exist which prohibit the regular and thorough testing of water samples for specific pathogens on a routine basis. As acknowledged by the ADWG and WHO-GDWQ, the detection of pathogenic organisms requires specific testing for, and identification of, the target organisms. This has contributed to the creation of several major deficiencies in the current body of literature that deals with pathogen detection in rainwater tanks. These include lack of health data to contextualise pathogen detection data (particularly within the context of non-drinking applications), lack of specificity in pathogen identification, over-dependence upon the use of indicator bacteria and the poor correlation of indicators with pathogens, and the lack of bacterial community profiling in rainwater tanks.

The first major deficiency is that almost all studies on pathogens in rainwater tanks have failed to provide information that enables the results to be contextualised within the broader context of human illness, making such data of only limited value and relevance. A relatively small number of studies have reported the detection of pathogenic bacteria in rainwater tanks in response to observed cases of illness. *Salmonella arechevalata* was isolated from a tap supplied by harvested rainwater at campsite in Trinidad at which a large number of campers developed gastroenteritis (Koplan *et al.*, 1978), while *Salmonella* Saintpaul was isolated from poorly maintained rainwater tanks in the northeast of Queensland, Australia, that was the site of a *Salmonella* outbreak (Taylor *et al.*, 2000). The isolation of *Campylobacter fetus* from the tank water of an elderly woman suffering recurring campylobacteriosis was attributed as the cause of the infections (Brodribb, 1995), and in NSW, *Clostridium botulinum* was isolated in a variety of household environmental samples, including rainwater tanks, of child patients suffering infant botulism (Murrel & Stewart, 1983). Cases involving illness will be more thoroughly reviewed in Section 9.2.2 – Health Risk Assessment.

However, very few studies have attempted to investigate rates of illness (or lack of) in response to the detection of pathogens. The result of this skew in the literature is that the correlation between pathogen presence and illness appears higher than is probably the case, as suggested by the results of epidemiological studies (Heyworth *et al.*, 2006). It is quite likely, therefore, that pathogens are frequently present in harvested rainwaters and do not cause illness to the users. This deficiency in the literature is further exacerbated by the lack of studies investigating pathogens that may be relevant to health risk via non-drinking routes of exposure, which is relevant within the context of urban rainwater harvesting. An example of this is the pathogen *Legionella pneumophila*, which infects the pulmonary tract and can be transmitted via showers and hotwater systems. *Legionella*-like organisms were isolated from three hotwater systems (operating below 52°C) supplied by harvested rainwater in the USA (Lye, 1991) and were detected in rainwater tanks by Broadhead (cited in Simmons, 1999) but were not detected by Albrechtsen (2002). There is a range of other non-drinking applications for tank waters potentially involving specific pathogens which have not been investigated.

The second major deficiency is that only specific species of a genus, and often only specific strains of pathogenic species, are actually capable of causing disease in humans. Despite this, studies often report the detection of pathogens as a result of identifying genera or species known to contain pathogenic organisms (e.g. Albrechtsen, 2002; Simmons *et al.*, 2001; Uba and Aghogho, 2000; Wirojanagud *et al.*, 1991; Lye, 1991). This leads to an overestimate of the predicted pathogen load on rainwater tanks. Albrechtsen (2002) reported the detection of *Aeromonas* in two of 14 tank water samples, *Campylobacter* in two of 17 samples and *Mycobacterium avium* in one of 14 samples though did not confirm the pathogenicity of the isolates. A number of studies have, however, detected known pathogens. In Thailand, *Salmonella* gr. E and gr. C were detected in 2% and 6% of samples, respectively, and *Vibrio parahaemolyticus* was detected in 3% of samples (Wirojanagud *et al.*, 1991). Fujioka (1991) identified low levels of *Clostridium perfringens* and *Salmonella* spp. in cisterns in Hawaii, while Canoy and Knudson (cited in Simmons, 1999) isolated *Shigella* spp. The isolation of multiple pathogenic species of Mycobacteria in tank waters was confirmed in Queensland, Australia (Tuffley & Holbeche, 1980), and Uba and Aghogho (2000) identified *Salmonella, Shigella* and *Vibrio* in roof runoff waters in Nigeria.

The distribution and identification of protozoan pathogens, namely *Cryptosporidium* and *Giardia*, have also not been well established. Despite their main reservoir being ruminants, these pathogens have been detected in rainwater tanks in the U.S. Virgin Islands (Crabtree *et al.*, 1996). While the majority of species of *Cryptosporidium* detected in this study were non-parvum, *C. parvum* oocysts were found in 20% of private cisterns (average 2.8 oocysts/100L), although the viability of these oocysts was not determined. *Giardia* was also identified in 23.2% of samples (average 1.12 cysts/100L). However, the likelihood of these pathogens being detected in urban rainwater tanks is not clear from the literature. Tank samples from a water-sensitive development in urban Australia were found to be free of both *Cryptosporidium* and *Giardia* (Coombes *et al.*, 1999), while in urban Denmark *Cryptosporidium* was detected in six of 17 samples, though the actual species of *Cryptosporidium* were not determine (Albrechtsen, 2002).

Lye (1991) tried to more directly relate microbial water quality data to human health risk by using an assay technique to determine the virulence factors of bacterial isolates from rainwater tanks in the USA. The study showed that a higher proportion of rainwater tank isolates demonstrated haemolytic, cytolytic or proteolytic activity than surface or treated drinking water isolates. A similar study by the same authors two years prior showed that surface waters contained the highest percentage of isolates expressing multiple virulence factors (Lye, 1989). While these studies provided an effective method for capturing potential pathogenicity of isolates in rainwater tanks, they did not establish correlations with actual health risk data.

A third major deficiency is that bacteria are the most commonly identified pathogens in rainwater tanks, although are often not accurately represented by faecal indicator organisms. Examples include Simmons *et al.* (2001), who found that while 56% of 125 rainwater tank samples exceeded the New Zealand Drinking Water Standards for faecal coliform bacteria (<1CFU/100mL), only *Aeromonas spp.* (16% of samples), *Salmonella typhimurium* (one sample), and an unidentified species of *Cryptosporidium* (2 of 50 samples) were detected in the tanks. *Legionella* spp., *Campylobacter jejuni*, and *Giardia* were not detected in any samples. In Germany, Hollander *et al.* (1996) found that while most of the 1600 samples of rainwater in storage tanks contained *E. coli* (average 26/100mL), only 12% contained *P. aeruginosa* and only one contained *Salmonella*. None of the samples contained *Staphylococci*, *Yersiniae*, *Shigellae*, or *Legionellae*.

The final inadequacy of the current literature is the lack of information on microbial community profiles in rainwater tanks. Understanding the typical microbial components of rainwater systems provides valuable insights into the sources of microbial inputs, the actual health risks of detected pathogens, and the microbial ecology within tanks. As with indicator organisms, there is a substantial deficit of literature relating to the presence of pathogenic organisms in rainwater tanks in urban regions and few studies have given consideration to pathogens that may be relevant to non-drinking routes of disease transmission.

2.2.3 Detection of Heavy Metals in RWH Systems

The concept of measuring indicator parameters is not generally applied for monitoring heavy metal contamination as it is with microbial contamination. Heavy metals are inorganic contaminants with a potential for contaminating urban rainwater harvesting systems. A range of specific metals are usually measured based on the likelihood of their presence in tankwaters or their public health importance. Perhaps the single most significant heavy metal contaminant of harvested rainwater is that of lead. Many studies have noted high levels of lead in tankwaters, and combined with the high toxicity of lead, particularly to children, it should be flagged as a parameter requiring close monitoring.

In New Zealand, Simmons *et al.* (2001) found that of 125 sampled rainwater tanks 17.6% exceeded one or more of the NZ guideline limits for chemical contaminants. Lead was the most common inorganic contaminant with 14.4% of systems exceeding the guideline limit of 10μg/L, with 2.4% of systems exceeding the 2mg/L limit for copper (Simmons *et al.*, 2001). In a comparative study on the effects of roof material and location on harvested water quality, Thomas and Greene (1993) found lead concentrations in roof runoff in industrial areas to average around 100μg/L with maximums up to 200μg/L. The authors found lower lead concentrations in urban areas (10–50μg/L) and undetectable levels in rural areas (Thomas & Greene, 1993).

Lead concentrations in 24 samples taken from concrete, galvanised iron and fibreglass tanks in the eastern Caribbean averaged 12μg/L and ranged up to 90μg/L (Haebler & Waller, 1987). Arsenic concentrations also exceeded ADWG limits in some samples, averaging 70μg/L and ranging up to 20μg/L. Other metals, including manganese, copper, aluminium, barium and cadmium were all below current ADWG limits (Haebler & Waller, 1987). In the northwest of China, lead concentrations in tank waters averaged 60μg/L in both the industrial town of Yuzhong and the less industrial town of Tongwei (Yang *et al.*, 1995).

While lead is a common contaminant in harvested rainwater supplies, not all investigations have resulted in the same conclusions. In Australia, Coombes *et al.* (2000b; 2003) found that neither lead, iron nor cadmium exceeded ADWG limits in tanks at two field sites in the urban/industrial city of Newcastle. Zinc was found in concentrations exceeding ADWG in one system (3.9mg/L) although guideline limits for zinc are based on aesthetic criteria rather than health risk. Similarly, Ghanayem (2001) did not detected elevated lead levels in cistern waters in Palestine. In Thailand, lead concentrations were below detection limits although manganese and zinc concentrations were approximately 4 mg/L and 10 mg/L, respectively, equating to an eight and a three fold exceedance of current ADWG limits (Wirojanagud *et al.*, 1991). Equally, in China, the low fluoride concentrations in harvested rainwaters (around 0.78mg/L) resulted in the reduction in the rates of endemic fluorosis amongst 8-15 year olds from 93% to 26% within 10 years of switching the main drinking water source from groundwater to harvested rainwater (Ling, 1997).

Hence, the literature presents only a limited amount of data relating to heavy metal contamination in urban rainwater harvesting systems, and significant variability exists within this data. Heavy metal contamination data is particularly sparse for urban systems and the large variability in metal concentrations between studies has shown that proximity to urban areas does not correspond closely with levels of heavy metal contamination.

2.3 Incidental Treatment Train Mechanisms

It is possible with modern technology to transform even the most contaminated waters into clean drinking water sources and many products are already available that can improve and disinfect harvested rainwater. A large number of studies have also suggested regular maintenance regimes be carried out by householders on their rainwater harvesting systems (Michaelides, 1989; Krishna, 1991), although it is abundantly clear that in very few cases either disinfection or maintenance strategies are actually employed (e.g. Plazinska, 2001). However, despite a lack of deliberate intervention by householders, evidence is emerging of the existence of a treatment train operating within rainwater harvesting systems resulting in the improvement of water quality (Scott & Waller, 1987; Coombes *et al.*, 2000; Fujioka & Chinn, 1987). Therefore, this thesis attempts to understand and evaluate those mechanisms which are independent of householder activity and which are 'base-case' mechanisms common to all rainwater harvesting systems.

2.3.4 Conceptualising the Treatment Train

Incidental treatment train mechanisms are defined in this thesis as mechanisms that improve water quality but have not been deliberately installed or established by the householder to do so. The ability of natural water bodies to undergo 'self-purification' is a phenomenon that has in the past been viewed with mystique, while at the same time being extensively relied upon. The concept of bioremediation is inherent in all natural systems including water courses, where chemical and biological processes lead toward an equilibrium unique to the specific circumstances of a given water body.

Natural systems have been exploited for millennia for their ability to remove or decrease pollutant levels, such as the disposal of sewage into rivers and ocean outfalls (Reed, 1975). The concept of the 'assimilation zone' in such systems is based on the principle that natural systems are able to incorporate, utilise or destroy waste products within a specific geographical space or time, preventing the area beyond the assimilation zone from being influenced by the waste input (Cullen, 1986; Cairns, 1981). A number of outbreaks from contaminated water supplies have been traced back to apparent failures of, or an overburdening on, this self-purification process, such as the Milwaukee *Cryptosporidium* outbreak (MacKenzie *et al.*, 1994). Much more is now understood about the complex ecological interactions of water within the environment and about the processes of self-purification.

Through our understanding of the mechanisms involved in natural bioremediation, humans have been able to engineer structures and processes to mimic and exploit such treatment mechanisms, as seen in modern water and wastewater treatment plants. Municipal water and wastewater treatment plants operate using the same treatment train principles, namely exploiting physical (primary treatment), biological (secondary treatment) and chemical/disinfection (tertiary treatment) mechanisms (Lester & Birkett, 1999; Tebbutt, 1998). Physical forces include straining large objects from the water supply followed by sedimentation of coarse particulate matter. Biological mechanisms include consumption of nutrients and organic matter from the bulk water phase by microorganisms through processes such as sand filtration, biofilm filtering and activated sludge. Final treatment of waters and wastewaters includes disinfection through the addition of chemical disinfectants (such as chlorine, ozone, colloidal silver, copper etc) or through the exposure of the water body to environmental stresses (such as UV, heat etc.).

Within rainwater harvesting systems, a limited number of studies have observed improvements in stored rainwater quality over time and at different points in the collection and distribution system suggesting that a degree of self purification also occurs. Within the storage tanks, this is indicated by general decreases in contaminant levels over time (e.g. Thomas & Greene, 1993). Through the collection and distribution system significant improvement in water quality has been reported at various points along the system (Scott & Waller, 1987; Coombes *et al.*, 2000).

Scott and Waller (1987) explicitly tested the hypothesis that the physicochemical quality of water improves through rainwater harvesting systems by monitoring water quality at various points along a system, including in roof runoff, at three depths within the tank water column and at the indoor tap. While the study did confirm changes in water quality were occurring throughout the system, the study focussed on basic physicochemical parameters including calcium, alkalinity, pH, potassium and phosphorus, and did not determine whether the same phenomena occurred for the high risk contaminants, namely microbial and heavy metal contaminants. The study also failed to suggest or elucidate any of the mechanisms responsible for such changes and did not explicitly acknowledge the existence of a treatment train.

Coombes *et al.* (2000) extended this research by including microbial and heavy metal parameters in a study which also found improvements in water quality through a rainwater harvesting system. While no investigation has elucidated the mechanisms responsible for improvements in water quality in rain harvesting systems, Coombes *et al.* (2000) speculated that the likely contributing factors may include sedimentation, flocculation, and bio-reaction processes. The process of sedimentation is clearly evident from simple visual observation of tank sludge, while processes of bioreaction, such as the absorption of contaminants into biofilms, starvation, and stratification may also prove significant but have not been experimentally tested. Several factors may influence the rates and extent of self-purification in tanks such as the quality of the incoming water, physical characteristics of the tank, pH, temperature and the addition of chlorinated mains water.

2.3.5 The Catchment Environment

The catchment environment, namely the roof, is a dynamic environment constantly undergoing the duelling processes of contaminant deposition and removal. The catchment is the first and the major entry point for both microbial and chemical contamination. At the same time, however, the treatment train begins on the roof, providing a hostile environment for microbial cells and rendering chemical contaminants vulnerable to photodecomposition and wind removal.

Various studies have shown roofing materials to influence survival rates of microbial cells. Yaziz *et al.* (1989) demonstrated higher levels of thermotolerant and total coliform levels in runoff from concrete tile roofs compared to galvanised iron roofs. Vasudevan *et al.* (2001) also found a significant improvement in the bacterial quality of water running off galvanised iron roofing than asbestos and plastic, with tile roofing providing water of the lowest microbial quality. This was thought to be due to the disinfecting effects of greater heating of the galvanised iron (Vasudevan *et al.*, 2001). A similar conclusion was reached by Ghanayem (2001) in Palestine where levels of thermotolerant and total coliforms were both lower in runoff from galvanised iron and asphalt compared to tile and concrete surfaces.

2.3.6 Storage

The second step in the treatment train is water storage, during which time a number of mechanisms are likely to be operating. The storage of water under passive conditions with no shear force or flow agitation is one of the most commonly practised methods of water purification. It is considered an important step in both municipal drinking water and wastewater treatment facilities (Tebbutt, 1998; Lester & Birkett, 1999) and is practised in many developing countries on a smaller scale (e.g. Hussein *et al.*, 1997).

Numerous examples exist in the literature where the quality of stored rainwater has improved over time. In Chima, Yang *et al.* (1995) found that concentrations of suspended solids, total coliforms and total bacteria were lower in water that had been stored for long periods. The authors also noted that the use of concrete tanks provided water with the lowest total coliform counts, and concluded that storage cellar material influences microbial water quality (Yang *et al.*, 1995). In another region of China suffering endemic fluorosis caused by fluoride-contaminated groundwater, Ling (1997) observed that the switch to tank water not only reduced the prevalence of fluorosis but that the storage of harvested rainwater for a 6 month period lowered fluoride concentrations in tank waters by greater than 50 percent.

In Bangladesh, Hussein (1997) found that water quality improved over a two week period after rainfall and exploited this by implementing a storage pit for new rainfall prior to transfer into a household jar for consumption. In Thailand, the pH of tank waters was found to be significantly higher than that of roof runoff resulting in reduced metal solubility (Wirojanagud *et al.*, 1991). The concentrations of heavy metals were also found to be lower in the surface layers of the tank water columns than in the deeper layers, which were speculated to be due to the action of adsorption and precipitation (Wirojanagud *et al.*, 1991).

In Japan, Kita and Kitamura (1995) and Kitamura *et al.* (1997) conducted experiments to monitor fluctuations in physicochemical parameters of rainwater stored in 300L polyethylene containers over three month periods. In both studies the authors observed decreases in turbidity over the initial two weeks, decreases in ammonium and nitrite after seven weeks (Kita & Kitamura, 1995), and stable levels of pH, COD, and colour over the duration of the experiments.

The mechanisms responsible for improving water quality during storage are presumed to be predominantly from sedimentation of particulate matter, biological consumption or uptake into biofilms, and starvation of microbial cells. These mechanisms have not been explicitly explored in previous studies, and current literature is limited to speculation or indirect inference.

2.3.7 Sedimentation

Possibly one of the most simple yet significant water treatment mechanisms is that of sedimentation. As particulate matter and larger organic contaminants enter rainwater tanks and settle to the bottom a base layer of sludge accumulates. This sludge layer is likely to contain high concentrations of a variety of microbial and heavy metal contaminants, although few studies have actually analysed this sludge layer in detail.

Scott and Waller (1987) analysed one sludge sample from a concrete rainwater tank and found it to contain relatively high levels of heterotrophic bacteria (20,000 CFU/mL). However, the authors did not analyse the sludge for anaerobic species nor indicated whether any attempt was made to disaggregate and break up the clumps of colonies that bacteria preferentially form, and hence this figure may have been a significant underestimate. Heavy metal concentrations were found to be high for lead (1,750 μg/g), iron (17,200 μg/g), aluminium (12,500 μg/g) and calcium (51,800 μg/g), and almost 60% of the sludge was volatile solids. A variety of algae were noted including Anabaena, Chlorococcum, Spirogyra, Chrysococcus, and Tabellaria, as well as non-aquatic mites, aphids, flies, and beetles, various flowers, seeds, and organic vegetation debris (Scott & Waller, 1987). Coombes *et al.* (2000) also reported that concentrations of metal, chemical and microbial parameters in samples taken from the sludge of a rainwater tank exceeded ADWG limits.

Many published reports have speculated on the influence of sludge on tank water quality (e.g. Plazinska, 2001), although none were found that had actually analysed and compared the physical and behavioural characteristics of tank sludges. There is a current deficit in the literature regarding many important aspects of tank sludge such as the spatial distribution of sludge within and between tanks, the spatial distribution of contaminants within sludges, sludge accumulation rates, and behavioural properties of the sludge such as settlement rates and potential for re-suspension. Furthermore, it is also possible that tank sludge, if resuspended, could act as a coagulant on suspended particulate matter, such as microbes, and enhance settlement of these suspended particles, although this also has not been investigated.

2.3.8 Biofilms

Another interesting phenomenon speculated to be playing an active role in influencing water quality in rainwater tanks are biofilms (Coombes *et al.*, 2000). Biofilms are layers of bacteria that develop rapidly on surfaces in low nutrient conditions (Costerton, 1995; Marshall, 1988). Coombes *et al.* (1999; 2000) speculated that variations in tank water quality were due to the role of biofilms, sorption and bio-reaction. Given that the vast majority of all bacteria exist within biofilms (Lappin-Scott & Bass, 2001) and that biofilms develop in almost every type of aquatic environment (Madigan *et al.*, 1997) it would be logical to assume some degree of biofilm development occurs on the walls of rainwater tanks. However, the current major deficit of research into rainwater tank biofilms has limited our understanding of their role in improving or degrading tank water quality.

2.3.8.1 Biofilm Structure and Function

Biofilms have evolved to become sophisticated and complex ecologies of micro-organisms and are responsible for two key processes. Firstly, biofilms provide shelter for microbes from environmental stresses such as disinfectants and nutrient limitations (Armon, 1997; Keevil, 1995). The second significant role of biofilms relates to their ability to remove various compounds from the surrounding water. These compounds are either co-metabolically degraded by various intercellular interactions or locked into the extracellular polymeric substance (EPS) (Szewzyk *et al.* 2000). The EPS is the slimy environment which encases the cells, composed primarily of a polysaccharide framework, with proteins, lipids, DNA, and sorbed compounds contributing to localised structures. To date, there have been no published investigations into the existence or role of biofilms in rainwater tanks, although the ability and inclination for microorganisms to colonise almost any aquatic surface means that they are no doubt present and functioning within rainwater tanks.

2.3.8.1.1 Cellular Protection in Biofilms

It is well known that biofilm-associated cells display resistance to disinfectants and antimicrobial substances. This has been viewed with some concern in a number of areas including the medical and health sciences (prosthetics, nosocomial infections, and dentistry) and industry (corrosion of ships, heat transfer loss, and flow resistance in pipelines). This concern is strongly indicated by the majority of research which has been directed at finding ways to limit or destroy biofilm development (Norton & LeChevallier, 2000; Taylor *et al.*, 2000b; Xu *et al.*, 1998). Within the water sector and particularly in centralised water systems, biofilms are regarded as a nuisance for their ability to protect resident cells from disinfection regimes by removing chlorine residual from the bulk water (Koudjonou, *et al.*, 1997; Watnick & Kolter, 2000). This has caused re-growth problems in some systems and has led to the need for multiple points of disinfection throughout these systems (LeChevallier, 1996).

2.3.8.1.2 Contaminant Removal Capacity of Biofilms

While biofilms are unwanted in mains water systems because of their ability to remove chlorine, they have been used successfully in wastewater treatment plants for removing unwanted nutrients and contaminants from sewage (LeChevallier *et al.*, 1996; Norton & LeChevallier, 2000; Sharp *et al.*, 2001). Within rainwater tanks, the influence of biofilms on water quality has never been investigated. However, the lack of systematic disinfection practices by householders with rainwater tanks means that the removal of any constituent from the water should be considered as beneficial, rendering biofilms a potentially important natural filter.

Biofilms have been shown to increase the adsorption of heavy metals (Scott & Karanjkar, 1998), nitrogen and phosphorus (Choi *et al.*, 2001). Their resistance to such things as toxic metals, organic toxins, and chlorine, have meant that these substances which are toxic to humans can be actively removed from water. Sorption sites within biofilms include EPS, cell walls, cell membranes and cell cytoplasm (Flemming, 1995). The advantage of such heterogeneous communities is that through symbiotic relationships and co-metabolic degradation, complex molecules can be broken down and used for metabolism and production of EPS. Conversely, substances toxic to the cells of a biofilm may lead to the temporary activation of the cells to produce more EPS in which to lock the toxins (Schmitt, 1995). Compounds bound into the EPS may enhance further contaminant removal due to the increase in adsorption sites (such as production of carboxyl groups in response to toluene stimulation which increases the adsorption rate of cations) exposed to the water phase (Schorer & Eisele, 1997).

Biofilm thickness is an important aspect for water treatment as it has been shown that thicker biofilms have an increased capacity for heavy metal, suspended solids, and nitrogen removal from water (Choi *et al.*, 2001). Thicker biofilms also increase the opportunities for the bacterial consortium to develop more sustainable heterogeneous ecologies. In the deeper layers of biofilms the conditions are often anaerobic, which if given the appropriate inoculations, could allow for the development of denitrifying species, such as *Bacillus licheniformis* and *Paracoccus denitrificans*, which would allow for the full processing of nitrogen. The establishment of other anaerobic bacteria in the thicker biofilms, such as sulphur reducers, would allow for the consumption of recalcitrant compounds and elements considered harmful to humans.

2.3.9 Tank Water Disinfection

Disinfection is the inactivation of disease causing bacteria and is practised by most water and wastewater treatment operations in the developed world. Disinfection through heating will be addressed separately in Chapter 10.

2.3.9.1 Tank Water Disinfectants

Strategies for disinfecting or improving water quality that involve the deliberate intervention of householders are not considered herein to be incidental treatment mechanisms and consequently do not fall within the focus of this thesis. However, a number of different methods have been suggested in the literature to disinfect tank waters. Briefly, these include UV treatment (Fujioka *et al.*, 1995; Joklik, 1995), Microdyn colloidal silver (Owen & Gerba, 1987), chlorination (Bo & Guangen, 2001), exposure to natural sunlight (Fujioka & Chinn, 1987), addition of burnt snail shell (Hussain *et al.*, 1997), micro-filtration (Bo & Guangen, 2001) and pasteurisation (Coombes *et al.*, 2000; Bo & Guangen, 2001).

2.3.9.2 Chlorinated Mains Top-Up Water

Under the suggested configuration by Coombes (2002), tanks will be slowly topped-up with municipally-supplied water. This mains water is typically expected to contain up to 1mg/L of free chlorine residual which may potentially have a substantial impact on tank water quality. As incoming mains water requires no intervention by the householder and is recommended as a widespread plumbing configuration for urban rainwater harvesting systems, the influence of chlorinated mains top-up water is considered here to be an aspect of the incidental treatment train mechanism. No published literature currently exists which has looked at the influence of mains water on tank water quality.

2.3.10 Delivery System Stresses

A less obvious treatment train mechanism may exist within the pump and pipework which delivers water from the tank to the house. A single publication has noted an improvement in water quality from the outlet of the tank to the door tap (Fujioka & Chinn, 1987). In this study, the authors found a decreases of up to three orders of magnitude in thermotolerant coliform and faecal streptococci concentrations between tank and tap samples in four separate systems. While no speculation was made as to the possible causes of these improvements in water quality by Fujioka and Chinn, the two most feasible explanations involve cellular disruption caused by pressure increases imposed by the pumps and copper toxicity incurred from leaching of the copper pipes.

2.3.11 Thermal Inactivation of Micro-organisms

While not all households opt to include hotwater in their suite of uses for harvested rainwater, for those that have, this final treatment train mechanism is possibly the single most significant in the system. The destruction of microorganisms through heating is an old practice and is commonly applied to many areas within the food and health industries. Sterilisation of medical and scientific equipment, disinfection of food products and pasteurisation of milk are some of the common applications of thermal disinfection. The principles of thermal inactivation have also been used for water disinfection in a variety of circumstances. During times of contamination of municipal water supplies or suspected outbreaks water authorities typically implement a widespread recommendation to boil water. This occurred in Sydney in 1998 during a highly publicised *Cryptosporidium* contamination. Heating water is a simple technique also used by travellers in developing countries to ensure the safety of local water supplies. Bandres *et al.* (1988) replicated the cooking and water heating practices of a number of third world hotels and found temperatures above 65°C appear to destroy most *E. coli*, *S. sonnei*, *Salmonella*, and *C. jejuni* cells. One of the most beneficial applications of thermal inactivation is from solar disinfection techniques used in many third world countries. Exposure of heavily contaminated waters to heat and sunlight has been shown to significantly reduce faecal coliform concentrations in waters held in transparent plastic bottles (Conroy *et al.*, 1996; Joyce *et al.*, 1996; Ciochetti & Metcalf, 1984).

2.3.11.1 Thermal Treatment of Rainwater

Very little data exists confirming the performance of domestic hotwater services in reducing bacterial numbers. The extent of the limited field studies have been conducted by Coombes *et al.* (2000; 2003) and have indicated that domestic hotwater systems operating at sub-boiling temperatures can greatly reduce concentrations of indicator organisms in rainwater supplies. The monitoring results from two demonstration projects have revealed that rainwaters treated via storage hot water systems (temperatures 50°C to 65°C) and instantaneous hot water systems (temperature 55°C) can produce water compliant with Australian Drinking Water Guidelines for index organisms and significantly reduce HPC concentrations (Coombes *et al.*, 2002; Coombes *et al.*, 2003). However, there are a number of types of hotwater systems that have not been monitored for their ability to disinfect rainwaters, including a variety of the increasingly popular solar hotwater systems. Furthermore, identification of species surviving hotwater systems has not been undertaken, nor is data available in the literature on thermal inactivation rates of relevant pathogens in conditions similar to those within domestic hotwater systems. These are significant topics of interest to both water engineers and public health officials and for which current research is lacking.

2.3.11.2 *Legionella* Control

While hotwater systems generally improve water quality, there are cases in the literature where the inappropriate operation of some hotwater systems has resulted in elevated health risk. These health concerns have resulted primarily from the bacteria *Legionella pneumophila*, which is known to inhabit warm water systems throughout the world. *L. pneumophila* is the aetiological agent of Legionnaires disease, an acute form of pneumonia, which most commonly infects the respiratory tract of immuno-compromised individuals. *L. pneumophila*-associated infections occur as a result of the inhalation of contaminated aerosols. Ingestion of high concentrations of *L. pneumophila* does not cause harm.

Hotwater systems originally received attention as posing a potential risk to public health after the isolation of *L. pneumophila* from several hospital hotwater systems implicated in nosocomial outbreaks of Legionnaires Disease (Ezzeddine *et al.*, 1989). These cases generally involved chlorinated municipal waters being supplied to hotwater systems operating at insufficiently high temperatures (<50°C) allowing *L. pneumophila* to multiply to concentrations considered dangerous (Darelid *et al.*, 2002; Goetz *et al.*, 1998). In Australia and other countries, subsequent recommendations were made (for example AS3500.4.2) stating that hotwater systems should operate at a minimum of 60°C in order to inhibit the growth of *L. pneumophila* (Standards Australia, 1997).

Within hotwater systems supplied by harvested rainwater, Lye (1991) found that three hotwater systems maintained below 53°C were positive for *Legionella*-like isolates. The two systems maintained above 60°C were negative for such isolates. When the operating temperatures of the three hotwater systems operating below 53°C were increased to above 60°C, *Legionella*-like isolates could no longer be isolated, confirming that operating temperature is significant for *Legionella* control.

While hotwater systems supplied by rainwater are not immune from *Legionella* contamination, there are numerous investigations in the literature into *Legionella* contamination of hotwater systems supplied by a variety of water sources. Many of these studies have investigated the influence of design and operating conditions on levels of *Legionella* contamination. The two major designs of hotwater system are storage tanks and instantaneous heaters. Martinelli *et al.* (2000) found that *L. pneumophila* were isolated much more frequently from hot water tanks (30%) as opposed to instantaneous hotwater systems (6.4%) in an area of Italy suffering an endemic outbreak of Legionellosis. The study concluded that temperature was critical, as the hotwater tanks in the study had been maintaining water at 50°C +- 5°C, while the tap water averaged 45°C and dropped to 40°C during flow. Instantaneous systems, however, always maintained temperatures above 60°C (Martinelli *et al.*, 2000).

Ezzeddine *et al.* (1989) found that the main reservoir for *L. pneumophila* were mixing tanks where hotwater at 60-65°C mixed with cold water to achieve 45°C. Maintaining temperatures of 60°C or accelerating flow rate were found to be the most effective methods of controlling *L. pneumophila* (Ezzeddine *et al.*, 1989). Lee *et al.* (1988) discovered that water temperatures in electrically heated tanks were significantly lower than in gas-heated tanks. They concluded that the presence of *L. pneumophila* was associated with systems maintained below 48.8°C and that city residences were more likely to be colonised than suburban residences (Lee *et al.*, 1988). Rogers *et al.* (1994) found that in hotwater systems maintained at 40°C *L. pneumophila* were at their most abundant. At 50°C the diversity of organisms was greatly reduced and numbers of *L. pneumophila* were reduced, and at 60°C *L. pneumophila* were absent from the system. Similarly, Wadowsky *et al.* (1985) detected *L. pneumophila* in water and sediment samples taken from hotwater systems maintained at 30-54°C, but not from systems maintained at 71-77°C. These studies are all consistent with the known optimal growth temperature of *Legionella* at around 36°C (Szewzyk *et al.*, 2000).

2.3.11.3 Pathogen Inactivation Rates

An extremely useful data tool for determining the theoretical microbial reduction capacity of domestic hotwater systems is the D-value. The D-value, or decimal reduction value, is defined as the time required to reduce a microbial population by one log-reduction at a given temperature. These thermal inactivation rates are specific to each bacterial species, enabling the more heat resistant species to be identified as well as providing insights into the stability of the heat resistance capacities of each species when challenged at various temperatures. This theoretical data is important for evaluating the disinfection efficiency of hotwater systems and for conducting risk assessment due to the fact that the inoculation of hotwater systems by particular pathogens is only a random occurrence. An isolated sample from a hotwater system may show a negative result for a given pathogen, but if the pathogen was not present, or present in low concentrations in the tank waters to begin with, then little insight has been gained into the ability of the hotwater system to achieve disinfection.

However, little information is available relating to the dynamics of bacterial death in a freshwater medium in the sub-boiling temperature ranges relevant to the evaluation of domestic hotwater systems. Past sterilisation practices have focussed on temperatures exceeding 100°C which naturally targeted the most heat resistant spore-forming bacteria, very few of which are relevant to potable water supplies. Unfortunately, the previous research on solar disinfection (Joyce *et al.*, 1996; Ciochetti & Metcalfe, 1984; Conroy *et al.*, 1996; Safapour & Metcalfe, 1999) and water heating for treating travellers diarrhoea (Bandres *et al.*, 1988) has lacked either precise species identification, temperature monitoring, or time keeping, resulting in the inability to derive actual inactivation rates for specific species. The limited amount of applicable research into thermal inactivation rates for water related species has been conducted by Stout *et a*l. (1986), Dennis *et al.* (1984) and Sanden *et al.* (1989), and has focussed primarily on *Legionella.* Stout *et al.* (1986) also investigated the thermal inactivation rates for *Pseudomonas aeruginosa* and *Staphylococcus aureus*. However, there are many other species relevant to harvested rainwaters for which no thermal inactivation data exists.

There is a large body of literature targeting thermal destruction rates of bacteria within perishable goods (Pagan *et al.*, 1999; Oteiza *et al.*, 2003; Juneja & Marmer, 1999). Several studies have assessed the thermal tolerances of a number of bacteria including *Aeromonas*, *Campylobacter*, *E. coli*, *Salmonella*, and *Shigella* in liquid nutrient and saline broths (Lee *et al.*, 1989; Oosterom *et al.*, 1983; Palumbo *et al.*, 1987) reviewed by ICMSF (1996). It has been clearly demonstrated that environmental variables, such as pH, water activity, and fat content of the medium, can greatly influence thermal inactivation rates (Gibson, 1973; Goepfert *et al.*, 1970; Humphrey & Lanning, 1987). Growth phase and nutrient availability are also known to have pronounced influences on heat resistance with cells in stationary-phase and starved cells demonstrating maximum resistant, due at least partly to the translation of the global regulator rpoS gene (Fujita *et al.*, 1994; Loewen & Hengge-Aronis, 1994; Miksch & Dobrowolski, 1995; Yildiz & Schoolnik, 1998). However, while these studies provide useful insights into relative heat differences and influential experimental variables, the results cannot be extrapolated to a water medium without explicit experimental testing.

2.4 Conclusions

While 17% of Australia's population now have a rainwater tank, very little is known about water quality within urban RWH systems and the mechanisms causing variations in water quality. The literature relating to water quality in urban and rural rainwater harvesting systems was reviewed and a number of data gaps were identified. At present, there is relatively little data available on water quality in urban rainwater tanks, even for basic microbial parameters such as the indicator organism groups. The majority of studies have focused on rural areas, often in developing countries where tropical to semi-tropical climates exist, and where systems are often poorly constructed and therefore not representative of modern urban RWH systems.

The knowledge gaps resulting from the lack of literature on urban rainwater harvesting is accentuated by the large degree of water quality variability noted in the studies. This has revealed a poor ability to generalise and extrapolate data both temporally and spatially between RWH systems. Urban tank water quality is also poorly understood due to the fact that few studies that have investigated the influence of having an integrated dual water supply of rainwater and treated municipal water. Furthermore, previous studies into tank water quality have focused on only a narrow band of contaminants prescribed in drinking water guidelines. This is understandable due to the primary focus of health risk being placed on specific bacterial groups. However, it has resulted in a deficit of knowledge on the majority of parameters making up total microbial water quality and limits our ability to understand the processes occurring within rainwater tank ecology.

The concept that RWH systems have an incidentally-occurring treatment train acting to improve water quality is also a novel scientific idea that has received almost no attention in the literature to date. A limited amount of literature suggests that spatial and temporal variations in water quality occur within single rainwater harvesting systems. Intra-system water quality variations were clearly noted by Scott and Waller (1987) and followed by Coombes *et al.* (2000) who first posed the hypothesis that RWH systems may incidentally contain microbial and chemical processes capable of water quality remediation. Several possible components of the treatment train have been identified from literature relating to water and wastewater treatment systems, though have not been confirmed experimentally as components of the RWH system treatment train.

The importance of understanding water quality issues relating to urban rainwater harvesting systems stems from the substantial increase in popularity of rainwater harvesting in urban areas as a means of improving urban water cycle management. Therefore, the research in this thesis will be focussed on investigating the water quality issues relevant to RWH systems operating in the urban environment. These investigations will focus on water quality at the various points-of-use, particularly hotwater taps, as well as within rainwater tanks to ensure that intra-system variability does not distort the water quality data. Furthermore, in selected rainwater tanks comprehensive water quality analysis will be conducted including establishing bacterial community profiles.

The general lack of data in this area has left significant scope for investigations into RWH treatment trains to be of significant and novel value. This thesis attempts to fill this large knowledge gap by undertaking a holistic research approach to identifying and confirming the presence of components of the treatment train and to investigate their impacts on water quality. Developing an understanding of the treatment train will enable a greater understanding of the variations of water quality within systems and ultimately to the safer and most optimal design, management and utilisation of this limited natural resource.

-------- SECTION I --------

Water Quality

CHAPTER 3

Water Quality

3.1 Introduction

This chapter comprises Section 1 and investigates the water quality in a number of rainwater harvesting (RWH) systems as well as exploring possible causes for inter-system water quality variations. As was noted in the literature review (section 2.2), water quality can vary significantly between RWH systems with the causes of these variations sometimes being attributed to specific design, environmental or geographical factors. However, as was also clear from the literature, our current level of understanding of the causes and processes contributing to the quality of harvested rainwater is not sufficient for predictions to be made about the water quality of RWH systems in locations not yet investigated.

Furthermore, considering the importance of rainwater harvesting within the urban environment for achieving water sensitive urban design (WSUD), data relating to the quality of roof-harvested water in urban environments is relatively sparse. In light of this, collaboration between the University of Newcastle, Brisbane City Council (BCC), Newcastle City Council (NCC) and other organisations resulted in the initiation of trial projects in two major Australian cities involving the retrofitting of existing houses or the inclusion in new developments of RWH systems together with extensive monitoring of these systems. From the literature review, a number of hypotheses relating to water quality were proposed. These include that the water in RWH systems would often not comply with Australian drinking water standards, that system design would impact on bacterial counts in stored water, and that water quality would vary at different points in each RWH system.

The aims of this chapter were therefore to, firstly, evaluate the quality of water in the rainwater tanks in the Brisbane and Newcastle studies in the light of drinking and non-drinking water quality guidelines, and secondly, to examine a range of system design variables that may influence water quality, including roofing and tank construction materials, tank size, tank volume to roof area ratio and overhanging tree coverage, as well as seasonal effects and rainfall. The studies were not aimed to allow direct comparison between regions but were designed to provide complementary data sets.

3.2 Experimental Design and Methods

3.2.1 Project Description

The two project sites were located in Brisbane and Newcastle, both major cities located on Australia's east coast. The Brisbane project was part of the '30 Homes' pilot project and included the monitoring of tanks at a number of sites across the Brisbane city district. After initial consultation on water quality monitoring strategies with the University of Newcastle, the project was managed by BCC. Most of the tanks monitored in the Newcastle initiative were part of the 'Kotara Roof to Creek' project and all water quality aspects of this project were managed and conducted by this author.

The two projects differed significantly in their experimental design and objectives but were designed to produce complementary data sets. The Brisbane 30 Homes project was designed to monitor a larger number of systems for compliance with ADWG for basic bacterial indicator groups and heavy metals to enable comparisons between systems to be made. Conversely, the monitoring of the Newcastle Kotara Roof to Creek project was designed to investigate a fewer number of tanks in greater detail, particularly to elucidate treatment train mechanisms operating within the systems, as will be discussed in Section II.

3.2.2 Brisbane: Design, Sampling & Data Analysis

The Brisbane project included 30 RWH systems located throughout Brisbane. Brisbane is Australia's third largest city with an estimated urban population of 1.8 million people residing in 640,000 houses (ABS, 2004). Brisbane is a moderately industrial city with an international airport located in the eastern suburbs near the coast. The Central Business District (CBD) of Brisbane is located approximately 20 km west of the coast, with the RWH field sites being confined to a perimeter 8 km north, 11 km east, 10 km south and 7 km west of the CBD.

A range of system designs were used within the Brisbane study. The rainwater tank sizes were generally small ranging in capacity from 3 to 5 KL and include 17 Aquaplate®, 6 polyethylene, and 7 galvanised iron tanks. The roofing materials were predominantly tile or metal with the exception of a single zinc roof, and ranged in area from 82 to 267 m^2. The number of residents in each house ranged from 1 to 6 with an average of 3.4 residents per dwelling. A summary of descriptive factors for the Brisbane systems is given in Appendix A.

The plumbing configurations of the rainwater tanks into the existing pipework were designed so that water from the tanks would always supply the designated uses for each particular house. The standard connections included outdoor uses and toilet flushing, with 21 rainwater tanks also connected to hotwater systems. When the water level in the tanks fell below a minimum water level (approximately 200–300mm), mains water would be used to slowly top-up the tanks to maintain that minimum level, as was depicted in the chapter 1 (Figure 1.1). This prevented the need for intervention by the householders and the trickle top-up contributed to reducing peak demand for mains water. At each site, samples of harvested rainwater (tank outlet tap) and mains water (kitchen tap) were taken, and for houses with tanks supplying hotwater systems, hotwater samples were also taken (discussed in Chapter 8).

Water sampling of the 30 RWH systems was conducted by staff of Brisbane Water on a monthly basis over the two-year study period. A number of physicochemical parameters were measured in the field, while bacterial and elemental analyses were conducted by Scientific Analytical Services (SAS), Brisbane. The SAS laboratory analysed total coliforms by membrane filtration onto LES-Endo agar, thermotolerant coliforms by membrane filtration onto M-FC agar with *E. coli* colonies confirmed with the use of the fluorescent protein MUG, while heterotrophic plate counts were determined on Plate Count Agar incubated at 35°C for 48 hrs. Metals were analysed at the Brisbane SAS laboratory using Inductively Coupled Plasma Optical Emission Spectroscopy.

Monthly results were recorded by BCC and sent to the University of Newcastle for data analysis. To fulfil the agreement of the collaboration between the University of Newcastle and BCC, preliminary reports were prepared (Spinks, *et al.*, 2004) on the results of the program after 12 and 18 months, and a final report was prepared (Spinks, *et al.*, 2005a) after the completion of the 2 year monitoring period.

3.2.3 Newcastle: Sampling, Testing & Data Analysis

Newcastle has in the past been a city supporting substantial heavy industry although has now become less industrial with the recent closure of a number of major processing plants including BHP steelworks and the Pasminco zinc smelter. The city still has an extremely active harbour, primarily operating in coal exportation. The population of Newcastle (including Lake Macquarie) is approximately 335,000 residing in 128,000 dwellings (ABS, 2004).

The major objective of the Newcastle project was to find RWH systems that could be monitored closely to detect and test 'anomalies' in water quality between different points within the systems (Section II). Fewer tanks were therefore focussed on and subjected to a greater degree of investigation. However, point-of-use water quality was also of major interest as it allowed tank waters to be evaluated against the ADWG as well as facilitating inter-system water quality comparisons.

The Newcastle project focussed on six RWH systems, five of which were located in Newcastle and one system located at Ourimbah approximately 60 km south of Newcastle. Three of the Newcastle RWH systems were part of Kotara Roof to Creek project, which in total included 15 retrofitted RWH systems. Of the other two Newcastle RWH systems, one was located in an inner city industrial area in close proximity to the harbour, and the other in a typical suburban area quite distant from heavy industrial activity.

The Newcastle rainwater tank sizes varied from 2 to 9 KL and included 3 Aquaplate® and 3 polyethylene tanks, while the Ourimbah system consisted of two concrete tanks joined by a pipe at the base to give a combined capacity of 34 KL. The Newcastle systems were designed and operated according to the same principles as those within the Brisbane study, where the tanks were connected to the existing plumbing of the house and constantly supplied water for the designated uses, with mains water trickle topping-up the tanks when water level fell below a predetermined capacity. All six systems were used for outdoor, toilet and laundry, with five of the six systems also connected to the hotwater systems, and three using harvested rainwater for all potable and non-potable purposes. A list of descriptive factors for the Newcastle systems is given in Appendix B.

The water quality monitoring program for Newcastle was conducted exclusively as a part of this PhD research. This included developing the monitoring strategy, sampling, microbiological analysis and data analysis. Heavy metal analysis was conducted either by technical staff at the University of Newcastle using Inductively Coupled Plasma – Mass Spectrometry (ICP–MS) or at Hunter Water Australia Laboratories using Atomic Absorption Spectroscopy (AAS). The microbiological analyses were conducted using membrane filtration (Millipore) with coliform and *E. coli* enumerated on mColiBlue® media, *Pseudomonas* on Pseudomonas Selective Broth® and HPC on mEndo® media, and inclubated as to the manufacturers specifications (Millipore). The samples in this project were defined as environmental waters, as opposed to finished drinking waters, and HPC were consequently incubated at 37°C. The data was compiled in Microsoft Excel and analysed statistically using StatSoft.

3.2.3.1 Summary of Relevant Guidelines

The tank water and mains water samples were compared to the Australian Drinking Water Guidelines (ADWG) and the formally proposed Water Quality Standards for Rainwater Cistern Systems – RCS (Krishna, 1993; Fujioka, 1993). Hotwater samples were also collected and evaluated against these guidelines though are presented in Chapter 8. The ADWG are designed for application to water supplies intended for drinking purposes and are not strictly relevant to rainwater harvesting systems intended for non-drinking uses. The intended non-drinking uses of harvested rainwater in these, and other similar urban rainwater harvesting projects also gave scope to the application of the Australian and New Zealand Guidelines for Fresh and Marine Water Quality – Guidelines for Recreational Water Quality and Aesthetics (GRWQA), which includes guidelines for swimming and bathing waters. Therefore, as the ADWG set higher water quality standards, comparisons to these were used as the primary point of comparison to satisfy a conservative health risk approach, with additional water quality assessment using the GRWQA.

3.2.3.1.1 ADWG (Drinking Water)

The ADWG are the official guidelines for drinking water supplies in Australia though they are not legally enforceable unless stated as an operating license requirement for water suppliers. The Australian guidelines are very similar to the WHO drinking water guidelines and have been widely adopted within Australia as a benchmark for water supplies. Table 3.1 presents some of the microbial and chemical standards in both the Australian and WHO guidelines. The microbial requirements are based on the absence of coliform indicator organisms in a minimum of 95% of samples, while specific maximum concentrations are prescribed for each chemical.

Table 3-1: Australian and WHO drinking water quality guidelines

	ADWG	WHO–GDWQ
MICROBIAL (CFU/100mL)		
E. coli	0 (>98%)	NS
Thermotolerant coliforms	0 (>98%)	NS
PHYSICO-CHEMICAL (mg/L)		
pH	6.5 – 8.5	–
Lead	0.01	0.01
Arsenic	0.007	0.01
Nickel	0.02	0.07
Cadmium	0.002	0.003

NS Not Stated

3.2.3.1.2 Rainwater Cistern Systems (Drinking Water)

The Water Quality Standards for Rainwater Cistern Systems formally proposed by Krishna (1993) with the endorsement of the International Rainwater Catchment Systems Association were designed for harvested rainwater supplies intended for human consumption. These guidelines describe three categories of water quality based on concentrations of thermotolerant coliform organisms, shown in Table 3.2. Classes I and II (i.e. ≤10CFU/100mL) were considered suitable for drinking applications.

Table 3-2: Formally proposed guidelines for rainwater cistern systems

Class	Thermotolerant coliforms (CFU/100mL)	Recommended Use
Class I	0	Drinking
Class II	1–10	Drinking
Class III	>10	Non-drinking

Krishna (1993)

3.2.3.1.3 GRWQA (Bathing Water)

The GRWQA cover the water quality requirements for water bodies that are subject to some degree of human use. The highest standard of water quality within these guidelines applies to water bodies used for 'primary contact'. Primary contact includes water used for swimming, bathing and other direct water-contact sports where water may enter body cavities including ears and eyes and may be accidental swallowed.

For primary contact waters, chemical parameters should not exceed the values given in Table 3.3, while the median bacterial content in samples of fresh and marine waters taken over the bathing season should not exceed:

- 150 faecal coliform organisms/100mL (minimum of five samples taken at regular intervals not exceeding one month, with four out of five samples containing less than 600 organisms/100mL).

Table 3-3: Chemical parameters of Australian bathing water guidelines (GRWQA)

pH	5.0 – 9.0
Lead	50µg/L
Nickel	100µg/L
Cadmium	5µg/L
Chromium	50µg/L
Aluminium	200µg/L

3.3 Results & Discussion

3.3.1 Brisbane Water Quality: Compliance with Drinking & Bathing Water Guidelines

The primary focus of the Brisbane study was to determine the microbial and physicochemical quality of harvested rainwater in a cross section of RWH systems and to evaluate this water quality using appropriate guidelines. A second major objective of the study was to determine the degree to which water quality is influenced when passed through domestic hotwater systems, the results of which are discussed in Chapter 8. While the results were assessed against drinking and bathing water guidelines, they were also compared to the local mains water supply in order to gauge the relative quality differences between the available supplies.

Water quality results based on sampling from the 30 sites within the Brisbane City Council district have been presented in Tables 3.4–3.13. The results presented here were based on the complete 24 months of monitoring which commenced July 2003 and concluded June 2005. A total of 1848 samples have been taken from the rainwater tanks (outdoor tap), hotwater systems, and mains water supply (kitchen tap). Tables 3.4 – 3.11 relate observed tank water quality to the ADWG, which were used at the time as the standards for municipal water supplies. Table 3.12 relates rainwater quality to water quality standards recommended for rainwater cistern systems intended for drinking supplies when piped supplies were not available. And finally, the results were evaluated against Australian bathing water guidelines in Table 3.13.

3.3.2 Brisbane Harvested Rainwater Compliance with ADWG

The water quality data in this section were grouped and evaluated in two distinct ways. The first, presented immediately below, consists of an aggregated data set, where data from all 30 RWH systems were compiled into a single data set and evaluated against water quality guidelines (Tables 3.4 & 3.5). Distinctions were therefore not made between systems in order to get a general overview of the performance of RWH systems in general. Within the aggregated data analysis, the failing of one system constantly would be statistically equated to the intermittent failure of several systems. The second way the data were categorised was into individual RWH system profiles, where each system was evaluated individually (Tables 3.6 & 3.7). This allowed specific systems that were performing poorly to be identified and allowed inferences to be made about the distribution and nature of chemical and microbiological contaminants.

Descriptive statistics of the aggregated dataset are presented in Table 3.4 and 3.5. Water quality within the Brisbane rainwater harvesting systems was found to vary over time and often did not meet ADWG. Approximately 55.6% of the 684 samples complied with ADWG for total coliform counts while 73.7% and 77.8% complied for thermotolerant coliforms and *E. coli*, respectively (Table 3.4). When all samples were assessed together, none of the bacterial parameters were found to comply with ADWG in more than 95% of samples, as recommended for drinking water supplies. Heterotrophic plate counts (HPC) were a measure of all aerobic bacteria and were included in the monitoring program although no guideline limit was set within the ADWG.

Table 3-4: Aggregated data from Brisbane RWH systems – Microbiological Parameters

(CFU/100mL)	N	Average	Min.	Max.	Compliance	ADWG
Total coliform	684	73.7	0	>800	55.6%	0 (95%)
Thermo. coliform	696	13.5	0	>600	73.7%	0 (95%)
E. coli	694	11.8	0	>600	77.8%	0 (95%)
HPC (CFU/mL)	695	16960	0	>60000	–	–

The physicochemical and inorganic parameters of the tank water were shown to be very good (Table 3.5). All of the heavy metals were in total compliance (100%) with the ADWG with the exception of iron and lead (73.9% and 95.3% compliance, respectively). Lead is an accumulative toxin and ADWG have based their standards on the ingestion of 1L per day causing no ill effects in young children (i.e. from a health perspective, drinking half a litre of water containing double the guideline level equates to the same level of risk). Given the high or total compliance rates for the metals and the supply of mains water for potable uses, health risks associated with heavy metals appeared negligible. Tank owners may have more concern for iron levels, which can impart a red-brown colour on water. The ADWG, which set only an aesthetic guideline limit for iron, were exceeded in approximately one quarter of all rainwater tank samples. pH complied in greater than 85% of samples with the occasional sample falling below pH 6.5. However, pH values are defined within the ADWG in order to protect pipelines from acid corrosion and ensure efficient disinfection and, as such, are not stated as health guidelines. The pH of the samples, averaging above 7.4, was surprisingly high given that rainwater is naturally acidic with a pH of around 5.6.

Table 3-5: Aggregated data from Brisbane RWH systems – pH & Inorganics

(mg/L)	N	Average	Min	Max	Compliance	ADWG
pH	696	7.39	5.1	8.7	86.1%	6.5-8.5
Sodium	234	19.58	<1	47.2	100%	180*
Aluminium	234	0.04	<0.005	0.12	100%	0.2*
Barium	234	0.02	<0.005	0.04	100%	0.7
Cadmium	234	0.0	<0.001	0.0	100%	0.002
Chromium	234	0.0	<0.002	0.01	100%	0.05
Copper	234	0.06	0.009	1.23	100%	2
Iron	234	0.04	<0.005	0.96	73.9%	0.3*
Manganese	234	0.01	<0.001	0.05	100%	0.5
Nickel	234	0.0	<0.002	0.01	100%	0.02
Lead	234	0.0	<0.005	0.03	95.3%	0.01
Zinc	234	0.21	0.008	1.79	100%	3*

*Aesthetic guideline only (no health guideline set)

RWH systems were then evaluated individually for compliance with ADWG. The ADWG acknowledge that it is unreasonable to expect any water supply to comply with every guideline parameter all of the time, and hence, the specification that compliance, and the acceptability of a water utility's performance, should be evaluated in terms of greater than 95% compliance for each water quality parameter. Each of the 30 houses undergoing water quality monitoring was assessed for the percentage of samples for which they achieved compliance with the ADWG over the 2 year period, as graphically illustrated in Figure 3.1 for *E. coli*.

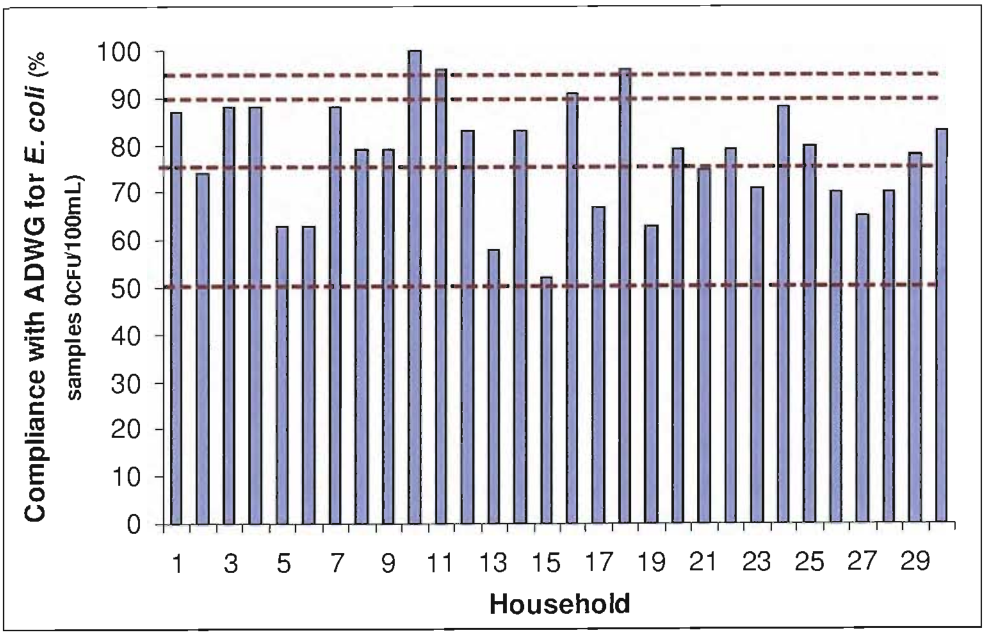


Figure 3-1: Percentage of samples in each house compliant with the ADWG standards for *E. coli* of 0 CFU/100mL. Three of the 30 houses (10%) were found to comply in more than 95% of samples, while four of 30 (13.3%) were compliant in more than 90% of samples. In all houses, more than half of all water samples contained zero *E. coli* organisms per 100mL, summarise in Table 3.6.

Tables 3.6 and 3.7 show the percentage of houses that complied with the ADWG in 0%, >50%, >75%, >90%, and >95% of samples. The >95% compliance category is shown in bold as this is the required standard set in ADWG. Every rainwater tank was found to comply with the ADWG at least part of the time for every measured water quality parameter. While none of the tanks failed any parameter all of the time, Table 3.6 shows that only 56.7% of tanks complied with ADWG more than 50% of the time for total coliform bacteria, with only one fifth of all tanks complying more than 75% of the time and no tanks complying more than 90% of the time. Despite the overall 73.7% and 77.8% compliance rates with ADWG for all tank samples for thermotolerant coliform and *E. coli*, respectively (Table 3.4), only 6.6% and 10% of the 30 tanks had greater than 95% compliance (Table 3.6). This highlights the fact that bacterial contamination was widespread and that the majority of systems were susceptible to some pathways of faecal contamination. Much greater compliance was seen for heavy metals, with all samples from all tanks complying with guidelines for sodium, aluminium, barium, cadmium, chromium, copper, manganese, nickel, and zinc (Table 3.7). Twenty one (70%) of the 30 rainwater tanks had greater than 95% compliance for lead, while 26 (86.7%) tanks had greater than 95% compliance for iron.

Table 3-6: Proportion of Brisbane RWH systems complying with ADWG for Index Organisms (0 CFU/100mL). The data presented shows the proportion of systems in each compliance-percentile.

Compliance 0 CFU/100mL	0%	>50%	>75%	>90%	>95%
Total coliform	0%	56.7%	20%	0%	**0%**
Thermotolerant coliform	0%	96.6%	46.6%	10%	**6.6%**
E. coli	0%	100%	63.3%	13.3%	**10%**

Table 3-7: Proportion of Brisbane RWH systems complying with ADWG for metals. The data presented shows the proportion of systems in each compliance-percentile.

Compliance with ADWG	0%	>50%	>75%	>90%	>95%
Sodium	0%	100%	100%	100%	**100%**
Aluminium	0%	100%	100%	100%	**100%**
Barium	0%	100%	100%	100%	**100%**
Cadmium	0%	100%	100%	100%	**100%**
Chromium	0%	100%	100%	100%	**100%**
Copper	0%	100%	100%	100%	**100%**
Iron	0%	100%	100%	86.7%	**86.7%**
Manganese	0%	100%	100%	100%	**100%**
Nickel	0%	100%	100%	100%	**100%**
Lead	0%	100%	96.6%	70%	**70%**
Zinc	0%	100%	100%	100%	**100%**

3.3.3 Brisbane Mains Water Compliance with ADWG

The Brisbane municipal water supply was a chlorine disinfected drinking water supply delivered through the city's pipe network. The same approach of data categorisation and evaluation as that for harvested rainwater was taken for the municipal water samples. From the aggregated dataset, mains water samples taken from kitchen taps (N=692-693) were compliant with ADWG in greater than 98% of samples for all parameters with the exception of iron, as shown in Tables 3.8 & 3.9. With the exception of a single sample containing two thermotolerant coliforms, mains water samples were also free of thermotolerant coliform and *E. coli* organisms.

Table 3-8: Water Quality of Brisbane's Municipal Supply (Kitchen Tap samples) – Microbiological Parameters

(CFU/100mL)	N	Average	Min.	Max.	Compliance	ADWG
Total coliform	692	0.1329	0	21	98.4%	0 (95%)
Thermotolerant coliform	692	0.0029	0	2	99.9%	0 (95%)
E. coli	693	0	0	0	100%	0 (95%)
HPC (CFU/mL)	692	94.5	0	>6000	–	–

The majority of heavy metals in mains water samples were in total compliance of ADWG, with the exception of iron and a single sample exceeding the guideline for lead (Table 3.9). The source of the iron was probably the pipes comprising the mains water distribution network.

Table 3-9: Water Quality of Brisbane's municipal Supply (Kitchen Tap) – pH & Inorganics

(mg/L)	N	Average	Min	Max	Compliance	ADWG
pH	694	7.9	6.9	9.1	99.9%	6.5-8.5
Sodium	232	36.27	25.0	51.56	100%	180*
Aluminium	232	0.0515	0.02	0.119	100%	0.2*
Barium	232	0.0268	0.01	0.036	100%	0.7
Cadmium	232	0.0	<0.001	0.0	100%	0.002
Chromium	232	0.0	<0.002	0.002	100%	0.05
Copper	232	0.0547	0.005	1.215	100%	2
Iron	232	0.0302	<0.005	2.831	86.6%	0.3*
Manganese	232	0.0094	0.003	0.071	100%	0.5
Nickel	232	0.0002	<0.002	0.013	100%	0.02
Lead	232	0.0002	<0.005	0.021	99.6%	0.01
Zinc	232	0.0164	<0.005	0.312	100%	3*

*Aesthetic guideline only (no health guideline set)

The individualised assessment of Brisbane's municipal water at each household provided revealing insight. The ADWG were designed specifically for managed water supplies intended for human consumption and allowed a non-compliance rate of less than 5% for each measured water quality parameter. It was therefore significant to report that, at the kitchen tap of a number of households, mains water did not meet this specification for three water quality parameters (total coliforms, iron and lead), as seen in Tables 3.10 and 3.11.

At two of the 30 houses, mains water contained coliform bacteria in more than 5% of samples, and at one house in more than 10% of samples, although all houses were free of *E. coli* in more than 95% of samples. The delivery of high quality water from the municipal supply was more consistent than that supplied by rainwater tanks for bacterial index organisms. However, the presence of any index organism in mains water was considered more alarming than their presence in harvested rainwater due to the increased probability that the index organism in mains water represented human or cattle faecal contamination. Human and cattle faecal material is known to contain significantly higher concentrations and diversities of human pathogens than the faecal matter of animals likely to contaminate house roofs and rainwater harvesting systems (discussed in Chapter 9).

Table 3-10: Proportion of Brisbane houses (of those in the study group) whose mains water samples (kitchen tap) comply with ADWG for Index Organisms. The data presented shows the proportion of houses in each compliance-percentile.

Compliance 0 CFU/100mL	0%	>50%	>75%	>90%	>95%
Total coliform	0%	100%	100%	96.6%	**93.3%**
Thermotolerant coliform	0%	100%	100%	100%	**100%**
E. coli	0%	100%	100%	100%	**100%**

Mains water samples contained two metals (iron and lead) for which not all houses complied with ADWG in greater than 95% of samples (Table 3.11). This was similar to rainwater samples which also contained two metals for which greater than 95%

compliance was not achieved by all houses. However, fewer houses were less than 95% compliant for iron and lead compared to rainwater tank samples.

Table 3-11: Proportion of Brisbane houses (of those in the study group) whose mains water samples (kitchen tap) comply with ADWG for metals. The data presented shows the proportion of samples in each compliance-percentile.

Compliance with ADWG	0%	>50%	>75%	>90%	>95%
Sodium	0%	100%	100%	100%	**100%**
Aluminium	0%	100%	100%	100%	**100%**
Barium	0%	100%	100%	100%	**100%**
Cadmium	0%	100%	100%	100%	**100%**
Chromium	0%	100%	100%	100%	**100%**
Copper	0%	100%	100%	100%	**100%**
Iron	0%	100%	100%	93.3%	**93.3%**
Manganese	0%	100%	100%	100%	**100%**
Nickel	0%	100%	100%	100%	**100%**
Lead	0%	100%	100%	96.6%	**96.6%**
Zinc	0%	100%	100%	100%	**100%**

Holistic assessment of mains water (i.e. as a single system) indicated compliance with minimal ADWG standards (Tables 3.8 & 3.9). This suggested the points of mains water contamination of the affected houses were at the tap or within the household piping and was not systemic throughout the mains water distribution network. This was supported by the finding that both lead and iron were detected in exceeding levels in mains water samples from the same house.

3.3.4 Brisbane Rainwater Compliance with Rainwater Catchment Systems (RCS) Guidelines

The proposed harvested rainwater quality guidelines (Krishna, 1993) recommended that the commonly used thermotolerant coliform standard of zero coliform organisms be increased to a maximum allowable concentration of ten thermotolerant coliforms, or *E. coli*, per 100mL sample. Table 3.12 shows the revised levels of tank compliance when assessed against the RCS guidelines. The bacterial index organism *E. coli* was found to be compliant with the RCS guidelines in greater than 90% of rainwater tank samples.

Table 3-12: Compliance with recommended Rainwater Cistern System Guidelines

(CFU/100mL)	RWT	Mains	RCS Guideline
Thermotolerant coliform	88.4%	100%	10 CFU/100mL
E. coli	90.8%	100%	10 CFU/100mL

3.3.5 Brisbane Rainwater Compliance with GRWQA

The application of water supplies for secondary uses includes outdoor watering, toilet flushing, and possibly hotwater uses. The application of drinking water guidelines for these uses confers a significantly over conservative health risk protection measure impeding the potentially safe and productive use of this water supply. Since the secondary use of harvested rainwater equates to an approximately equal (for showering) or lesser degree of contact as bathing waters, the application of Australian recreational and bathing water guidelines (GRWQA) was thought to be appropriate. The GRWQA standards for primary contact water quality were used to assess the quality of harvested rainwater, despite the fact that unheated rainwater does not generally have any substantial contact with humans.

Table 3-13: Brisbane Harvested Rainwater Compliance with GRWQA

Parameter	Compliance	GRWQA
Thermotolerant Coliforms	YES (Median 0 CFU) YES (99.1%)	Median <150 CFU/100mL, & >80% of samples <600 CFU/100mL
PH	100%	5.0 – 9.0
Lead	100%	50 μg/L
Cadmium	100%	5 μg/L
Nickel	100%	100 μg/L
Chromium	100%	50 μg/L
Aluminium	100%	200 μg/L

The quality of harvested rainwater was in total compliance with the GRWQA, as seen in Table 3.13, suggesting that the quality of harvested rainwater at least suitable for gardening, toilet flushing, laundry, swimming and bathing uses. The quality of harvested rainwaters after passing through domestic hotwater systems is evaluated in Chapter 8.

3.3.6 Newcastle Water Quality: Compliance with Drinking & Bathing Water Guidelines

While the major role of the Newcastle RWH monitoring program was to explore intra-system water quality variations, the Newcastle database was subjected to the same statistical analysis as the Brisbane database and is presented accordingly. However, more stringent selection criteria were used relating to the amount of data collected for each system before selecting the systems to be included in the analysis. Some of the systems not included here make up important case studies for exemplifying specific aspects of the treatment train and are presented in Section II. None of the RWH systems were excluded from this chapter due to water quality criteria and no attempt was made to influence the results by selection bias.

3.3.7 Newcastle Harvested Rainwater Compliance with ADWG

As only six systems were included in the statistical analysis from the Newcastle study, the data analysis was conducted on aggregated data only. Too few houses were used to derive any statistically meaningful conclusions from separate analysis of individual RWH systems. Rather, RWH systems within the Newcastle study with obviously outlying data will be presented on an individual basis. Individual Newcastle household systems have provided the basis for a number of case studies for specific operational phenomena and will be examined in more detail in the following chapters.

The microbial quality of the Newcastle RWH systems can be seen in Tables 3.14. While it was not the aim of this research to compare bacterial loads between the two regions, the extent of contamination in the samples taken from the Newcastle tanks was clearly far greater than that of the Brisbane systems. The average concentrations of coliform and *E. coli* contamination in the Newcastle systems was more than ten fold higher than those in Brisbane. The number of samples containing coliforms and *E. coli* were also much greater, as demonstrated in the correspondingly lower rates of compliance with ADWG.

Concentrations of *Pseudomonas* were not monitored in the Brisbane study as they were generally not taken into consideration when assessing health risk, although they do provide a useful environmental category of bacteria to compliment the coliform and total bacteria groups and were therefore monitored in the Newcastle study. The levels of *Pseudomonas* averaged more than three fold the total coliform concentrations, though did not comprise the majority of the total bacteria counts.

It was probably that a significant proportion of the variation observed in microbial and chemical water quality between the Newcastle and Brisbane studies was related to experimental design. The single most fundamental difference in the design of the two studies was the trigger for sampling. The Brisbane sampling regime was based on a monthly sampling protocol, irrespective of rainfall. Conversely, the Newcastle sampling regime was based around rain events, and while not all samples were taken immediately after a rain event, a large number were taken soon after. Consequently, the Brisbane study probably includes a larger number of samples taken from tanks holding a significant proportion of disinfected mains water, while the Newcastle study in many ways represents a worst-case scenario.

Table 3-14: Aggregated water quality data from Newcastle RWH systems – Microbiological Parameters

(CFU/100mL)	N	Average	Min.	Max.	Compliance	ADWG
Total coliform	87	1352	0	25000	2.3%	0 (95%)
E. coli	87	192	0	6800	27.6%	0 (95%)
Pseudomonas	86	4463	0	75000	–	–
HPC (CFU/mL)	84	1910	0	35000	–	–

The chemical quality of stored rainwater was excellent for most parameters, with the most significant exception for lead, as seen in Table 3.15. While the majority of metal and physicochemical parameters were in complete compliance with ADWG, the low compliance rates for lead (58.9%) were a significant compromise of drinking water quality. However, lead samples exceeding acceptable levels were concentrated at one site, where all 15 samples exceeded the ADWG limit for lead. Relative to the other field sites in the Newcastle region, this RWH system was not located in close proximity to heavy industry or main roads, suggesting the source of contamination may have been from within the RWH system itself. When this site was excluded from the analysis, the compliance rate for lead increased significantly to 76%. The proportion of samples compliant for pH (88%) was very similar to that of Brisbane (86.1%), though unlike Brisbane the levels of iron in Newcastle rainwater tanks did not exceed ADWG in any samples.

Table 3-15: Aggregated water quality data from Newcastle RWH systems – pH & Inorganics

(mg/L)	N	Average	Min	Max	Compliance	ADWG
Temp.	50	18.5	11	26		
PH	58	6.6	4.5	8.2	88%	6.5-8.5*
Chlorine	8	0	0	0	100%	5 / 0.6*
TDS	28	0.048	0.0006	0.22	100%	500*
Turbidity	25	0.296	0	1.1	100%	5 NTU*
D.O.	30	5.89	1.11	9.8		>85%
NH_4	25	0.06	0.02	0.14	100%	0.5*⁺
NO_3	26	22.86	1.16	102	96.2%	50
Cadmium	64	0	0	0	100%	0.002
Copper	72	0.046	0	0.58	100%	2
Iron	22	0.118	0.0045	0.28	100%	0.3*
Nickel	42	0	0	0	100%	0.02
Lead	73	0.025	0	0.16	58.9%	0.01
Zinc	22	0.197	0.0037	0.64	100%	3*

*Aesthetic guideline only (no health guideline set)

⁺Guideline value for ammonia (NH_3)

3.3.8 Newcastle Mains Water Compliance with ADWG

A number of random water samples were taken from the chlorinated municipal system supplied by Hunter Water Corporation. These samples were taken from five different locations including the University of Newcastle, two house taps, and two park taps. As with Brisbane, these samples were used as the basis for making an assessment of harvested rainwater quality with the reality of the quality of the alternative supply. From the limited number of samples taken, the microbial quality of this supply was shown to be very good and in full compliance with ADWG, as shown in Table 3.16.

Table 3-16: Water Quality of Newcastle's Municipal Supply – Microbiological Parameters

(CFU/100mL)	N	Average	Min.	Max.	Compliance	ADWG
Total coliform	9	0	0	0	100%	0 (95%)
E. coli	9	0	0	0	100%	0 (95%)
Pseudomonas	9	0.4	0	1	–	–
HPC (CFU/mL)	9	0.9	0	3	–	–

The chemical quality was also high apart from a single exceedance of lead in one sample, shown in Table 3.17. The sample with the lead exceedance was taken from a park tap which appeared to be significantly aged. The lead concentrations in all other samples were well below the ADWG limit, suggesting the park tap was the possible cause of the lead contamination probably resulting from corroding pipes or lead soldering.

Table 3-17: Water Quality of Newcastle's Municipal Supply – pH & Inorganics

(mg/L)	N	Average	Min	Max	Compliance	ADWG
pH					100%	6.5-8.5
Cadmium	8	0	0	0	100%	0.002
Copper	9	0.18	0.009	1.43	100%	2
Nickel	8	0	0	0	100%	0.02
Lead	9	0.005	0	0.025	89%	0.01
Arsenic	8	0	0	0	100%	0.007

*Aesthetic guideline only (no health guideline set)

3.3.9 Newcastle Rainwater Compliance with RCS Guidelines

The proportion of samples complying with the more relaxed RCS standards was still modest, with only slightly more than half of all cold rainwater samples containing fewer than 10 *E. coli* organisms per 100mL (Table 3.18). This implies that most harvested rainwater supplies in the Newcastle study were unfit for drinking purposes, if no further treatment of the waters was provided.

Table 3-18: Compliance with recommended RCS Water Quality Guidelines

	RWT	Mains	RCS Guideline
E. coli	54%	100%	10 CFU/100mL

3.3.10 Newcastle Rainwater Compliance with GRWQA

The harvested rainwater quality in the Newcastle study was found to easily comply with the GRWQA for thermotolerant coliforms, shown in Table 3.19. The median thermotolerant coliform concentration in the harvested rainwater samples was 7 CFU/100mL, significantly lower than the maximum allowable median value prescribed by the GRWQA of 150 CFU/100mL. Frequency of compliance with the upper thermotolerant coliform limit of 600 CFU/100mL (GRWQA >80%) was also achieved with 83% of samples containing less than this value. While samples were generally compliant for pH, lead levels exceeded the bathing water limits in 18% of samples. However, as noted in section 3.3.7, consistently high lead concentrations in one system significantly skewed the data set. Of the 14 samples analysed for lead from this system, 13 exceeded the bathing water limit for lead of 50μg/L with a maximum lead concentration of 155μg/L. The cause of this lead contamination was the lead flashing on the roof, discussed more fully in the following chapter. When data from this house was excluded from the analysis, 100% of samples complied with the GRWQA.

Table 3-19: Newcastle Harvested Rainwater Compliance with GRWQA

Parameter	Compliance	GRWQA
Thermotolerant coliform	YES (Median 7 CFU) YES (83%)	Median <150 CFU/100mL, & >80% of samples <600 CFU/100mL
pH	95%	5.0 – 9.0
Lead	82%	50 μg/L
Cadmium	100%	5 μg/L
Nickel	100%	100 μg/L
Arsenic	100%	50 μg/L
Zinc	100%	5 mg/L
Nitrate	100%	10 mg/L
Copper	100%	1 mg/L
TDS	100%	1000 mg/L

The water quality results from both the Brisbane and Newcastle studies were therefore found to vary quite significantly. While the unheated harvested rainwaters were found to regularly exceed ADWG for microbial parameters, they were easily compliant with bathing water guidelines, suggesting the common uses of garden watering, toilet flushing and laundry were suitable applications of harvested rainwaters.

3.4 Factors Affecting Water Quality

A number of design variables were distinguished between houses which may potentially influence water quality. The database from the Brisbane study was used as the focus of statistical analyses as it included houses with a cross section of RWH system designs. The variables examined in this chapter were those related to the design, local environment and rainfall. Variables relating to changes in water quality either spatially through the systems or over time are explored in section II. The statistical power of the analyses of such variables could be maximised when specific hypotheses were set up prior to commencement of such investigations, allowing the sampling regime to be tailored to answer the specific hypotheses. However, due to the fact that several design variables were to be investigated, the statistical analyses were conducted in an exploratory fashion. The major design variables examined included roofing material, tank material, tank capacity, rainwater tank volume to roof area ratio, the proximity of trees to catchments, and rainfall intensity and temporal distribution.

Analysis of variance was conducted using the *Statsoft Inc.* statistical program *Statistica.* Additional *posthoc* analysis was conducted on parameters with significant omnibus F values to determine between which groups the significant differences lay. The *posthoc* analysis was conducted using the Tukey Honest Significant Difference (HSD) test, a commonly used *posthoc* test which confers a moderate degree of conservatism (protection against type I error) and is able to maintain the level of confidence specified in the initial ANOVA ($p<0.05$) during the *posthoc* pairwise analysis.

3.4.1 Roof Material

In rainwater harvesting systems, the household roof is the catchment surface and is therefore an important component of the system. The catchment surface may be a site of both contaminant deposition and removal. The 30 houses in the Brisbane study were analysed to determine the influence of roofing material on microbial and physicochemical water quality. The Brisbane houses included three different roof types: METAL (55%), TILE (41%) and ZINC (3%), with one roof being unidentified.

The significant results of the Tukey HSD test (assuming unequal number of samples) for the independent variable ROOF MATERIAL have been summarised in Table 3.20. Significant differences were seen for pH, iron and zinc between METAL and TILE roofs, with a highly significant difference for zinc. No significant differences were observed between ZINC roofs and METAL or TILE roofs. It is possible that a number of water quality parameters were influenced by zinc roofing material but were filtered out in the analysis due to the much smaller sample size from ZINC roofs (ZINC roofs=1). This idea is supported by the fact that statistically significant differences were observed for some parameters between METAL/TILE roofs but not between METAL/ZINC or TILE/ZINC roofs, despite the mean concentration of the parameters from the ZINC roof being further apart than those contrasting the METAL/TILE roofs.

Table 3-20: Tukey HSD Test (unequal N) – The mean concentrations (M) of water quality parameters and degree of confidence for separation of groups based on roof material. Statistically significant (P<0.05) differences were only seen for pH, iron and zinc concentrations between {METAL} and {TILE} roofs.

	{METAL} ROOFS = 16	{TILE} ROOFS = 12	{ZINC} ROOFS = 1
pH	M = 7.33	M = 7.47	M = 7.69
{METAL}		**0.039**	
Iron (mg/L)	M = 0.028	M = 0.061	M = 0.01
{METAL}		**0.045**	
Zinc (mg/L)	M = 0.320	M = 0.076	M = 0.223
{METAL}		**0.000022**	

The observation of higher pH values in runoff waters from TILE surfaces than METAL roofs was consistent with the literature (Haebler & Waller, 1987). This was likely due to the leaching of calcium hydroxides from the concrete tiles adding alkalinity to the roof runoff. The higher concentrations of zinc in METAL roof runoff was not surprising given that zinc was typically used as the sacrificial corrosion coating on galvanised iron roofs. However, the lower concentration of iron in METAL roof runoff than TILE roof runoff was surprising. This was possibly due to the young age and good condition of the METAL roofs with the zinc coating still conferring protection on the METAL roofs from corrosion. However, the source of the higher levels of iron in runoff from the TILE roofs is not clear. Previous studies have also found corroding galvanised iron roofs to be a source of zinc contamination (Van Metre & Mahler, 2003; Coombes *et al.*, 2003) as well as cadmium and possibly nickel contamination (Van Metre & Mahler, 2003).

Lead was not found to be significantly associated with roofing material in the Brisbane study. This in itself was a significant finding given that lead is perhaps the most prevalent toxic heavy metal associated with harvested rainwater contamination. This result is further significant given that the literature has presented inconsistent findings on this subject and often assessed roofing materials of little relevance to modern urban dwellings. Many previous studies have assessed materials that are rarely used in Australia today, such as asbestos (Uba & Aghogho, 2000), shingles (Chang *et al.*, 2004; Van Metre and Mahler, 2003) and thatched roofs (Uba & Aghogho, 2000). Mixed results have been presented from assessments conducted on relevant materials, such as galvanised iron, with some studies finding associations with lead contamination (Chang *et al.*, 2004) and others finding no association (Van Metre & Mahler, 2003).

A case study of one house in the Newcastle study suffering chronic lead contamination was conducted to determine the cause of the contamination (discussed in Chapter 4). It was found that the source of lead in this system was the lead flashing located on several areas of the roof at critical rainwater runoff collection points. Therefore, while the predominant roofing material may not contribute to lead contamination, materials that are commonly used in conjunction with lead flashing (such as concrete and terracotta tiles) may indirectly encourage lead contamination through the use of this inappropriate flashing material.

3.4.2 Tank Material

The influence of tank material on water quality was then analysed for significance using the same approach as that described above for roof material. As evident from Table 3.21, the GALVANISED IRON tanks demonstrated significant differences in the concentration of a number of parameters compared to POLYETHYLENE and AQUAPLATE® tanks. The only significant difference noted between POLYETHYLENE and AQUAPLATE® was for coliform bacteria, with AQUAPLATE® tanks containing waters with significantly lower levels of coliform contamination. This may have been the case for two reasons.

Table 3-21: Tukey HSD Test (unequal N) – The mean concentrations (M) of parameters and degree of confidence for separation of groups based on tank material. Statistically significant (P<0.05) differences were seen for a nnmber of water qnality parameters.

	{POLYETHYLENE} TANKS = 6	{AQUAPLATE®} TANKS = 17	{GAL. IRON} TANKS = 7
Coliforms (CFU/100mL)	M = 124.8	M = 59.9	M = 70.6
{POLYETHYLENE}		**0.022**	
PH	M = 7.5	M = 7.5	M = 7.2
{POLYETHYLENE}			**0.0004**
{AQUAPLATE®}			**0.0002**
Conductivity (μs/cm^{-2})	M = 233	M = 251	M = 178
{POLYETHYLENE}			**0.014**
{AQUAPLATE®}			**0.00007**
Calcium (mg/L)	M = 12.6	M = 14.1	M = 9.0
{AQUAPLATE®}			**0.003**
Magnesium (mg/L)	M = 6.7	M = 7.8	M = 4.8
{AQUAPLATE®}			**0.004**
Sodium (mg/L)	M = 18.9	M = 22.0	M = 14.0
{AQUAPLATE®}			**0.003**
Potassium (mg/L)	M = 1.87	M = 2.13	M = 1.26
{AQUAPLATE®}			**0.003**
Barium (mg/L)	M = 0.320	M = 0.076	M = 0.223
{AQUAPLATE®}			**0.045**
Cadmium (mg/L)	M = 0.00007	M = 0.00004	M = 0.00023
{AQUAPLATE®}			**0.033**
Lead (mg/L)	M = 0.0009	M = 0.0012	M = 0.0034
{POLYETHYLENE}			**0.013**
{AQUAPLATE®}			**0.01**
Zinc (mg/L)	M = 0.095	M = 0.159	M = 0.434
{POLYETHYLENE}			**0.00002**
{AQUAPLATE®}			**0.00002**

Firstly, the development of biofilms on submerged surfaces was almost inevitable and such biofilms have been known to influence water column microbial levels by both removing and adding bacteria to the water column (Donlan, 2002; Marshall, 1988). The different types of rainwater tanks in the Brisbane study may have influenced the extent of biofilm development and activity on each different material, leading to altered water column microbial levels. The development and function of rainwater tank biofilms is investigated in detail in Chapter 6.

Secondly, rainwater tank material was related to tank capacity, which has a significant influence on coliform levels as discussed below. POLYETHYLENE tanks were on average the largest of the three types of tanks (average 4.8KL) and corresponded to the highest level of coliform contamination. GALVANISED IRON tanks were the second largest (average 4.1KL) and contained the second highest level of coliform contamination, while the smallest AQUAPLATE® tanks (average 3.7KL) contained the lowest coliform levels. It therefore needs to be acknowledged that there is a certain amount of confounding in the experimental design and that, while the appropriate statistical analyses have been conducted, not all parameters were held equal for the comparison of tank material or roof material.

A number of non-toxic elements (Ca, Mg, Na, K) were found in higher concentrations in AQUAPLATE® tanks than GALVANISED IRON tanks, while a number of heavy metals were found in higher concentrations in GALVANISED IRON tanks than AQUAPLATE® (Ba, Cd, Pb, Zn) and POLYETHYLENE tanks (Pb, Zn). While the majority of houses containing GALVANISED IRON tanks also contained metal roofs (83%), roofing material was not found to be the source of contamination, demonstrated in Table 3.20. Furthermore, tank size did not follow the same distribution for metal contamination as it did for coliform bacteria. It therefore appears that the cause of the higher heavy metal concentrations in the GALVANISED IRON tanks was leaching of impurities from the GALVANISED IRON tanks themselves, which may have been accentuated by the slightly lower average pH in these tanks. Previous investigations by Haebler and Waller (1987) found that galvanised iron rainwater tanks can be a source of zinc and iron. The findings of this thesis go further than previous research and identify that along with zinc and iron, contamination from leaching of galvanised iron may extend to include barium, cadmium and lead, though generally not to concentrations exceeding guideline limits.

3.4.3 Tank Size

For the analysis of rainwater tank capacity, tanks were categorised into four ordinal groups based on capacity. These included GROUP 1 with tanks of 3 – 3.5 KL capacity, GROUP 2 with tanks 3.5 – 4 KL, GROUP 3 with tanks 4 – 4.5KL and GROUP 4 with tanks 4.5 – 5KL. There was a clear increase in coliform concentrations with increasing tank size, although this was only significant between the smallest and largest sets of tanks, as seen in Table 3.22. This was contrary to the findings of Plazinska (2001) who found decreasing levels of bacteria with increasing tank size. Plazinska speculated that this was due to larger tanks undergoing more dilution by cleaner waters captured in the latter stages of rain events and also due to greater potential resuspension of sediment in smaller tanks.

The two major differences between the Plazinska study and this study were that the largest tank capacities in this study were up to 5KL, which in the Plazinska study would have constituted the smallest tank group, with the largest group in the Plazinska study having capacities >15KL. Furthermore, and perhaps more intrinsically linked to the results of this study, was the absence of mains water supplementation. It seems logical that the smaller tanks in this study would empty more quickly and hence spend a longer period of time holding a higher proportion of chlorinated municipal water, which was shown in section 3.3.3 to contain lower microbial concentrations than harvested rainwaters.

A number of other parameters were found to be significantly different between groups although this was not clearly related to size. This was perhaps a little surprising given that the explanation for the correlation between coliform levels and tank size would yield a similar result, or inverse result, for elements predominantly originating from either harvested rainfall or mains water. The elements originating from mains water, including calcium, magnesium, sodium, potassium and also electrical conductivity, did appear to follow the pattern of dilution up until the largest tank size, where concentrations appeared higher than expected. The other relevant, though unmeasured, variable is water demand. Six of the nine properties in this largest tank group were utilising their tank water in their hotwater systems as well as for toilet, laundry and outdoor applications, while the remaining three properties did not supply hotwater services. Although showering and other water using behaviours in these properties were not monitored, a higher water demand would have effectively negated the larger tank capacity. If so, this would have resulted in a higher proportion of mains water in the larger tanks than in the smaller tanks with less demand and would have explained why concentrations of calcium, magnesium, sodium and potassium were higher than expected in the largest sized tanks.

Table 3-22: Tukey HSD Test (unequal N) – The mean concentrations (M) of parameters and degree of confidence (P<0.05) for separation of groups based on tank capacity.

	{3 – 3.5 KL} TANKS = 12	{3.5 – 4 KL} TANKS = 4	{4 – 4.5 KL} TANKS = 5	{4.5 – 5 KL} TANKS = 9
Coliforms (CFU/100mL)	M = 50.98	M = 61.47	M = 85.71	M = 105.97
{3 – 3.5 KL}				**0.023**
pH	M = 7.52	M = 7.06	M = 7.14	M = 7.51
{3 – 3.5 KL}		**0.00001**	**0.00003**	
{3.5 – 4 KL}				**0.00001**
{4 – 4.5 KL}				**0.00004**
Conductivity (μs/cm^{-2})	M = 260	M = 181	M = 176	M = 245
{3 – 3.5 KL}		**0.002**	**0.0002**	
{3.5 – 4 KL}				**0.021**
{4 – 4.5 KL}				**0.003**
Calcium (mg/L)	M = 14.75	M = 9.23	M = 9.00	M = 13.37
{3 – 3.5 KL}		**0.029**	**0.008**	
Magnesium (mg/L)	M = 8.12	M = 5.28	M = 4.80	M = 7.26
{3 – 3.5 KL}			**0.014**	
Sodium (mg/L)	M = 22.96	M = 15.18	M = 13.78	M = 20.39
{3 – 3.5 KL}			**0.009**	
Potassium (mg/L)	M = 2.23	M = 1.38	M = 1.25	M = 2.00
{3 – 3.5 KL}			**0.01**	
Cadmium (mg/L)	M = 0.00006	M = 0.0000	M = 0.0003	M = 0.00005
{3 – 3.5 KL}			**0.014**	
{3.5 – 4 KL}			**0.004**	
{4 – 4.5 KL}				**0.008**
Lead (mg/L)	M = 0.0015	M = 0.0022	M = 0.0033	M = 0.0006
{4 – 4.5 KL}				**0.011**
Zinc (mg/L)	M = 0.173	M = 0.212	M = 0.476	M = 0.114
{3 – 3.5 KL}			**0.00002**	
{3.5 – 4 KL}			**0.0007**	
{4 – 4.5 KL}				**0.000008**

The cause of the relationship between coliform concentrations and tank capacity, therefore, was probably not exclusively related to the effects of dilution *per se* but also to the action of free chlorine residual in the mains water. Mains water entering the tanks will only influence the elemental constitution of the tank water in direct proportion to the amount of mains water entering the tank, whereas, a small volume of chlorinated mains water entering a tank may have a significant impact of microbial levels. This hypothesis is investigated in more detail in Chapter 5, where it was found that the initial action of chlorine appeared to significantly reduce coliform levels even though free chlorine residual was not detectable within a short time period after top-up.

3.4.4 Tank Volume to Roof Ratio

As speculated by Plazinska (2001), smaller tank storage capacities result in greater proportions of more highly contaminated initial roof runoff stored in the tank, resulting in greater levels of tank water contamination. This effect was therefore hypothesised to be exacerbated by larger roof areas. To investigate this phenomenon, a tank volume to roof area ratio (T:R) was regressed against the measured water quality parameters to determine correlations. Table 3.23 summarises the parameters that were found to have correlation to the designated confidence level ($p<0.05$). However, the R^2 values for coliform bacteria, cadmium and zinc indicate that the influence of the T:R ratio on water in reality was negligible and contributing very little to overall water quality in the tanks.

Table 3-23: R^2 values for parameters correlating with the Tank to Roof ratio

Parameter	Coliforms	Cadmium	Zinc
R^2	0.01	0.03	0.04

3.4.5 Overhanging Tree cover

The accumulation of microbes on the roof catchment has previously been thought to be the main source of microbial contamination of rainwater harvesting systems, as opposed to bacterial multiplication within tanks (Fujioka *et al.*, 1991; Lye, 2002). The presence of vegetation overhanging or in close proximity to the roof catchment was hypothesised to be the major driver for contamination of roof catchments in this study. To test this hypothesis, houses were classified into three groups according to whether, firstly, vegetation hung directly over the roof surface (HANGING), secondly, vegetation was located within 5m of the roof (WITHIN 5m}, or thirdly, vegetation was not located near the house (NONE}, as shown in Table 3.24. The low statistical power of the NONE group resulted in an inability to statistically compare differences with the other groups, although the higher values for many parameters in the NONE group suggest that further work is warranted to explore this phenomenon.

Table 3-24: Tukey HSD Test (unequal N) – The mean concentrations of parameters and degree of confidence (P-value) for separation of groups based on proximity to vegetation.

	{OVERHANGING} HOUSES = 16	{WITHIN 5m} HOUSES = 13	{NONE} HOUSES = 1
pH	M = 7.3	M = 7.5	M = 7.7
{OVERHANGING}		**0.005**	
Conductivity	M = 209	M = 252	M = 302
{OVERHANGING}		**0.002**	
Magnesium	M = 6.02	M = 7.72	M = 11.04
{OVERHANGING}		**0.042**	
Sodium	M = 17.2	M = 21.7	M = 31.1
{OVERHANGING}		**0.047**	
Potassium	M = 1.61	M = 2.13	M = 3.04
{OVERHANGING}		**0.03**	
Aluminium	M = 0.033	M = 0.040	M = 0.041
{OVERHANGING}		**0.024**	
Barium	M = 0.015	M = 0.018	M = 0.024
{OVERHANGING}		**0.046**	
Copper	M = 0.050	M = 0.052	M = 0.193
{OVERHANGING}			**0.002**
{WITHIN 5m}			**0.002**

It was somewhat surprising to find no significant differences between the groups for any microbial parameter. This was also the case when the number of samples was assumed to be equal (less conservative analysis) and when the more liberal Duncan *posthoc* test was applied. The hypothesis in this study, that overhanging vegetation would account for a substantial proportion of the microbial load on the catchment surface, was therefore found to be false. The rejection of this hypothesis may be expected for the coliform, thermotolerant coliform and *E. coli* populations whose sources were likely to be faecal or soil based. However, HPC concentrations were also not influenced by the presence or absence of local vegetation. The Brisbane study unfortunately did not include the monitoring of *Pseudomonas,* a group of environmental bacteria that may have been more representative to the presence of vegetation.

An alternative hypothesis to explain the distribution of microbes in the rainwater tanks may be that wind and other meteorological factors more strongly influenced bacterial deposition than did overhanging vegetation. This hypothesis is supported by the findings of Tuffley and Holbeche (1980) who identified the same strains of Mycobacteria in rainwater tanks as in surrounding soil samples. The authors speculated in this study that contamination of the rainwater tanks had occurred due to wind blown dust deposition and was unrelated to the presence of overhanging vegetation. Stronger evidence of this cause of contamination was supplied recently by Evans *et al.* (2006) who found strong correlations between bacterial concentrations in roof runoff and local weather patterns, namely wind speed and direction.

While it appears that the role of overhanging vegetation in providing a source of microbial contamination may be much smaller than previously thought, vegetation may still indirectly encourage microbial contamination by providing access to the catchment for small animals, reptiles and birds. Such animals have at times been found to carry opportunistic/pathogenic bacteria. For example, reptiles and frogs have been considered possible vectors for *Salmonella* (Taylor *et al.*, 2000). Wild birds and poultry are also well known carriers of *Salmonella* spp. and *Campylobacter jejuni* and have been flagged as possible sources of pathogenic contamination of rainwater systems (enHealth, 2004). The protozoan pathogen *Cryptosporidium parvum* has its main reservoir in cattle, however, Grazcek *et al.* (1997) found cross-transmission of *Cryptosporidium* was possible through waterfowl and gulls feeding near sewage effluent. The proximity of rainwater tanks to sewage outfalls or agricultural land may therefore potentially add marginal risk to tank water contamination. Hence, in regards to microbial contamination of rainwater harvesting systems, it appears that while faecal and pathogenic organisms may enter the system indirectly via overhanging vegetation, the role of local vegetation in contributing environmental bacterial loads is minimal.

Unlike for microbial deposition, the influence of local vegetation on chemical deposition resulted in a number of statistically significant findings, as summarised in Table 3.24. Vegetation was found to influence pH, with pH decreasing as the proximity of trees to roofs became closer. This was probably due to the formation of organic acids from decaying leaves and vegetation that had entered the system. Conductivity and the mean concentrations of the other elements also exhibited a clear relationship with the proximity of trees, with significantly higher concentrations found in tanks belonging to the WITHIN 5m group compared to tanks within the OVERHANGING group. The reason for the lower concentrations in the group with overhanging trees is unclear, though may have either been influenced by confounding factors or may have been related to some form of catchment protection from wind deposition. The influence of a confounding factor seems probable in the case of varying copper concentrations, due to the NONE group containing only one household which may have contained copper sheeting or copper fittings within the catchment or collection system.

However, the effects of tank material and tank volume as confounding factors does not appear to explain the lower levels of conductivity and metals in tanks with trees located more closely to the roof. The average tank capacity in the OVERHANGING group was 3.9KL compared to 4.05 in the WITHIN 5m group. The distribution of POLYETHYLENE and GALVANISED IRON tanks was a little more skewed, with the OVERHANGING group containing 6 GALVANISED IRON and only 2 POLYETHYLENE tanks, while the WITHIN 5m group contained only 1 GALVANISED IRON and 4 POLYETHYLENE tanks. Both of these groups contained 8 AQUAPLATE® tanks and the single NONE group contained one 4.85KL AQUAPLATE® tank. However, apart from conductivity, differences were not noted in the concentration of the relevant parameters between POLYETHYLENE and GALVANISED IRON tanks as seen in Table 3.21.

As seems likely the case with microbial deposition, the influence of wind or other meteorological conditions may also substantially influence chemical deposition. In China, Yang *et al.* (1995) found that the concentrations of magnesium, selenium, potassium, calcium and total alkalinity in tank waters were related to the constituents of the local soil and dust. Forster (1996) also found chemical contamination of urban roof surfaces to be significantly related to the atmospheric deposition of pollutants produced in the local environment. It therefore appears from the data that the presence of overhanging trees reduces the overall concentrations of ions and metals in tank waters either through protection of the roof catchment from wind/atmospheric deposition or through some alternative mechanism.

3.4.6 Seasonal Fluctuations of Water Quality

A number of measured parameters, including rainfall, bacteria, and some elemental constituents, showed clear seasonal trends in concentration variability. Daily rainfall data were obtained from the Bureau of Meteorology (BOM) for Brisbane City (site 40913) and is shown in Figure 3.2. The summer months had significantly higher rainfall, as well as higher levels of bacteria in the rainwater tanks, shown in Figure 3.3. Lead levels in all water supplies also demonstrated seasonal variations, with higher levels during summer.

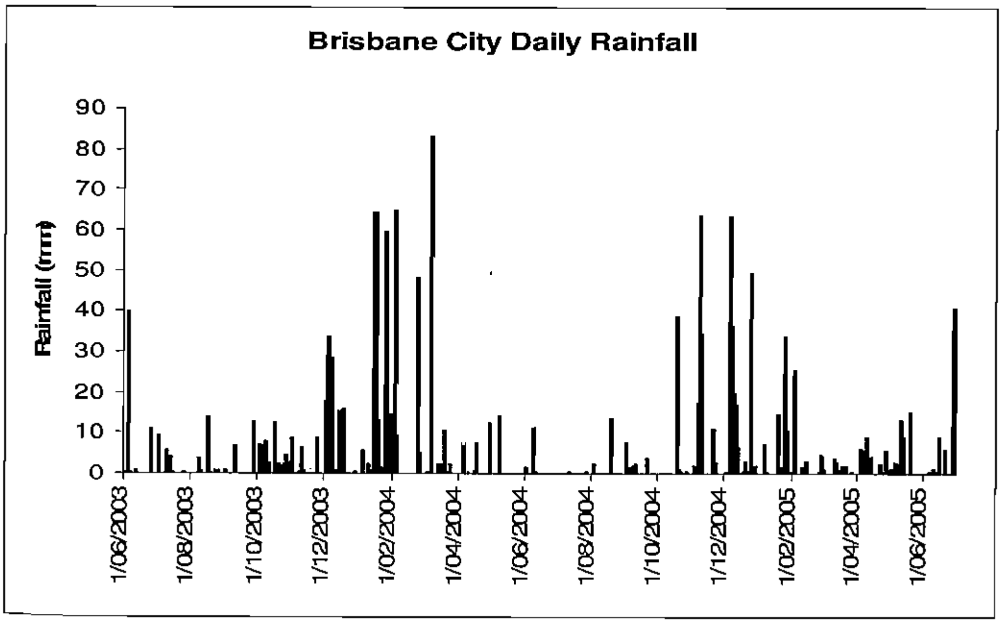


Figure 3-2: Daily Rainfall recorded at Brisbane City (Site 40913) by BOM

Calcium, magnesium, potassium, sodium, and barium also exhibited a strong temporal distribution pattern, although for these elements maximum concentrations were observed during winter months and minimum concentrations during summer, shown in

Figure 3.4. This is the reverse pattern as that displayed by lead, suggesting that rainfall may be the source of lead input, while mains water, which is higher in Total Dissolved Solids (TDS), is the probable source of the other elements which enter the tank during the dry winter months when tanks are topped up with mains water.

The relationship between bacterial parameters and rainfall appears strong on a seasonal scale, evident by comparing Figures 3.2 and 3.3. However, it is also probable that the involvement of confounding factors which relate to season but not necessarily rainfall, such as the growth and flowering of vegetation and animal feeding behaviours could contribute to this seasonal microbial effect.

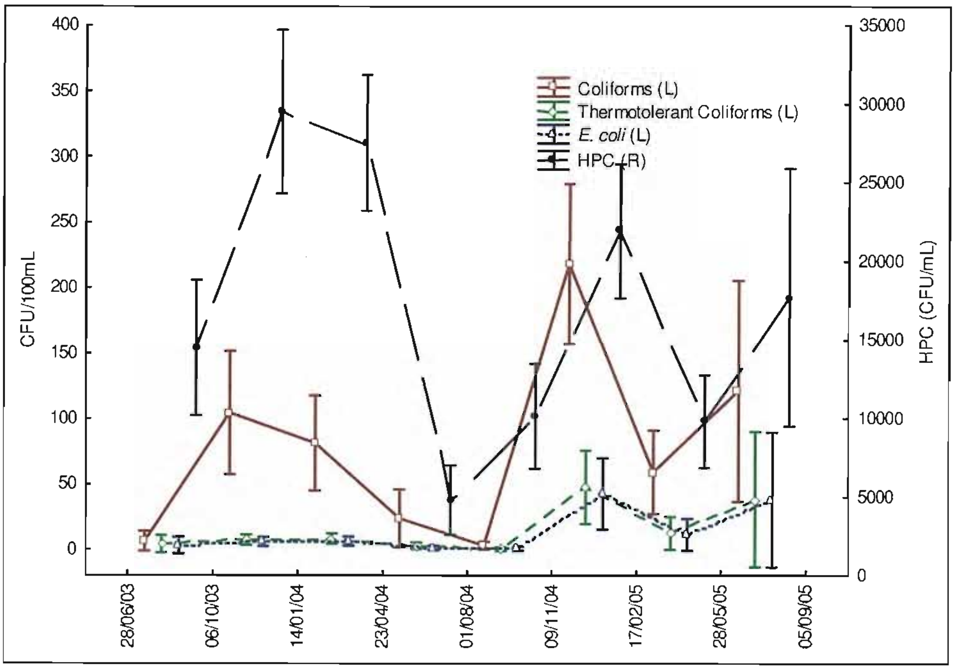


Figure 3-3: Seasonal variations show higher concentrations of HPC (right axis), coliform, thermotolerant coliform and *E. coli* (left axis) in rainwater tanks during summer months.

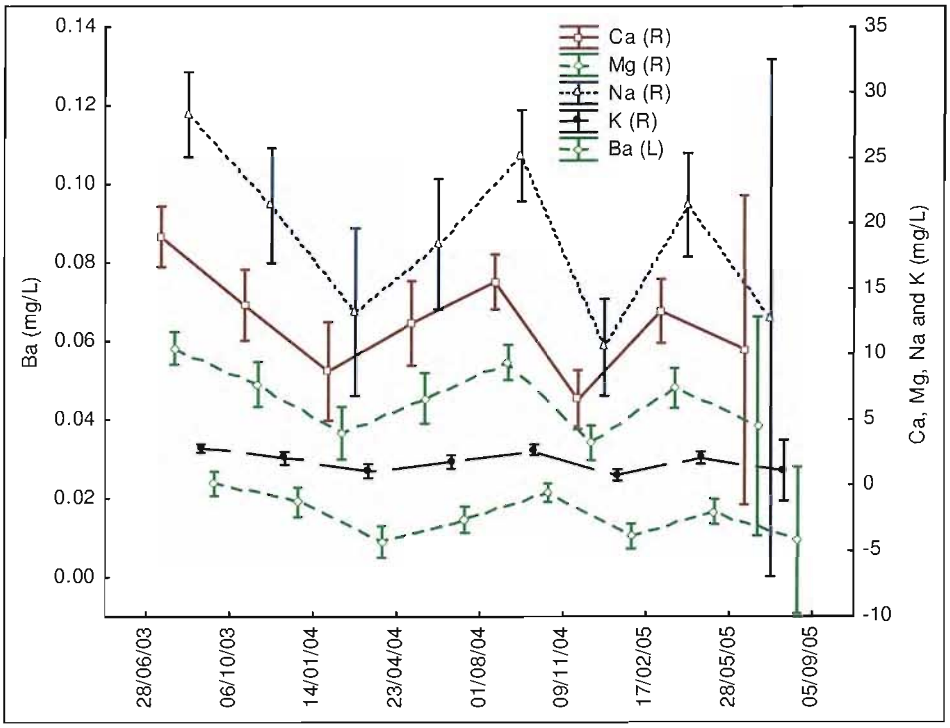


Figure 3-4: Seasonal trends exhibited by Calcium, Magnesium, Sodium, Potassium and Barium

3.4.7 Influence of Rainfall on Water Quality

Rainfall was also hypothesised as having an impact on water quality in the Brisbane and Newcastle RWH systems. The influence of rainfall on water quality was analysed in two distinct ways in an attempt to determine the significance of rainfall volume on water quality along with the significance of the length of time between rain events and sampling.

The first analysis evaluated the relationship between the concentrations of various water quality parameters and rainfall volume (defined here as total rainfall in previous 24hrs). Water quality parameters were regressed with rainfall volumes in discrete 24 hour periods, with each day of rainfall being treated as an independent variable. The parameters were regressed against the recorded rainfall for the 24 hours immediately prior to sampling (Rain 0), against the recorded rainfall for the previous 24-48 hours prior to sampling (Rain 1), against the recorded rainfall from the period 48-72 hours prior to sampling (Rain 2), etc. A seasonal influence appeared from the correlation matrices between rainfall and bacteria resulting in the need to separate the seasonal influence. Tables 3.25 and 3.26 show the results of correlations (r^2-value) determined for summer and winter months between rainfall and bacterial, pH, and electrical conductivity parameters, with correlations having a confidence interval of greater than 99% ($P<0.01$) shown in bold.

Table 3-25: Correlation matrix for relationship (r^2) between bacteria and quantity of rainfall in days preceding sampling during summer (Oct – Mar)

	Rain 0	Rain 1	Rain 2	Rain 3	Rain 4	Rain 5	Rain 6	Rain 7
Tot coliform	**0.08**	**0.04**	-0.001	0.006	0.01	**0.04**	**0.03**	0.001
Th. Coliform	**0.09**	**0.03**	0.002	0.004	0.001	0.02	**0.08**	0.001
E. coli	**0.09**	**0.03**	0.003	0.005	0.001	**0.02**	**0.09**	0.001
HPC	0.003	**0.04**	**0.05**	**0.03**	**0.09**	**0.05**	0.002	0.001
pH	-0.005	-0.01	-0.02	**-0.05**	**-0.07**	**-0.02**	-0.01	-0.01
Conductivity	-0.02	**-0.04**	**-0.03**	**-0.04**	**-0.04**	**-0.03**	-0.01	-0.02

The correlations between rainfall and bacterial levels for summer months (Table 3.25) were statistically significant for a number of previous days rainfall, however, the strength of the correlations was extremely weak (low r^2-values). In other words, there is a high degree of confidence that rainfall has an extremely small degree of impact on water quality at the 24hr time scale, although for practical purposes the size of this impact could be considered as zero. Table 3.25 indicates that rainfall volume accounts for approximately 10% or less of the variation seen in bacterial concentrations in rain events within 24hrs of sampling. The proportion of bacterial variability attributed to rainfall volume in events more than 24 hrs prior is even lower.

The winter correlation matrix (Table 3.26) exhibited an even weaker relationship between rainfall and bacterial levels with fewer significant correlations. This was probably confounded by the higher inputs of chlorinated mains water into tanks during winter, negating the impacts of longer antecedent dry periods between winter rain events which would have otherwise been expected to contribute higher levels of bacterial contamination. Correlations between rainfall and heavy metals appeared negligible and no trends can be seen in either summer or winter correlation matrices.

Table 3-26: Correlation matrix for relationship (r^2) between bacteria and quantity of rainfall in days preceding sampling during winter (April – Sept)

	Rain 0	Rain 1	Rain 2	Rain 3	Rain 4	Rain 5	Rain 6	Rain 7
Coliform	**0.04**	0.02	0.00	0.001	0.004	0.005	**0.03**	0.002
Th. Coliform	0.02	-0.001	-0.001	-0.001	0.006	0.01	**0.03**	0.01
E. coli	0.01	-0.001	-0.001	-0.001	0.005	0.01	**0.03**	0.01
HPC	**0.02**	0.01	**0.02**	0.01	0.005	**0.02**	0.01	-0.001
pH	-0.003	0.01	-0.004	-0.02	-0.02	-0.01	-0.002	-0.001
EC	**-0.03**	0.002	-0.01	**-0.03**	**-0.06**	-0.01	-0.01	-0.006

The lack of a clear correlation between rainfall intensity and contaminant concentrations at the 24hr time scale may have been a result of the two opposing processes. The first process is based on the hypothesis that higher intensity rain events impart more energy on the roof surface and are therefore capable of mobilising greater amounts of deposited matter. Hence, the relationship between rainfall intensity and contaminant concentrations would be expected to be positive, with higher rainfall intensity resulting in higher contaminant concentrations. However, larger rain events simultaneously provide a greater harvestable yield of rainwater, effectively diluting the concentrations of mobilised contaminants to a greater degree than during smaller rain events. These juxtaposed processes are therefore thought to both contribute and mask the relationship between rainfall intensity/volume and contaminant concentration. Patterns of roof runoff quality are known to vary within rain events and between systems. It is generally acknowledged that runoff quality improves over the course of a rain event in a sub-24hr time scale, providing the basis of the first flush disposal principle (Gardner *et al.*, 2004; Millar *et al.*, 2003; Coombes, *et al.*, 2000; Klein & Bullerman, 1989). A much shorter time-scale sampling regime would have been required to achieve the resolution required to observed patterns of roof runoff quality at the Brisbane households, however, this has been the subject of much already published research and was therefore not defined as a major objective of this study.

The second analysis evaluated the relationship between water quality parameters and the length of time between the previous rain event and the sampling date. This is not a measure of the antecedent dry period before the rain event but the lag period between the previous rain event and the sampling date. Rain events were defined here as days on which greater than 5mm of rainfall was recorded. Table 3.27 shows the r^2 values of bacterial and physicochemical parameters when regressed against the number days

Table 3-27: Correlations between the number of days since rain (>5mm) and the concentrations of water quality parameters

	Parameter	r^2-value	Best-fit Trendline
Brisbane			
	Total coliform	0.82	Logarithmic
	E. coli	0.43	Logarithmic
	HPC	0.75	Polynomial
	pH	0.71	Power
	Conductivity	0.77	Power
	Lead	0.72	Polynomial
Newcastle			
	Total coliform	0.62	Exponential
	E. coli	0.12	Linear
	HPC	0.02	Power
	pH	0.71	Polynomial
	TDS	0.74	Polynomial
	Lead	0.24	Exponential

since the previous rain event. The r^2 values for these correlations (Table 3.27) were significantly higher than those for rainfall intensity (Tables 3.25 and 3.26) indicating that the length of time between rain events and sampling accounts for much more of the variation in bacterial concentrations than the intensity or volume of rain events. For bacterial parameters and for lead, the correlations were negative, indicating that as the number of days since the previous rain event increased, the concentrations of microbes and lead decreased. These 'decay' curves indicate that some form of self-purification is occurring within the tanks, discussed in section II.

A variety of trendline types were found to provide the best fit for different water quality parameters within each data set, as well as between the Brisbane and Newcastle data sets for the same parameters, as shown in Table 3.27. This indicates that each parameter responds uniquely to storage in tanks and that the influence of mains water on tank water quality will vary depending on the particular parameter. The 'unaccounted for' variation (i.e. $1 - r^2$) may also affect the response of each parameter to storage, which may have included regional influences between the two cities. The correlations for the Brisbane data set were generally stronger than those of Newcastle, shown by the higher r^2-values in Table 3.27. The Newcastle data have been shown for comparison, although these tanks were not sampled using the same systematic approach employed in the Brisbane study. The adaptive sampling approach employed in the Newcastle study resulted in specific tanks with water quality problems being targeted which effectively skewed the data towards the higher concentration ranges. Consequently, the actual relationships between time and water quality may have varied from those presented here. Scatter plots of the Newcastle data are present in Appendix C.

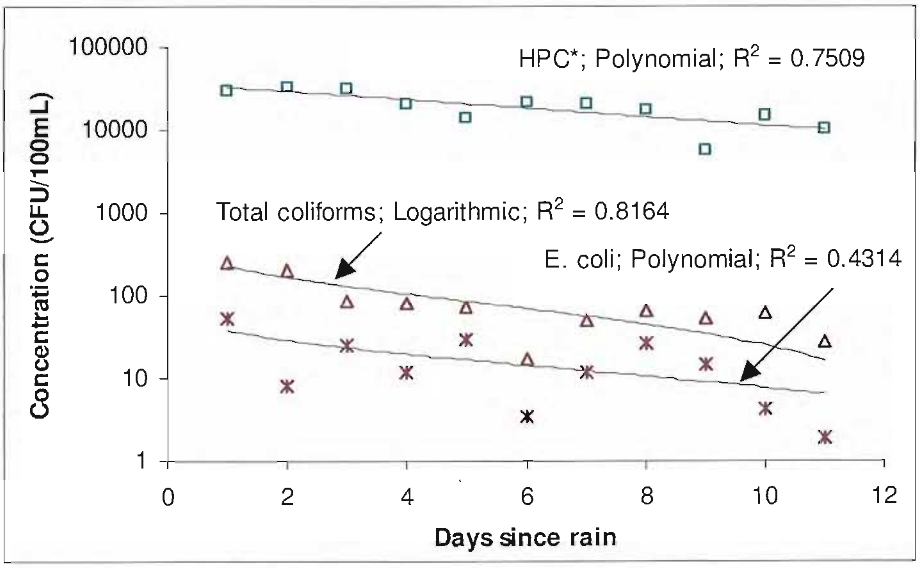


Figure 3-5: Concentrations of bacterial parameters in Brisbane RWH systems as a function of the length of time between the previous rain event and sampling date (*HPC concentrations in CFU/mL)

Figures 3.5 and 3.6 are scatter plots of the average concentrations of bacterial and physicochemical variables in each time category (i.e. number of days since rain event) fitted with the trendline producing the highest r^2-value. The scatter plot of bacterial concentrations is graphed semi-logarithmically, with a linear time scale and a logarithmic concentration scale, shown in Figure 3.5. The reduction in microbial concentrations in the Brisbane tanks was found to be rapid in the initial days following rain events and reducing over time, which are best described by polynomial and logarithmic trendlines. This non-linear reduction is more clearly evident in Appendix D, where bacterial concentrations, as well as pH and electrical conductivity, are plotted on scatter plots with linear axes.

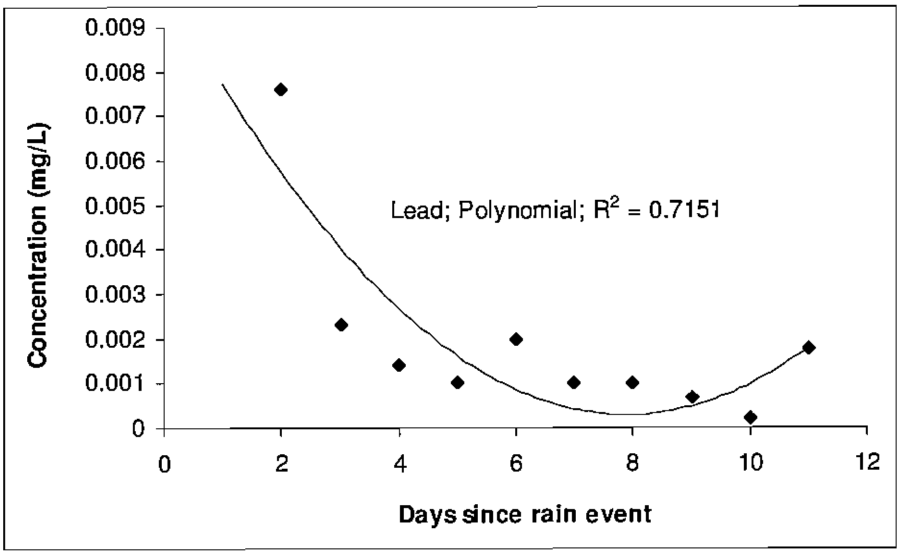


Figure 3-6: Lead concentrations in Brisbane RWH systems as a function of the length of time between the previous rain event and sampling date

The reduction in lead concentrations was also more rapid during the initial period immediately following rain events. Lead concentrations, best represented by a polynomial trendline, were observed to decrease for approximately eight days following rain events, after which concentrations began to increase slightly. This coincided with the estimated time required to trigger mains water top-up in the Brisbane systems (approximately 7.1 days) given the average tank sizes, average number of residents per house and levels of rainwater usage. The average concentrations of lead in tanks eight days after rain events were estimated to be around 0.001mg/L while lead concentrations in mains water were estimated to average as low as 0.0002mg/L (calculated assuming samples below the detection limit were 0mg/L). However, the actual detection limit was significantly higher (0.05mg/L) and lead concentrations in mains water closer to this detection limit may have represented an increase in rainwater tank lead concentrations. It is therefore possible that the concentration of lead in tank water was higher than mains water in the days immediately following rain events though decreased to below mains water levels within approximately 8 days.

3.5 Conclusions

A number of significant findings arose out of this study relating to tank water quality and its relationship with design, environmental and rainfall variables. The harvested rainwater quality in both the Brisbane and the Newcastle studies often did not meet the microbial standards of the ADWG. However, tank water quality in both studies did meet the requirements of the GRWQA for all parameters (with the rare exception of lead and pH in the Newcastle study). Mains water was clearly of a higher microbial standard than harvested rainwater, although this water supply was also vulnerable to exceeding the ADWG on occasions. Lead is the heavy metal of most concern in tank waters, with the majority of other heavy metals being present in trace concentrations usually below detectable limits. The fact that rainwater is not advocated for primary consumption but is intended for secondary purposes suggests that the quality of this supply is acceptable for uses requiring cold water such as toilet flushing, laundry and gardening. This conclusion is reinforced by the almost complete compliance of harvested rainwater with Australian bathing and swimming water quality standards.

A number of design variables were investigated for their influence of water quality, although surprisingly few were found to have any explainable significance. The influence of roof material was seen to influence pH, iron and zinc concentrations. Tank waters collected from METAL roofs had lower pH and higher concentrations of zinc than TILE roofs, though surprisingly contained lower concentrations of iron. One highly important finding relating to roof material was the inappropriateness of lead products within the catchment, particularly lead flashing, which was found to contribute significantly to chronic lead contamination in one system.

Tank materials were found to influence coliform levels, although this was most likely related to the sizes of these tanks. AQUAPLATE® tanks on average contained the lowest coliform levels but also averaged the lowest storage capacity. GALVANISED IRON tanks averaged the second highest coliform concentrations and averaged the second largest capacities, while POLYETHYLENE tanks averaged the largest capacities and contained the highest levels of coliform contamination. GALVANISED IRON tanks were also found to be a source of barium, cadmium, lead and zinc contamination, though did not cause tank waters to exceed ADWG limits.

Tank storage capacity was found to be a significant design variable particularly in relation to microbial quality. This was thought to be related to the proportion of mains water being stored in the tanks, with smaller tanks containing lower coliform concentrations probably due to the higher proportion of stored mains water. Non-microbial parameters did not follow the same pattern of reduction in smaller tanks, suggesting that the action of chlorine entering the tanks during top-up plays a significantly role in reducing bacterial levels. The influence of the tank volume to roof area ratio had a negligible influence on water quality, while the presence of overhanging vegetation was found to decrease ion and metal concentrations, though no logical explanation for this could be found. Surprisingly the proximity of trees to roofs did not influence the levels of bacterial contamination for the groups of bacteria monitored in the Brisbane study.

Clear seasonal trends were observed in the Brisbane study for rainfall along with two separate groups of elements. The high summer rainfall and low winter rainfall distribution pattern correlated with the distinct seasonal distribution pattern of coliform, thermotolerant coliform, *E. coli*, HPC and lead concentrations in tanks, suggesting that these contaminants are transported into the tank through rainfall. The converse seasonal distribution patterns displayed for calcium, magnesium, potassium, sodium and barium strongly indicate that mains water is the major source of these.

Rainfall intensity/volume exhibited surprisingly weak correlation with microbial and chemical parameters. The downpour volumes of rain events within 24hrs of sampling accounted for approximately 10% of the variation in microbial concentrations during summer. The contribution of rainfall volumes to explaining microbial and chemical levels in rain events beyond 24hrs and in rain events during winter were negligible. It was thought that this was due to the opposing processes of contaminant mobilisation and dilution during rain events resulting in a mixed response between contaminant concentration and rainfall intensity. Of much more significance to water quality was the relationship between elapsing time after rain events and water quality. As the number of days between rain events and water sampling increased, the concentrations of bacteria and lead decreased. This was a demonstration of the temporal dynamics of stored rainwater and provided clear evidence of the self-purification of harvested rainwaters.

The single chapter in this section has evaluated water quality in two large urban rainwater tank retro-fit projects by evaluating water quality under the assumption of it having a homogenous and static nature. A number of design variables were then assessed for their contribution to explaining *inter*-system water quality variations. In section II (chapters 4–8), water quality is treated as a spatially and temporally dynamic phenomenon. *Intra*-system water quality variations will be explored and the incidental treatment mechanisms behind these variations investigated. This investigation will begin in the next chapter by examining the role of two specific roof catchments in two rainwater harvesting systems suffering serious and chronic water quality problems.

-------- SECTION II --------

The Incidental Treatment Train

CHAPTER 4

The Roof Catchment

4.1 Introduction

The beginning of all rainwater harvesting systems is the roof catchment. The roof represents both the first component of the treatment train as well as the first, and potentially most significant, point of contaminant entry into the system. The roof catchment contributes to rainwater contamination through the accumulation of environmental contaminants and through leaching of the catchment materials, while exposure to wind, heat, desiccation and ultra violet rays concurrently reduce microbial and organic loads. These parallel processes of contaminant deposition and removal occur simultaneously and are interconnected with the dynamics of the surrounding environment, as summarised in Figure 4.1. The net effect of these processes can be evaluated by looking at the quality of water in the roof runoff and in the receiving tanks.

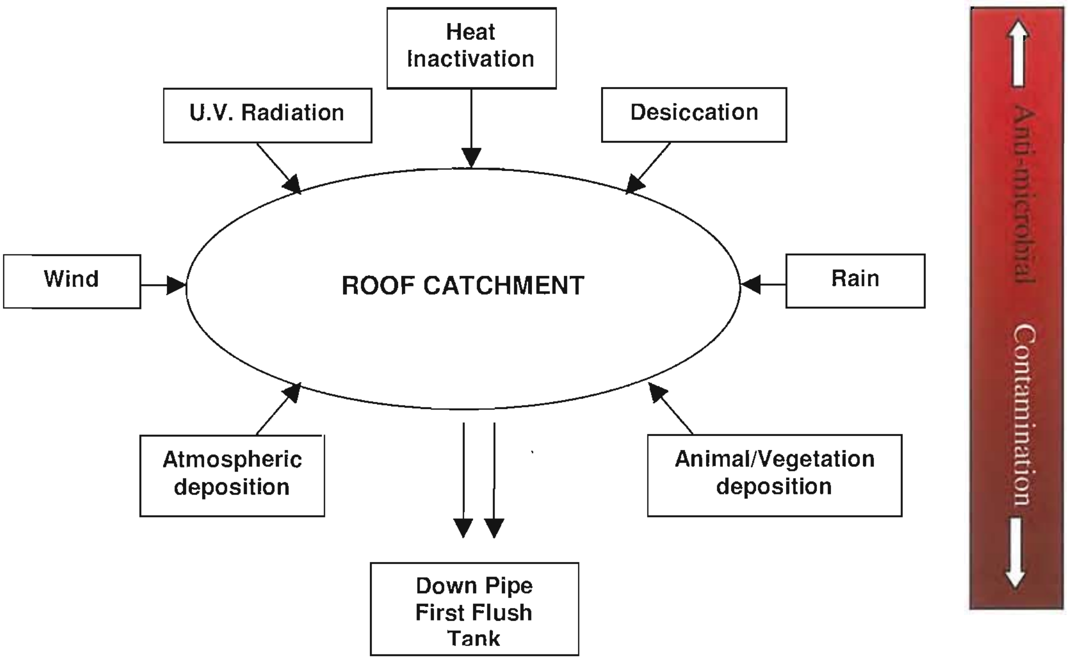


Figure 4-1: Simultaneously occurring pro- and anti-microbial processes on the roof catchment

Roof catchments, and the quality of the runoff waters they produce, are influenced by a number of environmental, design and maintenance factors. Overhanging trees may provide a source of organic contamination and environmental bacteria while also providing access for local fauna and the possibility for subsequent faecal contamination (enHealth, 2004). Local wind and rain conditions have also been shown to influence the microbial loads on roof runoff waters (Evans *et al.*, 2006) while catchment materials can be a source of metal contamination. Lead flashing has been suggested as a source of lead contamination and is generally not recommended for use on roofs that are used for collecting rainwater, though few studies have specifically identified lead flashing as a source of lead contamination in rainwater tanks or have attempted to quantify the levels of lead leaching from flashing (enHealth, 2004). A number of design factors may also influence roof runoff quality, including the aspect and slope of the roof, as well as roof cleaning and maintenance practices.

This chapter presents two case studies of RWH systems that were producing poor quality water and investigates the role of the roof catchment in contributing to the water quality problems. Both case studies involved houses with extreme or chronic water quality problems and the investigations conducted were an attempt to relate tank water quality with specific characteristics of the systems. A number of factors were investigated in an attempt to make these links, including environmental, design and maintenance factors. The aims of this chapter were, firstly, to identify design and component factors of roofs that adversely affect water quality, secondly, to evaluate the contribution of these factors to water contamination, and finally to propose feasible solutions to overcome these issues.

4.2 Experimental Design

Two rainwater harvesting systems were identified in the Newcastle study area due to the atypically high level of contamination in their rainwater tanks and were selected for further investigation. As the general designs of the systems and the types of rainwater tanks used in these two systems were common to many other systems that were free of serious water quality problems, the catchment environments were identified as a key point of interest. The roofs and catchment surrounds were surveyed for overhanging vegetation, proximity to major industries or bushlands, and roofing materials and roof design, including aspect, size and slope.

A number of hypotheses were developed for each site relating to the likely causes of the poor water qualities. The presence of significant overhanging vegetation was noted at both sites and this was proposed to contribute to the high microbial loads and aesthetic problems with the harvested rainwater. Further to this, inappropriate catchment materials (i.e. lead flashing) were found on the roof surface at site 1, hypothesised to be contributing to lead contamination. Design factors were hypothesised to be contributing to the production of poor water quality at site 2, namely the low slope of the roof and the ineffective gutter guard system.

Sampling of the tanks was conducted as previously described in section 3.2.3. Water samples were also taken from various points on the roof catchment at site 1 during rain events by collecting roof runoff overflowing from the roof tiles and by siphoning gutter-water through small pre-sterilised polyethylene tubes. Microbial and heavy metal analysis of the samples was also conducted in accordance with the procedures used in Chapter 3. Laboratory experiments were also conducted to determine the rates of lead leaching from lead flashing. These experiments have been described in detail in section 4.3.1.3 below.

4.3 Results & Discussion

4.3.1 CASE STUDY 1 – Silky Oaks, Ferns and Lead Flashing

The subject of the first case study was an existing house retro-fitted with a rainwater tank. The house was located in an urban area of Newcastle and water from the 5KL polyethylene tank supplied outdoor, toilet, laundry and hotwater uses. During the project a number of water quality issues emerged for this system. Initially, the tank water was found to be of aesthetically poor quality, with the householder complaining of colouration and odour. Microbiological and heavy metal issues also emerged and each of these provided insights into the sources of contamination and the treatment train of the rainwater harvesting system.

4.3.1.1 The Silky Oak Tree

The tank was installed in June 2003 and monitoring of this system commenced 3 months after installation. For the first 6 months of operation a large Silky Oak tree (*Grevillea robusta*) from the adjoining property overhung the western aspect of the roof catchment. During this period degradation of the aesthetic quality of the tank water occurred with some colouration and odour reported by the householder. The Silky Oak is the largest of the Grevillea family and was observed flowering over the house during spring (Sept – Nov, 2003). The householder also noted that each night while the tree was flowering a significant number of fruit bats from Blackbutt Reserve, a nearby bush reserve that is habitat for a large population of fruit bats, would roost in the Silky Oak and feed on its flower. It was hypothesised that leaf and organic debris from the Silky Oak along with faecal deposition by the fruit bats were the primary cause of the colouration and odour as well as providing a majority of the bacterial load.

In late November, 2003, the adjoining property was demolished and the Silky Oak was felled. Consequently, the shading of the roof provided by the Silky Oak was reduced and the roof was exposed to significantly more direct sunlight. A large 2-storey house was built 3 months later which provided shade for the western aspect of the roof during the late afternoons in summer and for most of the day in winter.

For microbiological analyses, three water samples were taken from the rainwater tank over a six-week period while the large Silky Oak tree was overhanging the roof. These samples were compared to three samples taken over a six-week period immediately after the tree was felled. The three post-felling samples were taken before the construction of the neighbouring house and the samples in the two groups (OAK and NO OAK) were matched for number of antecedent dry days prior to sampling (2, 4 and 5 days).

Table 4.1 shows the microbial quality of the tank supply with and without the presence of the overhanging tree. The felling of the Silky Oak was found to have a significant impact on water quality, resulting in reduced microbial concentrations and noticeable improvements to the aesthetic quality of the water. The householders no longer observed colour or odour from the tank supply and for the duration of the program (2 years) found the water to be of acceptable aesthetic quality. A significant reduction in the total bacterial load (HPC) was observed, as well as for total coliform and *Pseudomonas*. Interestingly, the concentrations of *E. coli* increased dramatically after the felling of the tree although this was not found to be statistically significant due to large variance within the post-felling group.

Table 4-1: Average bacterial concentrations (CFU/100mL) and standard deviations (SD) in a rainwater tank before and after the felling of a large overhanging Silky Oak tree.

	N	HPC	TC	*E. coli*	Ps
OAK					
	3	666,700	15,467	23	36,167
		(±368,600)	(±6,394)	(±14)	(±15,605)
NO OAK					
	3	17,700	2,867	2,919	2,967
		(±9,600)	(±1,159)	(±1,743)	(±1,463)

The improvement in the aesthetic and microbial quality of the tank water was probably due to a combination of effects produced from the felling of the tree. The tree was a likely source of bacterial contamination, from tree debris and indirectly from bat faeces, as well as a nutrient supply that may have facilitated bacterial growth and odour production within the tank. While it was not determined whether the majority of the bacterial concentration was a result of direct inoculation by the tree and bat faeces or from growth within the tank, the high tank water temperatures (up to 30°C) observed during summer months suggested that water temperatures were sufficient for the growth of many bacterial species. The removal of the tree further provided greater direct sunlight on the roof catchment resulting in magnified microbicidal stresses for bacteria inoculating the roof surface. The subsequent greater heat, desiccation and UV potential presumably also increased photochemical degradation of organic contaminants facilitating easier removal by wind shear forces.

The increase in *E. coli* concentrations after the removal of the tree was unexpected. The Silky Oak and the bat faecal depositions were not thought to be a direct source of *E. coli* contamination as *E. coli* are an enteric bacterium rarely capable of environmental growth and are rarely carried by fruit bats due to the absence of a caecum in their intestinal tract (Gordon & Cowling, 2003). However, the removal of the tree was not expected to increase the loading of *E. coli* on the system. In subsequent investigations, described below, it was found that the source of *E. coli* was quite localised, traced to gutters near a tree-fern on the opposite roof aspect to the late Silky Oak. It was possible therefore that the actual inoculum concentrations of *E. coli* were consistent but that some form of suppression of the *E. coli* population had occurred during the presence of the Silky Oak. This may have involved the destruction or inhibition of *E. coli* cells in the tank, or a reduction in their cultivability in the laboratory, due to the presence of antagonistic constituents sourced from the silky oak/bat faeces. Many bacteria and protozoa are known to be bacterial predators or capable of producing antibacterial agents and the reduction in HPC may have reduced competitive pressures on the *E. coli* population. It is also possible that the increased *E. coli* concentrations were a result of significant inoculation events occurring post-felling and were unrelated to the felling of the tree.

4.3.1.2 Localised Sources of Roof Contamination

Further investigations of the roof catchment were motivated by chronic lead contamination and extremely high *E. coli* to total coliform ratios (EC:TC) in the tank water column, summarised in Table 4.2. Lead contamination persisted through the entire monitoring period with none of the tank water samples complying with the ADWG of 10μg/L. The source of the lead contamination was thought to be from either, or combinations of, the down pipes (stormwater-grade pipes containing lead etching), the roof catchment or atmospheric deposition. *E. coli* concentrations varied over the monitoring period though regularly comprised the majority of the total coliform count, a phenomena that was not observed in any other system.

Table 4-2: Average concentrations of lead and *E. coli* in the water column of the rainwater tank

	N	Average	Range	EC:TC
Lead				
(μg/L)	14	95	34 – 155	–
E. coli				
(CFU/100mL)	21	632	0 – 6800	73:100

Inspection of the roof catchment revealed the presence of numerous pieces of lead flashing located on the western side of the roof, including a long strip of lead flashing running directly along a strategic collection contour, depicted in Figure 4.2. From the runoff drainage lines it was estimated that up to 25% of total roof runoff (front-western aspect) would flow over this one specific piece of lead flashing that had been used in the extension of the roof many years earlier. A large tree-fern overhung the roof and gutter on the eastern side, with the roof catchment essentially free from other interfering vegetation since the removal of the Silky Oak tree.

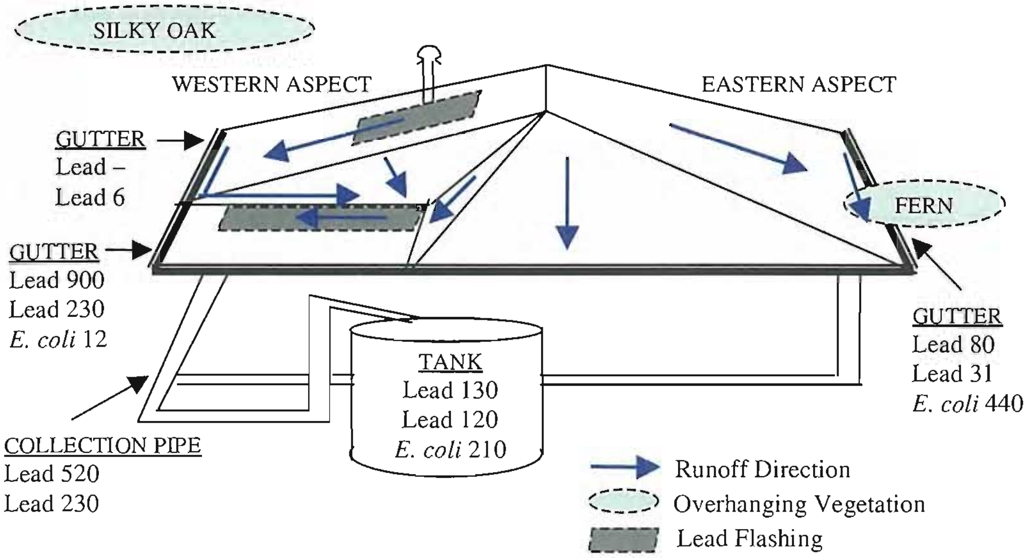


Figure 4-2: Localised sources of lead (µg/L) and *E. coli* (CFU/100mL) contamination. The spatial distribution of lead concentrations from two rain events clearly indicated that lead was leaching from the lead flashing, while *E coli* concentrations were associated with an overhanging tree fern

During two separate rain events samples were taken from five points in the system; three points in the gutters (front/back western; front eastern), from the drowned collection pipes at the point where water harvested from the front halves of the western and eastern portion of roof was mixed, and from the tank where water from the entire roof is mixed. The results were incorporated into Figure 4.2, where it can be seen clearly that the points of lead and *E. coli* contamination were extremely localised and originated from opposite sides of the roof.

The front-western aspect of the roof catchment, which contained the most lead flashing, was found to contribute the clear majority of lead contamination, indicated by the samples which were taken downstream of the prevalent lead flashing. The implication of the front-western portion of the roof as the major source of lead contamination was identified by examining the levels of dilution through the system. Samples taken from the submerged collection pipe that mixed water from the front halves of the western and eastern sides showed concentrations of approximately half that of samples taken from the front-western aspect only. Samples from the tank, mixed with water from the entire roof, were approximately half the concentration again. This suggested that lead flashing was a significant source of elevated lead levels, with contributions from the stormwater pipes and atmospheric deposition being negligible by comparison.

In contrast, *E. coli* concentrations in samples taken from the gutters on the front-eastern aspect were almost 40 times higher than those from the front-western aspect. Concentrations in the tank were approximately half that of concentrations from the gutters on the eastern side. The only feasible source of this contamination was found to be the large overhanging tree-fern located on the eastern side, which would provide a high source of nutrients and constant shade for a section of the eastern gutter. It is likely that small mammals or birds feeding off the fern-tree inoculated the gutters with *E. coli* which were largely protected by the presence of the fern.

4.3.1.3 Lead Flashing Runoff Quality

The consistently high lead levels in the tank were a concern and given the widespread use of lead flashing on roof tops a more explicit investigation of runoff quality from lead flashing was conducted. Although many authors have sited lead flashing as a possible source of lead contamination, no data could be found quantifying the likely contribution of lead flashing to roof runoff quality. A series of laboratory experiments were conducted to examine the leaching rates of lead from lead flashing under differing conditions. Tested variables included runoff pH, runoff flow rate, rainfall impact velocity, and level of oxidation of lead flashing.

An old, heavily oxidised, piece of lead flashing and a new un-oxidised piece of lead flashing were employed in the experiments, shown in Figures 4.3 and 4.4. Each piece had a length and width of 200mm and 100mm, respectively, and was clamped using a retort stand at a slope of 15°. Fifty litre volumes of distilled water were buffered and set to the designated pH using hydrochloric acid and alkaline phosphate buffer. 25L of the buffered waters were then placed into a 40L water dispenser. The water dispenser was set above the top end of the lead flashing to drop water from the heights of 0.1m (LOW) and 2m (HIGH). Flow rates (SLOW=0.4L/min: MED=1.5L/min: FAST=5.5L/min) were initially adjusted by the tap with the remaining 25L of buffered water added to the dispenser over the course of each run to maintain a consistent head pressure. Three 200mL samples were taken during the course of each run from the 1st, 5th and 10th litres of lead flashing runoff. Samples were analysed for lead by Hunter Water Australia (NATA accredited) within 24hrs using atomic absorption spectrometry.

Figure 4-3: Determination of lead mobilisation off heavily oxidised flashing from slow flow (0.4L/min) runoff

Figure 4-4: Determination of lead mobilisation off new un-oxidised lead flashing from fast flow (5.5L/min) runoff

The concentrations of lead in the samples from the 1st, 5th and 10th litre of lead flashing runoff showed no clear trend of increasing or decreasing concentration so data were presented as averages. Figure 4.5 shows the summarised data from the LOW impact (0.1m) experiments where a number of trends could be observed. Perhaps the most striking observation was the difference between lead leaching rates between the old oxidised lead flashing and the new un-oxidised lead flashing. Concentrations of lead in the runoff off old lead flashing were 2–30 times higher than runoff from new lead flashing at the SLOW flow rate, with the effect decreasing slightly for the MED and FAST flow rates (2–10 times higher).

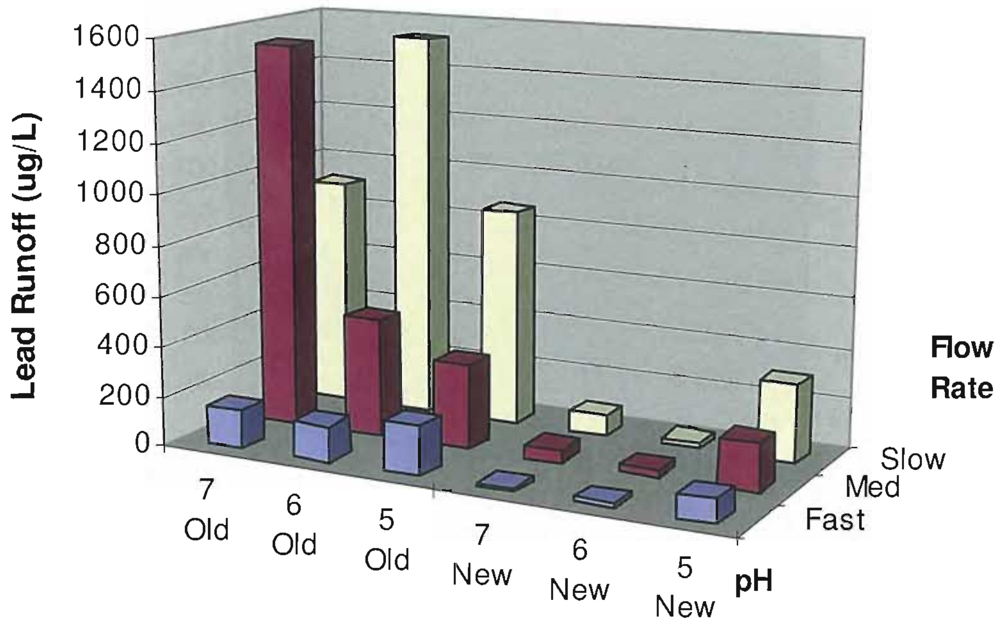


Figure 4-5: Lead concentrations in runoff from old (oxidised) and new (un-oxidised) lead flashing in waters of differing pH and flow velocity.

Flow rate was also seen to be a significant variable, with slower flow rates resulting in higher concentrations of lead. This relationship was particularly pronounced for old oxidised lead flashing ($r = -0.73$, $p < 0.05$) where lead concentrations in the SLOW flow runoff were 4 to 11 times greater than in the FAST flow runoff. A similar but less pronounced effect (not statistically significant) was seen for new un-oxidised lead flashing with SLOW flow runoff containing 2 to 7 times greater lead concentrations than FAST flow runoff. The explanation for the lower lead concentrations in the FAST flow experiments is likely related to the effects of contact-time and dilution. The FAST flow experiments contained appeared to have contained a larger proportion of water that had experienced relatively short contact times with the lead flashing. Shorter contact times were assumed to result in less lead suspension/dissolution, which therefore resulted in the lead concentrations in the FAST flow experiments being subject to a greater degree of dilution.

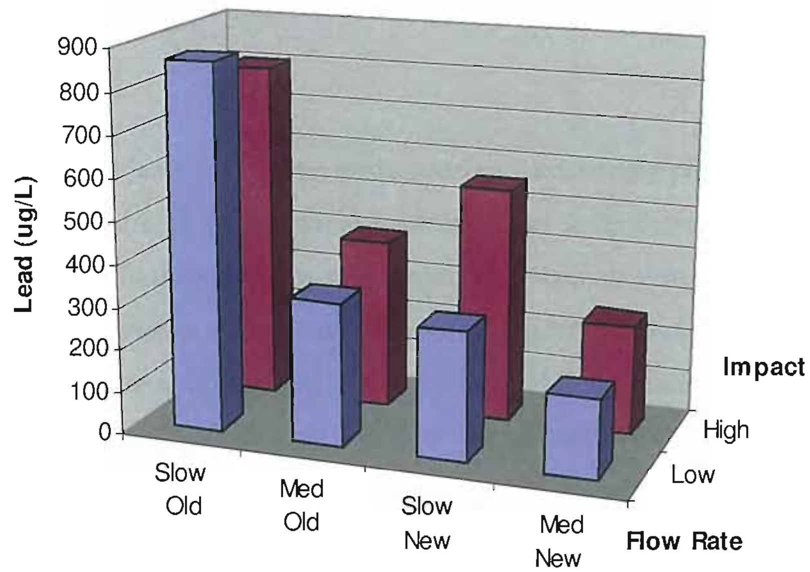


Figure 4-6: Lead concentrations in runoff (pH5) from old and new flashing with slow and medium flow rates comparing high and low initial water impacts.

From these data, it was proposed that the temporal distribution of rainfall will influence lead contamination, with a series of low intensity rain events resulting in significantly more lead contamination than a high intensity event delivering the same amount of water. The new lead flashing was influenced to a far greater extent by the pH of the runoff water, with more acidic waters leaching greater amounts of lead. A clear increase in lead levels in runoff waters with pH 5 was observed across all flow rates. This effect was not seen for old lead flashing where flow rate was the predominantly controlling variable.

The influence of the higher velocity impact (2m drop) generally resulted in slightly higher (though not statistically significant) mobilisation of lead from the flashing, shown in Figure 4.6. The result was seen consistently for the new flashing and for the medium flow rate from the old flashing. However, the flow rate was again identified as the dominant variable, with slow flow rates resulting in higher lead concentrations in the runoff. In reality, the terminal velocity of rain would be higher than that simulated in this experiment and may therefore have a more pronounced influence on runoff concentrations than shown here. However, given the relatively minor surface area occupied by lead flashing compared to the total roof area, the majority of rainfall would not strike the flashing but would flow over it, rendering flow rate as the major potential influencing variable.

Although the use of lead flashing on roofs is being increasingly phased out in Australia, the practice of retrofitting older houses with rainwater tanks increases the likelihood of encountering lead flashing in urban rainwater harvesting systems. Lead flashing is generally recommended not to be used on roofs intended for harvesting rainwaters (e.g. enHealth, 2004). The evidence from case study 1 and the findings of the laboratory experiments were in full support of this recommendation. The extent of lead contamination from lead flashing is directly related to the amount of lead flashing used and the location of the flashing within the roof catchment. Obviously, the greater the relative surface area of the lead flashing on the roof the more rainfall will come into contact with the flashing. Location of lead flashing is therefore also important. The placement of flashing along the ridge lines may be far less detrimental to water quality than at locations downstream where greater volumes of runoff will come into contact with the flashing. The location of the lead flashing along major collection contours in case study 1 was probably the worst possible location for lead flashing, resulting in maximum exposure to roof runoff and high levels of tank water contamination.

4.3.2 CASE STUDY 2 – Gum Trees, Flat Roofs and Maintenance Practices

The subject of the second case study was a house located next to Blackbutt Nature Reserve that had been retrofitted with a 2.4KL polyethylene tank. Severe aesthetic and microbial water quality issues emerged related to a number of design and maintenance factors. The house was considered a worst-case-scenario due to a number of location, design and maintenance flaws.

a)

b)

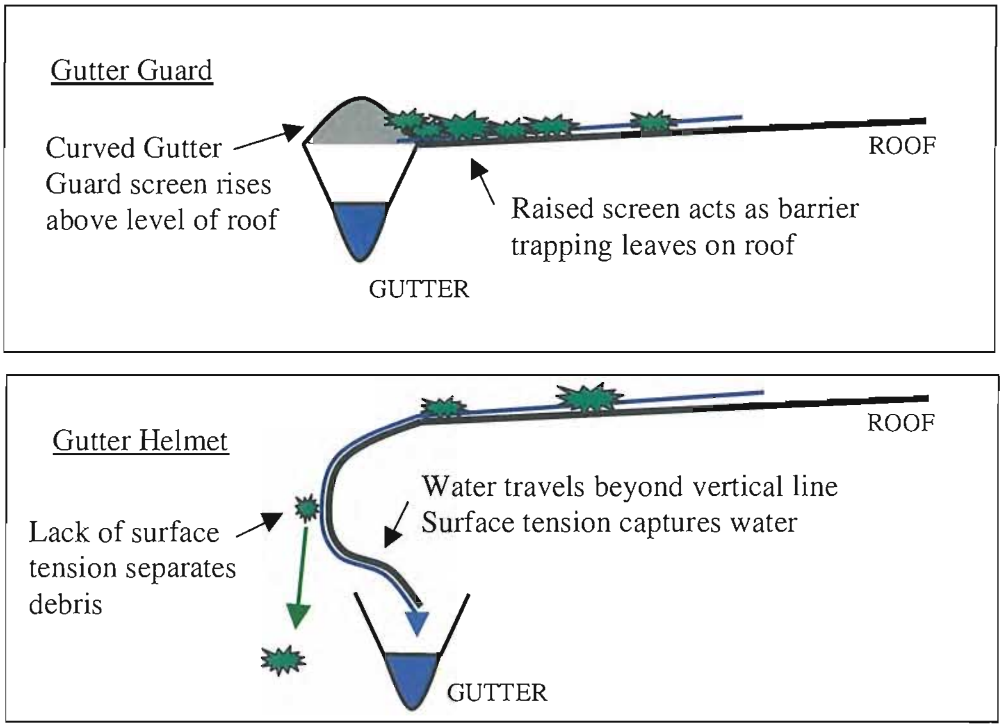


Figure 4-7: a) Traditional gutter guard on flat roofs facilitates the build up of vegetation and organic matter, b) Gutter Helmet is proposed to separate the majority of roof debris from harvested rainwater due to differences in surface tension capacities.

The house had been noted in a preliminary survey to be a likely problematic site due to the presence of eight large overhanging gum trees. The close proximity of the site to Blackbutt Reserve ensured the gum trees were frequented by a range of birds and marsupials and that the roof catchment was accessible to reptiles and bats droppings. One of the major design problems was the low slope of the Colorbond® roof which accumulated large amounts of vegetation. Gutter guard had been installed in an attempt to reduce the levels of organic matter entering the system. However, the raised gutter guard inadvertently prevented much of the vegetation from blowing off the roof and acted as a leaf trap accumulating vegetation directly behind it, as illustrated in Figure 4.7a.

The tank was installed and connected in October, 2003, and with the first major rain event severe odour and colouration were reported by the householder. The water was of such unacceptable aesthetic quality that it was completely disconnected from all indoor usage and limited to garden watering. Microbiological analyses were conducted on two samples from the system during this time, with the results presented in Table 4.3. The physical and microbial quality of the tank water were clearly very poor, with *E. coli* and total coliform concentrations several orders of magnitude above those typically observed in other systems. The waters also contained an extremely low pH and dissolved oxygen concentration. The second sample was taken 5 days after a significant rain event where 105mm of rain was recorded in 24hrs.

Table 4-3: Microbial Concentrations in Putrefied Rainwater

	E. coli CFU/100mL	TC CFU/100mL	Ps CFU/100mL	HPC CFU/mL	Temp. °C	pH	DO mg/L
19.01.04	>1 500	>100 000	1 700	4 000	–	–	–
02.03.04	75 000	150 000	8 000	44 000	19.6	3.9	1.8

A thorough inspection of the entire RWH system was conducted and revealed that a significant amount of heavily decomposing organic matter had accumulated in the gutters, shown in Figure 4.8. Interviews with the householders revealed that they had cleaned the roof surfaces with a pressure hose prior to the installation of the gutter guards and the tank. The gutter sludge appeared to be easily mobilised and averaged 10–20mm in depth. It seems logical that the practice of cleaning the roof catchment with a pressure hose without subsequently cleaning the gutters simply resulted in the mobilisation of a large quantity of organic debris that would otherwise have remained bonded to the roof surface. The following rain events suspended this debris into the water train and transported it to the tank causing putrefaction.

A number of remediation strategies to overcome the numerous defects within the system were considered. Thoroughly cleaning the gutters would have temporarily improved water quality, however, the gutter guards were considered ineffective due to the low slope of the roof and would likely have resulted in poor long-term water quality. The chosen solution involved the removal of the gutters and replacement with Gutter Helmet®. Gutter Helmet is a debris removal method which exploits the high surface tension properties of water. In the Gutter Helmet design, shown in Figure 4.7b, water travels greater than 90° over the eve and is collected in gutters located behind the vertical line of the eve. Debris, which does not contain the same surface tension capacity as water, is separated from the runoff water by gravity when the water begins flowing back under the eve beyond the vertical drop line.

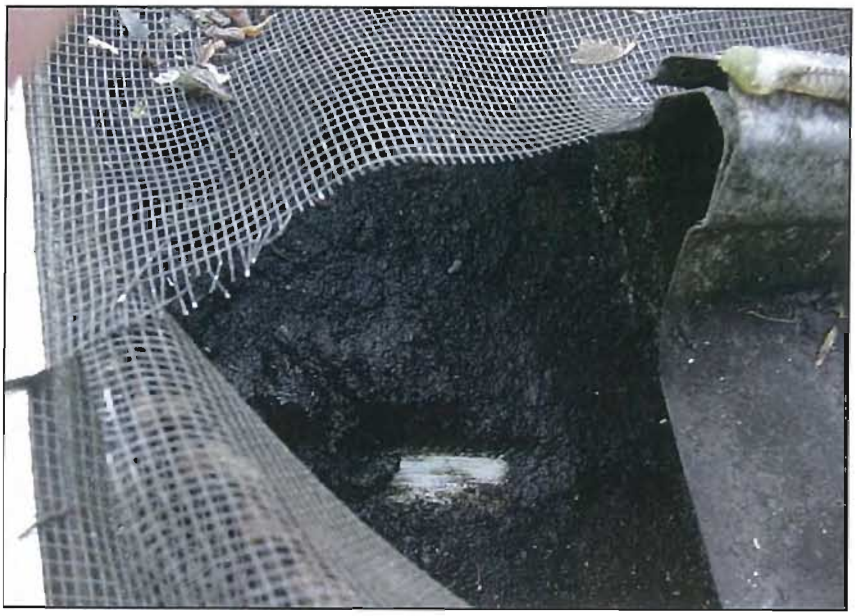


Figure 4-8: Mobilisation of organic matter into a gutter leading to putrefication of tank water

A number of unrelated limitations has delayed the installation of the Gutter Helmet and at the submission of this thesis was yet to be installed. However, the Gutter Helmet appears to be an innovative solution overcoming the problem posed by gutter guards on flat roofs. Replacing of the gutters removed the source of the mobilised particulate matter, although had this not been conducted, cleaning of the gutters and emptying and cleaning of the tank would have achieved this.

The single largest fault with this system was the inappropriate roof cleaning procedure employed by the householder before the tank was installed. In this case, it was highly probable that the cleaning procedure added a significant amount of contamination to the system by mobilising hardened organic matter and that tank water quality may have been far better had the roof not been cleaned at all. A clear lesson from this case study is that maintenance practices must be thorough, removing matter from the system rather than relocating it within the system. Inadequate cleaning may simply risk mobilising contaminants within the system and degrading water quality. This paradox was also noted by Lye (1991) who found that systems with no maintenance had on average 10 fold less bacteria than systems which employed at least one of the following: first-flush diverter, cleaning or disinfection. The author observed that some maintenance practices may actually cause an increase in bacterial concentrations.

4.4 Conclusions

These two case studies highlighted that both the local environment and the roof catchment can be sources of contamination and that contamination can potentially be made worse by inappropriate maintenance procedures. Sources of contamination were also found to be extremely localised within the roof catchment, with small sections of gutters often responsible for the collection of the majority of the catchments contaminants.

Overhanging vegetation was clearly identified as having a substantial influence on the microbial and aesthetic quality of the harvested rainwaters. The main vegetation in these studies included a silky oak, a fern, and several gum trees, which probably provided a direct source of environmental bacteria as well as providing nutrients for potential microbial growth. The trees also likely provided a route for local fauna to access the roof surface enabling faecal deposition on the roof surfaces. The role of bats and bat faeces was not quantified though was possibly also a major influence of water quality. The removal of the Silky Oak tree significantly improved both the microbial and aesthetic quality of the tank water in the first case study, though the removal of surrounding trees is often not a desired solution.

The chronic lead contamination in the first case study was directly related to the extensive use and strategically inappropriate location of lead flashing on the roof. The lead flashing resulted in tank waters consistently exceeding ADWG limits for lead. Laboratory experiments found that oxidised lead flashing contributes significantly more to lead contamination than newer unoxidised flashing and that low flow rates entrain the highest concentrations of lead. This case study supports the recommendation that lead flashing should either not be present or should be painted with an appropriate non-lead-based paint on roofs used for rainwater harvesting.

Selection of the most appropriate method of protecting gutters from organic debris was believed to be related to the slope of the roof. The flat roof in the second case study initially employed gutter guard that trapped vegetation and degraded water quality. Replacement with Gutter Helmet was identified as a more site-sensitive design and is probably more suitable to flat roofs in general. Self-cleaning systems are generally more effective than designs requiring maintenance as very few householders regularly inspect or clean their systems in an appropriate way.

Finally, partial cleaning practices were found to significantly compromise tank water quality. The cleaning of the roof without subsequent cleaning of the gutters was found to mobilise contaminants and resulted in putrefied and unusable tank water. It is important that householders either conduct thorough cleaning of the catchment, entailing the removal of sediment from the system, or conduct no cleaning at all.

This chapter has investigated two rainwater harvesting systems suffering chronic or severe water quality degradation and examined the role of the roof catchments in contributing to the contamination. The remaining chapters in section II will explore treatment mechanisms that provide a benefit to water quality within rainwater tanks and hotwater systems. The following chapter will examine the dynamics of water quality within the water column of rainwater tanks.

CHAPTER 5

The Water Column:

Water Quality Dynamics and Ecology

5.1 Introduction

In many ways, the rainwater tank is the melting pot where a variety of constituents from various environments are brought together to form a unique ecology. The tank is a constrained yet dynamic environment shaped by the physical, chemical and microbial processes occurring within it which in turn are governed by local meteorological conditions, catchment processes and householder behaviour. However, rainwater tanks can still largely be regarded as a 'black box', with little understanding of their internal environments. The experimental design of the majority of previous investigations into tank water quality have effectively treated the internal tank environment is a homogeneous and static environment void of ecology and potential incidental treatment processes.

Recently, however, researchers have begun identifying significant variations in water quality within rainwater tanks and raised the possibility of the presence of a functioning rainwater tank ecology. Coombes *et al.* (2000) found significant variations in bacterial concentrations throughout a rainwater harvesting system, with variations also noted within the water column of the tank, summarised in Table 5.1. The low levels of bacteria observed in rainwater samples at the Figtree Place water-sensitive-urban-design project site were found to significantly increase after coming into contact with the roof catchments. The subsequent passage of the harvested rainwater through the tank and supply pipes resulted in the progressive reduction in concentration of these bacterial populations. The concept of the rainwater tank operating as a bioreactor was then proposed, potentially incorporating incidental treatment mechanisms such as biofilms, sedimentation and stratification (Coombes *et al.*, 2000; Spinks *et al.*, 2003).

Urban rainwater tanks have accessible pathways for a variety of environmental organisms to create an ecosystem in which nutrients and contaminants can be sequestered from the water column and utilised as a substrate for microbial metabolism. Rainwater tanks may also contain a range of environmentally hardy or chlorine resistant bacteria through the input of treated mains water. The uniqueness of urban RWH systems as opposed to rural RWH systems is the existence of a plumbing configuration in which disinfected municipal water is mixed with harvested rainwater to maintain a minimum water level. This provides the means for the creation of a diverse rainwater tank ecology, potentially capable of having a beneficial impact on harvested rainwater quality. The regular introduction of low levels of free chlorine residual into rainwater tanks may also have a substantial impact on microbial populations, though to the authors' knowledge this is yet to be examined.

Table 5-1: Spatial variations in water quality through rainwater harvesting systems at Figtree Place (Coombes *et al.*, 2000)

	Rainfall	Roof runoff	Tank surface	Tank outlet
Microbial (CFU/100mL)				
Thermo coliform	0	218	119	20
Total coliform	0	542	834	166
Pseudomonas	5,200	78,690	6,768	7,544
HPC	300	181,200	325,600	33,100
Physical (mg/L)				
Suspended solids	8.4	4.9	1.4	1.6
Dissolved solids	21	98	98	114
pH	6.0	5.6	6.2	6.1
Chemical (mg/L)				
Lead	<0.01	0.014	<0.01	<0.01
Iron	<0.01	<0.01	0.10	0.10
Cadmium	<0.002	<0.002	<0.002	<0.002

While the water quality dynamics of small decentralised water supplies have been largely overlooked by the recent centralised water paradigm, the benefits of understanding the water quality dynamics of the internal rainwater tank environment are numerous. Elucidation of these processes is important for understanding these water storage systems with a view of optimising maintenance strategies and effectively appraising health risks. A thorough understanding of the processes in rainwater tanks will assist in providing more accurate water quality comparisons between systems and will highlight the need for standardised sampling procedures. It will also provide insights into the need and effectiveness of current maintenance practices and provide the scientific basis for suitable maintenance recommendations. Furthermore, with future research, systems may be strategically designed to optimise the action of such beneficial processes in order to improve water quality.

The objective of this chapter was to investigate water quality variations in rainwater tanks and hence establish the tank as part of the treatment train. The specific aims were, firstly, to confirm spatial and temporal changes in water quality, secondly, to survey microbial communities within rainwater tanks and to investigate the potential benefits of having a diverse microbial ecology within the tank, and finally, to examine the influence of the addition of chlorinated municipal supplies on stored water quality.

5.2 Experimental Design

The Newcastle water quality monitoring program included surveying water quality at multiple points in several separate RWH systems in order to investigate spatial variations in water quality. The sampling points included the collection and distribution pipes as well as varying depths in the water columns of the tanks during periods when the tanks were close to full. The sampling depths of the water column were 100mm from the base (bottom), 50mm from the surface (top) and at the mid point (middle). These systems were also monitored at various times during and after rain events to elucidate temporal trends in water quality.

The field sampling and laboratory analysis protocols were essentially the same as those presented in section 3.1.2. Water samples taken from the top layer of the water column were plunge-sampled while the mid and bottom levels of the water columns were sampled by siphoning through polyethylene tubes. Slow flow rates (<50mL/min) were used during siphoning to avoid agitation of the water column and sludge. The sampling tubes were flushed before collecting sample by draining the first three siphon-tube volumes of tank water to waste.

A survey of the heterogeneous bacterial communities in four rainwater tanks was also conducted. The survey was designed to serve multiple purposes, including to gauge the level of microbial diversity within the tank water columns, to identify species of interest that may pose either health risks or water remediation potential, and to serve as inoculum for heterogeneous thermal inactivation experiments (chapter 8). The samples from the four rainwater tanks were enriched in Brain Heart Infusion (BHI) broth and incubated for two consecutive periods of 24hrs at 24°C and 37°C, described in detail in section 8.1.2.1. Serial dilutions of the samples were then cultured on nutrient agar plates at 24°C and 37°C for 72hrs before selected colonies were isolated for identification. Colonies were differentiated and selected based on unique colony morphology. The selected colonies were identified using Polymerase Chain Reaction (PCR) of the 16S rRNA gene using a universal primer (Wilson *et al.*, 1990). The amplified products were sequenced by Hunter Sequencing Laboratories and species identified in GenBank using the BLAST 2.1 program (http://www.ncbi.nlm.nih.gov/BLAST/).

The influence of the addition of chlorinated mains on microbial concentrations was examined by regularly sampling a selected rainwater tank over a two week period of continual mains water top-up. In order to separate the effect of free chlorine on microbial concentrations from other incidental treatment processes (such as sedimentation, biofilms, starvation), the same tank was evaluated after two separate rain events. After the first rain event, the tank was left full and sampled over the following days, with bacterial reductions being attributed to incidental treatment processes. After the second rain event, the tank was emptied immediately to the point of triggering mains water top up and consequent reductions in microbial concentrations were attributed to a combination of chlorination and dilution from mains water input as well as incidental treatment mechanisms. The tank was drained by siphoning the excess water until the water level had fallen to the level required to activate the top-up mechanism. Visual observation confirmed that the sludge layer at the base of the tank had not been agitated or re-suspended.

Meters on the tank inflows and outflows were monitored to calculate water usage and the volume of mains water being added to the tank. Free chlorine residual was measured by colorimetry (Standard Methods 4500-CI G, 1998) using the Hanna field colorimeter and N,N-diethyl-p-phenylenediame (DPD) powder reagents (Hach). This required the addition of the reagent to 10mL of sample, which facilitated a colour reaction between the reagent and the free chlorine residual which was then quantified in the colorimeter. Changes in the microbial water quality were assessed by monitoring *E. coli*, total coliform, *Pseudomonas* and Heterotrophic Plate Counts (HPC).

5.3 Results & Discussion

5.3.1 Spatial Water Quality Variations

The vertical distribution of bacteria in five rainwater tanks was investigated by sampling at varying water depths within the water columns of the rainwater tanks. On a number of occasions consistent variations were observed in microbial concentrations at different depths in the tanks. As shown in Figure 5.1, the average concentrations of all measured bacterial parameters in the 15 samples taken from the five monitored tanks were greater in the surface layer of the water columns than in the lower levels. This trend was more consistently seen for HPC and *E. coli* than for *Pseudomonas* and total coliforms (TC). The concentrations of HPC in the lower regions of the water columns averaged 63.7% (SD ±16%) of the concentrations observed in the surface layer (100% = 1,670CFU/mL), while the proportions of *E. coli* in the lower regions averaged 81% (SD ±9.5%) of the surface layer concentrations (100% = 26.5CFU/100mL). Microbial stratification was less consistently observed for *Pseudomonas* and total coliforms, with average concentrations in the lower levels of the tanks 82% (SD ±22.5%; 100% = 1,753CFU/100mL) and 94% (SD ±16%; 100% = 1,843CFU/100mL) of the surface layer concentrations, respectively.

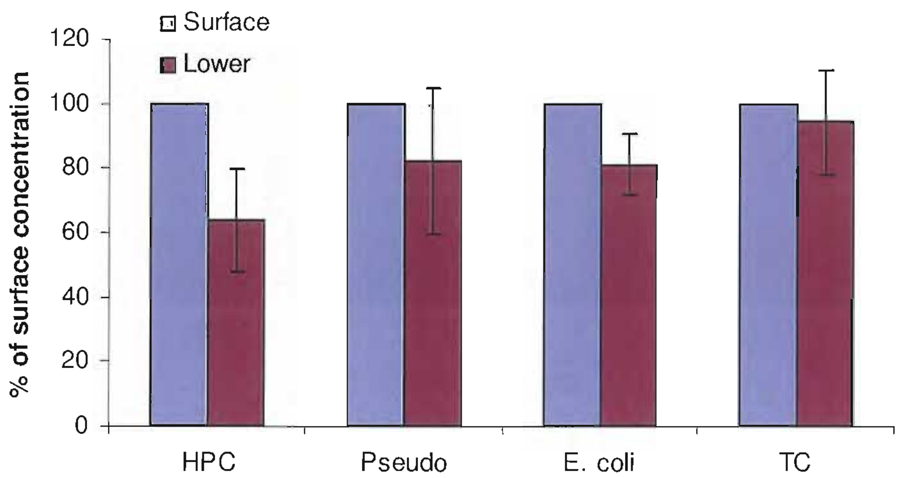


Figure 5-1: Average concentrations with standard deviation bars (I) of bacterial groups in the lower levels (0.1 – 0.2m from base) of the water columns in five rainwater tanks as a percentage of the surface layer concentrations.

Trends of microbial stratification were not always obvious in the monitored systems, with the spatial variations in microbial concentrations at times appearing to be random within the tanks. While rainfall would provide an obvious disruption to microbial stratification, the five tanks were monitored at consistent intervals after rainfall to control this variable. The degree of stratification appeared to be more strongly related to specific tanks. Figure 5.2 shows microbial stratification in one system which was consistent for all bacterial parameters. The consistent trend of stratification in this system resulted in higher concentrations of microbes near the tank surface and lower concentrations in the lower levels of the water column. Concentrations of bacteria in the mid level of the water column were 15-74% of concentrations in the top layer, while concentrations in the bottom layer were 31-74% of the top layer. It is possible that the physical shape of the tank may have accentuated this effect. This rainwater tank was of comparable height (2.1m) to the tallest of the five tanks involved in this specific investigation and had the smallest diameter (1m), resulting in the tank containing the most elongated cylindrical shape of those monitored.

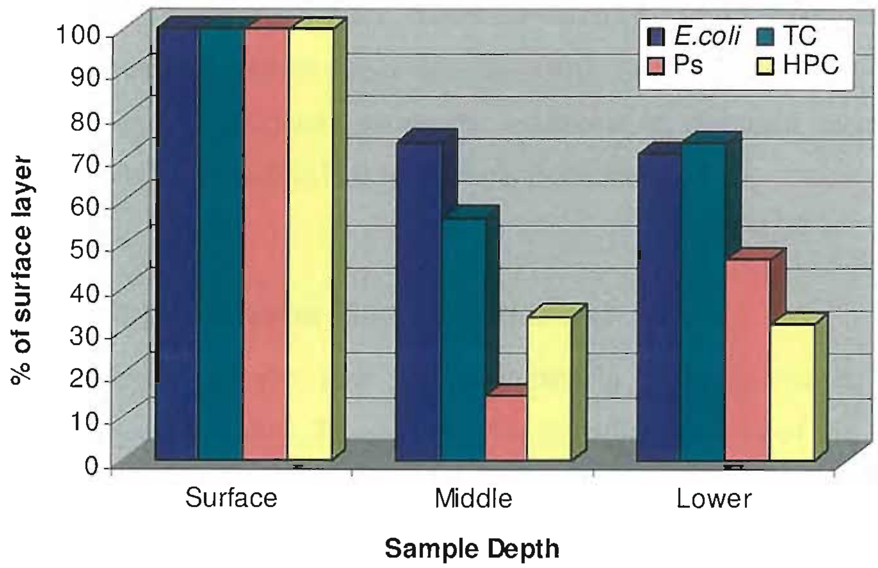


Figure 5-2: Microbial stratification in one rainwater tank resulting in higher concentrations of bacteria in the upper water layer and lower concentrations in the middle and lower levels of the water column

Coombes *et al.* (2000) noted that a surface micro-layer exists in rainwater tanks containing elevated concentrations of microbial cells. This surface micro-layer would incorporate other floating organic matter and, being at the surface of the water column, would also contain the water with the highest temperatures in the tank. Stratification of a tank water column has been shown for physicochemical properties in a much earlier study (Scott & Waller, 1987). Thermal stratification of stagnant water bodies is a well known phenomena and it is possible that the physical characteristics of water bodies influence the spatial distribution of microbial populations (Hollibaugh *et al.*, 2001; Owens *et al.*, 1986). The benefit of microbial stratification in rainwater tanks employing the mains water top-up system is that the surface layer in such tanks never falls to the level of the outlet, and hence is never part of the available supply.

Water column stratification may be disrupted by a number of physical processes. Inflowing rainfall would cause a degree of water column mixing and result in the lack of stratification for a subsequent period of time. Horizontal thermal gradients caused by heating of tank walls would also possibly create slight currents in the water column overturning stratification. Microbial stratification is also likely influenced by the relative buoyancy of the bacterial cells as well as their ability to adhere to particles that may have a specific gravity less than one and hence remain suspended in the water column. While it will be demonstrated in the following chapters that bacteria and heavy metals were not evenly distributed within rainwater tanks, microbial stratification of the water column does not inherently prove the existence of treatment mechanisms. Temporal changes in water quality may be stronger evidence of this.

5.3.2 Temporal Water Quality Variations

Variations in water quality over time were examined in RWH systems in both the Brisbane and Newcastle studies. To examine this, statistical analysis of the Brisbane dataset was conducted to determine relationships between the concentrations of specific water quality parameters and the length of time between sampling and the previous rain event. The method of statistical analysis reflected the systematic sampling approach employed in the Brisbane study with RWH systems sampled once per month regardless of rainfall. The approach taken in the Newcastle study was a more interrogative approach designed to more explicitly address the hypothesis that water quality improves over time with storage.

As discussed in Chapter 3 (section 3.4.7), specific water quality parameters in the Brisbane study were regressed against the length of time since previous rain events. Reductions in all microbial parameters were observed in the days following rain events, shown previously in Table 3.27. These microbial decay curves were best represented by polynomial and logarithmic trendlines and were found to statistically explain the majority of the variation in microbial concentrations (HPC $r^2 = 0.75$, TC $r^2 = 0.82$, *E. coli* $r^2 = 0.42$), suggesting that storage time plays a significant part in determining water quality. The decay of contaminants was not limited to microbial parameters as lead concentrations were also shown to decline in the days following rain events ($r^2 = 0.72$), shown previously in Figure 3.6.

The five rainwater tanks in the Newcastle study were also sampled over the days following rain events to determine whether water quality was influenced by storage time. The tanks were sampled from the lower region of the water column (100mm from base) to standardise any spatial variations that may have been observed. The water quality within a number of Newcastle rainwater tanks was also found to change over time. The nature of these fluctuations was largely dependent on the specific parameter, though most parameters displayed consistent increasing or decreasing trends.

In the five Newcastle tanks the major microbial and metal parameters were found to reduce in the days following rain events resulting in significant improvements in water quality. Table 5.2 shows the concentrations of microbial parameters in five rainwater systems in the days following rain events. *E. coli* and coliform concentrations consistently decreased over time after rain events. HPC and *Pseudomonas* also often displayed a decreasing trend, although at times concentrations were seen to increase during non-rain periods.

Within 48hrs of the onset of rain, *E. coli* concentrations were found to range between 0 and 410 CFU/100mL, while total coliform concentrations ranged between 125 and 2,050 CFU/100mL. However, during the first week of post-rainfall storage, concentrations of *E. coli* and total coliform were reduced by approximately 50 – 99% in the five RWH systems. *E. coli* concentrations had reduced to between 0 and 18 CFU/100mL while total coliform concentrations were reduced to between 1 and 345 CFU/100mL.

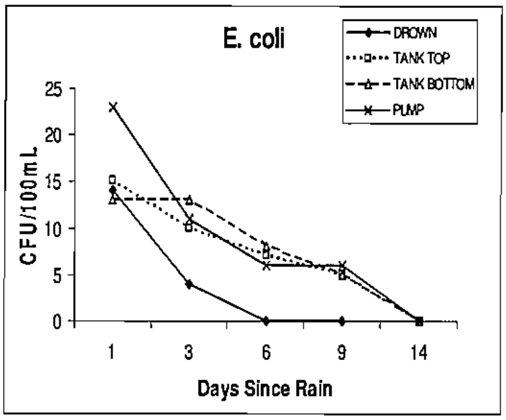
Table 5-2: Changing microbial water quality in days proceeding rain events

Tank	Rainfall (mm)	Days since rain	HPC	*E. coli*	Total coliform	Pseudo
BB	18.4	1	5600	13	265	1900
		3	–	13	226	1720
		6	–	8	140	1570
		9	50	5	135	1610
		14	3	0	2	460
	77	1	20	410	790	14300
		4	10	30	63	460
		6	3000	36	80	40
		8	3200	7	24	900
27G	104.3	2	3550	0	1600	12500
		8	20	0	2	0
W	104.3	2	590	19	550	17600
		8	60	0	1	70
	123.4	1	920	8	2050	4100
		8	100	0	11	90
S	104.3	2	350	6	125	30000
		8	96	2	51	30000
	123.4	1	380	2	460	2920
		8	105	0	180	2670
L	104.3	2	40	42	700	820
		8	210	18	345	8500

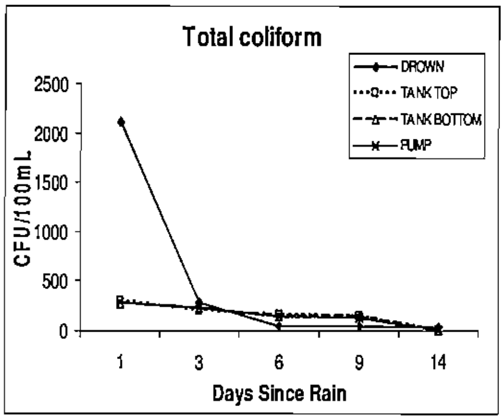
In six of the eight studied rain events, HPC concentrations also decreased during subsequent storage. Immediately after these rain events, HPC ranged between 350 and 5,600 CFU/mL and were reduced over the following week to 20 – 105 CFU/mL. Reductions in *Pseudomonas* concentrations also occurred after six of the eight rain events. *Pseudomonas* populations reduced from 1,900 – 17,600 CFU/100mL immediately after rainfall and decreased to 0 – 2,670 CFU/100mL approximately one week later.

Reductions in the concentrations of the monitored bacterial groups were also seen in different locations within the RWH systems. Figures 5.3a–c show the reductions in *E.*

coli, total coliform and *Pseudomonas* concentrations in one system in the days following a rain event. The samples were taken from different locations within the RWH system, including the permanently submerged collection pipe (also referred to as charged for drown system), the upper and lower levels of the rainwater tank, and the garden tap. The only real exception to the reduction trend was an increase in *Pseudomonas* concentrations between days 3 and 14 after a rain event in the submerged pipe (corresponding to the data in Table 5.2).



a



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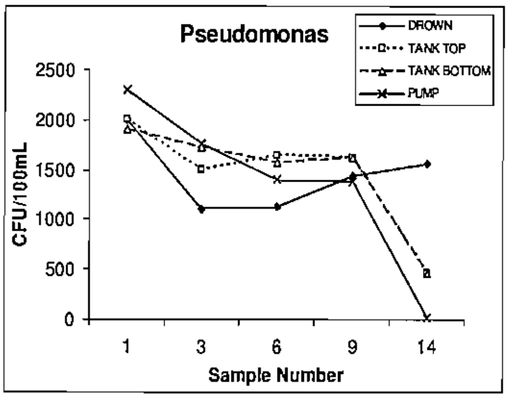


c

Figure 5-3a-c: Reductions in the concentrations of *E. coli*, total coliforms and *Pseudomonas* in days following a rain event in the submerged collection pipe, tank water column (top and bottom) and garden tap (supplied by tank water) of a Newcastle RWH system.

In contrast to the general microbial reduction trend observed in most systems after rain events, the concentrations of HPC and *Pseudomonas* after two of the six rain events were observed to increase during post-rainfall storage. The reason for the occasional increase in HPC and *Pseudomonas* concentrations during storage is unclear. It was probable that *E. coli* and coliform bacteria, being essentially enteric bacteria, were either inactivated more quickly in the water column or were more inclined to attach to biofilms or to settling particulate matter than the majority of HPC and *Pseudomonas* bacteria. The heterotrophic and *Pseudomonas* bacterial communities, being essentially environmental bacteria, are generally more adapted for survival in the environment than enteric bacteria. These bacteria were therefore more likely to persist in the water column without the immediate need for protection in biofilms or large rapidly settling clumps.

However, this does not explain the increases in HPC and *Pseudomonas* concentrations observed. Cellular growth within rainwater tank water columns is generally restricted by nutrient limitations, although the growth of some species has been reported (Lye, 2002) and may also be occurring within the tank biofilms, as discussed in Chapter 6. It was equally likely that the increase in concentrations of these bacteria was due to the disaggregation of bacterial clumps. Under starvation conditions, some bacterial species are known to reduce production of extracellular polysaccharides (cell binding slime) leading to increased cellular dissociation (e.g. Allison *et al.*, 1998) resulting in the appearance of higher plate counts.

If this were the case, this would indicate that bacterial concentrations were not actually increasing but that an underestimation of bacterial concentrations was occurring in the initial samples. The presence of bacterial clumps in rainfall entering the tanks is logical given that environmental stresses (e.g. heat, UV rays, desiccation) on the roof catchment would advantage the survival of bacteria capable of accessing the protection of bacterial clumps on the roof and in gutters. The process of drying also promotes physical adhesion, which would further facilitate the binding of microbes to organic and cellular material, which is often present in gutters. The extent of clump formation may be specific to the roof catchment, the quantity and type of organic and cellular matter in the gutters, and the meteorological and environmental conditions during the antecedent dry period, which would explain the inconsistent observation of this trend.

Reductions in heavy metal concentrations were also observed in the days after rain events. While cadmium, nickel and arsenic concentrations were generally below detectable levels, reductions in lead concentrations were seen across a number of systems, shown in Table 5.3. Immediately after rainfall (<48hrs), lead levels across the systems averaged 22µg/L and exceeded the ADWG (10µg/L) after five of the eight rain events. Within one week of storage, the average concentration of lead in the tanks had decreased to 8µg/L. Lead levels in all but one of the systems had reduced to below the guideline limit, representing an average reduction of 62% (27 – 91%). Lead levels were never observed to increase during storage. Conversely, copper levels were much more regularly observed to increase over time. The opposite trends observed for copper and lead may have been related to their differing dissolution properties. The increased acidity of the rainwater may have been sufficient to facilitate the gradual dissolution of sediment-bound copper over time, though may not have been sufficiently acidic to dissolve greater amounts of lead than were precipitating or settling out of the water column.

Table 5-3: Changing metal concentrations in days following rain events

Tank	Rainfall (mm)	Days since rain	Pb (μg/L)	Cu (μg/L)
BB	123.4	1	80	9
		8	34	13
27G	104.3	2	8	7
		8	5	31
W	104.3	2	11	8
		8	4	30
	123.4	1	9	8
		8	3	22
S	104.3	2	11	110
		8	8	62
	123.4	1	35	6
		8	3	79
L	104.3	2	3	6
		8	3	6
	123.4	1	16	4
		8	5	4

Sporadic cases in the literature have reported temporal changes in the water quality of stored rainwater, with some claiming water quality improves while others claiming it deteriorates over time. Bambrah and Haq (1997) claimed that in Kenya stored rainwater supplies deteriorate over time due to putreficiation of organic material and microbial growth, although these claims were not supported with scientific data. Lye (1991) also found that the growth of some bacteria occurred during storage of harvested rainwaters.

Conversely, other studies have found water quality to improve with storage. Michaelides (1989) noted that microbial concentrations reduced during the first few days storage, while Yang *et al.* (1995) observed that total bacteria and total coliform concentrations were lower after four months of storage. Other studies have also shown physical and chemical improvements in water quality during storage (e.g. Hussein *et al.*, 1997; Kita & Kitamura, 1995; Kitamura *et al.*, 1997).

Gould and Nissen-peterson (1999, p.50) supported these conclusions by commenting that the deterioration of water quality during storage was a 'popular myth'. The quality and robustness of these various studies can be difficult to determine, though it is probable that significant variation occurs in the dynamics and ecologies of stored rainwater in systems of different design, local environmental condition and geographical location. However, the results of this study have clearly established that storage of harvested rainwater in 2 to 17KL polyethylene, Aquaplate®, stainless steel and concrete rainwater tanks located in urban centres along the Australian East Coast resulted in the improvement of water quality.

The significant reductions in concentration of several microbial and chemical parameters, including *E. coli*, total coliforms, *Pseudomonas*, HPC and lead, demonstrate that rainwater tanks have the capacity to act as natural bio-reactors. The improvements in water quality in the absence of deliberate human intervention highlight the existence and efficiency of incidental treatment mechanisms, defining a new understanding of tank eco-dynamics and highlight the need for a further and fuller understanding of the behaviour of rainwater tank ecologies.

5.3.3 Microbial Ecology

The general perception of microbial water quality is that the lower the concentration of total bacteria the higher the water quality. While the ADWG and WHO–GDWQ do not specify maximum HPC limits for drinking water supplies, some national guidelines do, such as the Japanese drinking water guidelines which prescribe a limit of 500 HPC/mL. However, there are a range of bacteria that may be beneficial to the overall quality of the water supply, either indirectly through their contribution to the maintenance of a beneficial tank ecology or through a specific individual ecological function. To examine the hypothesis that the presence of a heterogeneous population of bacteria is beneficial to water quality, individual species were identified and their likely ecological roles, and hence their influence on water quality within rainwater tanks, was speculated through the evaluation of current literature.

To develop a profile of the microbial communities in the water columns of rainwater tanks, water samples from four systems were enriched to facilitate the growth of environmental and enteric organisms. Cultured plate colonies were then isolated and identified by PCR and gene sequencing. Figure 5.4 presents a list of the bacterial species identified in at least one of the four rainwater tanks examined.

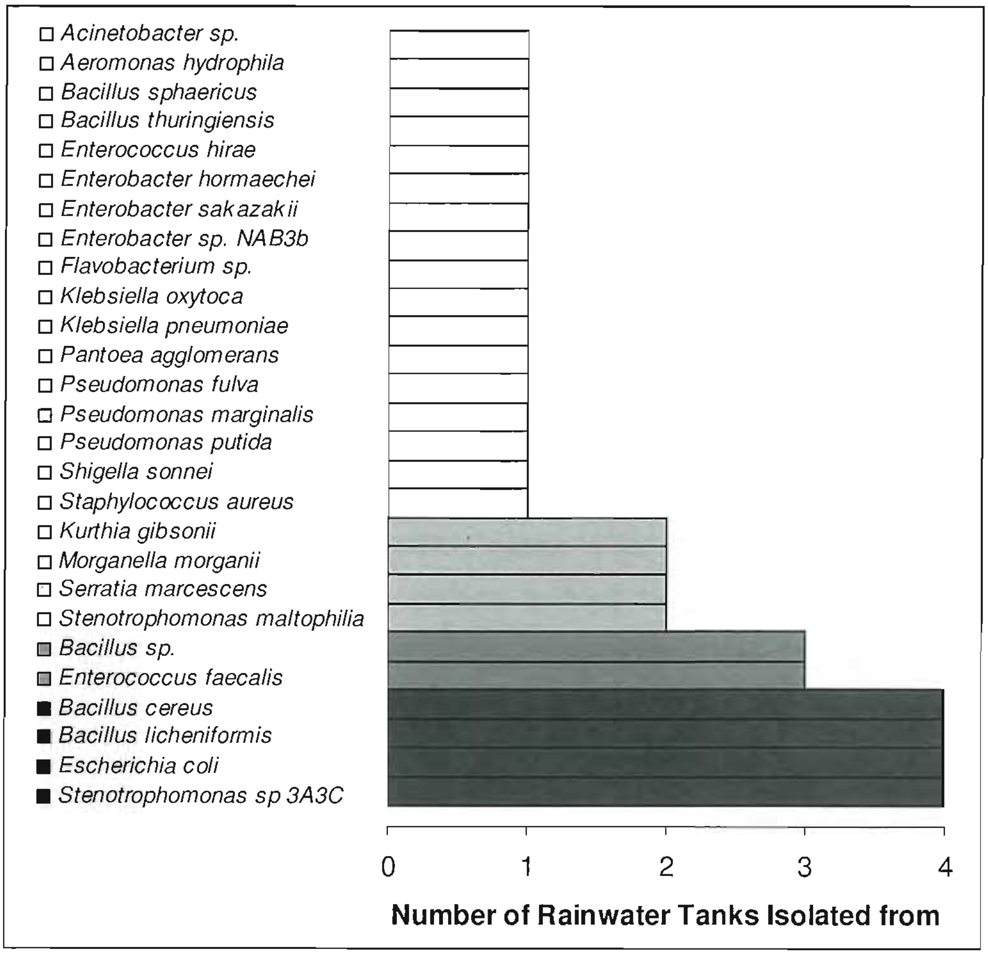


Figure 5-4: Bacterial species identified in four Newcastle rainwater tanks. The majority of species were only identified in one tank, while four species were identified in all of the rainwater tanks.

A range of environmental, enteric and spore-forming bacteria were detected in the harvested rainwaters. While the majority of species were identified in only one of the four tanks, a core group of bacteria appeared to be common inhabitants of rainwater harvesting systems. The core group of bacteria, defined as those being isolated from at least three of the four tanks, included *Bacillus*, *Enterococcus*, *E. coli* and *Stenotrophomonas*. These bacteria have all been commonly isolated in the environment, being sourced from soil and the enteric tracts of animals. *Bacillus* are ubiquitous in the environment and, according to the Australian Drinking Water Guidelines, are commonly found in Australian surface waters though pose minimal human health risks. *E. coli* and *Enterococcus* are common faecal indicator bacteria and, apart from rare strains, are not pathogenic.

Due to the enrichment procedure used to cultivate and isolate these species, the concentrations of individual species could not be quantified. However, the contributions of general bacterial groups to the total microbial population can be determined by the results of the water samples analysed through membrane filtration. The microbial populations in the four tanks were comprised of approximately 0.8% total coliforms, 0.1% *E. coli* and 3% *Pseudomonas*, leaving the major constituents of the population undetermined. This profile was similar to the averages from the entire Newcastle study (TC = 0.7%, *E. coli* = 0.1%, *Ps* = 2.3%) and was comparable to the results from the Brisbane data as well as numerous other studies for the total coliform and *E. coli* proportions (e.g. Coombes *et al.*, 2003; Crabtree *et al.*, 1996; Bo & Guangen, 2001). The small contributions of faecal bacteria to total bacterial populations was also reflected in low faecal streptococci counts (<0.06%) in tanks in Hawaii (Rijal & Fujioka, 1995).

Greater variations may be encountered for *Pseudomonas*, with Evans *et al.* (2006) reporting that *Pseudomonas* comprised 42% of the bacterial composition of tank waters at the Figtree Place development. Given the low contributions of faecal bacteria and the minor proportions of *Pseudomonas* to total microbial load, it seems likely that one or more alternative groups of environmental bacteria constitute the remaining majority of tank microbial populations. It seems probable that *Bacillus* account for a significant portion of this given their widespread distribution in tanks, the detection of multiple species in tanks and their high capacity for survival in various environmental conditions. Furthermore, the ubiquity of *Bacillus* in the environment, and particularly in soil, provide the potential for high inoculum loadings of these bacteria on tanks.

5.3.3.1 Adverse Impacts of Microbial Communities

An assessment of the potential benefits of having a heterogeneous microbial population present in tank waters must first be balanced by evaluating its potential negative impact. This can be achieved by identifying the potentially pathogenic components of the microbial community. From Figure 5.4 it can be seen that the majority of species were present in only one of the four sampled rainwater tanks. Amongst these isolates, some pathogenic and opportunistically pathogenic organisms were detected, including *A. hydrophila*, *K. pneumoniae*, *S. sonnei* and *S. aureus*. Data relating to these species does not implicate them as significant water-related pathogens (WHO, 2006), though greater association of these bacteria with illness is seen in hospitalised and immuno-compromised individuals (Ko *et al.*, 2002; Szewzyk *et al.*, 2000; Rello *et al.*, 1990).

While *S. sonnei* and *S. aureus* can cause severe illness in humans, the transmission of disease caused by these pathogens through water supplies has not been confirmed in Australia. Diseases caused by these bacteria are much more typically associated with alternative transmission routes, such as nosocomial infections (Nguyen *et al.*, 1999; Steinberg *et al.*, 1996). Illness caused by *Aeromonas* and *Klebsiella* is much less severe and less common than that caused by *Shigella* and *Staphylococcus*. Data relating to the pathogenicity of *A. hydrophila* is mixed, with some studies associating it with gastroenteritis while others have not (e.g. Jana & Duffy, 1988; Gracey *et al.*, 1982). *K. pneumoniae* has previously been associated with a range of infections, though given the high levels of general exposure to this ubiquitous species infection rates are relatively low. The presence of a number of opportunistic or pathogenic bacteria in harvested rainwater is not surprising given that a number of species, including *Klebsiella*, *Aeromonas*, *Campylobacter*, *Pseudomonas*, *Legionella*, *Yersinia*, and *Salmonella*, are known to have at least one environmental (i.e. soil or water), small animal, or bird reservoir (Szewezyk *et al.*, 2000).

A number of the other species detected in the tanks have, on rare occasions, been associated with clinical infection. These include *S. marcescens*, *P. agglomerans*, *M.*

morganii, *E. hormaechei*, *E. cloacae*, *S. maltophilia* and *Acinetobacter* sp., which were typically associated with secondary infections relating to injuries such as gunshot wounds, snake bites, thorn cuts, foot wounds, nosocomial infections, use of contact lenses, ventilators and nebulisers, etc. (Corrigan *et al.*, 2001; Cunha, 2002; Valdezate *et al.*, 2001; Jorge *et al.*, 1994; Davin-Regli *et al.*, 1997; O'Hara *et al.*, 1989; O'Hara *et al.*, 2000; Kratz *et al.*, 2003; Ostrowsky *et al.*, 2002; Kim *et al.*, 2002; Lin *et al.*, 1997). These types of injuries obviously pose little relevance to health risk from harvested rainwater use. However, more common ailments, such as burns and damaged eyes, may increase the risk of infection by these bacteria if the wounds are exposed to tank waters or if tank waters are used to wash personal or medical devices, such as contact lenses, ventilators, nebulisers etc. It should also be noted that drinking water, including mains water, is neither regarded as suitable for treating/washing burns or damaged eyes. Chapter 9 further discusses health risk assessment in relation to tank water quality and specific secondary uses.

5.3.3.2 Beneficial Impacts of Microbial Communities

Typically only a small minority of bacteria in rainwater harvesting systems are faecally derived and pathogenic (e.g. Evans *et al.*, 2006; Simmons *et al.*, 2001), which infers that the majority of bacteria in these populations do not negatively impact on water quality. The actual function of these bacteria in rainwater harvesting systems and their potential to impact positively on water quality is, however, rarely investigated. Through a number of direct and indirect means, heterogeneous microbial populations can have a beneficial impact on harvested rainwater quality. Firstly, through the utilisation of nutrients, non-pathogenic bacteria can increase competitive stresses on pathogenic organisms in the water column and accelerate starvation and encourage migration into biofilms and sediment. Secondly, the establishment of multi-species biofilms on the walls of tanks can maximise contaminant removal. And thirdly, the bio-insecticidal properties of some species (e.g. *B. thuringiensis* and *B. sphaericus*) can have benefits to the surrounding environment by controlling pest (e.g. mosquito) populations.

The metabolic utilisation of nutrients by microbial populations is a water treatment process commonly exploited in community scale systems. While the levels of nutrients in rainwater supplies are usually insufficient to be considered as contaminants themselves, the utilisation of nutrients by non-pathogenic organisms increases the competitive pressures on pathogenic organisms which reduces survival rates. Reduced survival rates of the enteric bacteria *E. coli* and *Salmonella typhimurium* has been shown in estuarine waters due to competition from the autochthonous ('indigenous') heterotrophic bacterial community (Chandran & Hatha, 2005; Rhodes & Kator, 1990). These studies also showed that bacterial predators, such as protozoa and coliphages which are known to graze on bacteria, increase the removal rates of *E. coli* and *S. typhimurium* from natural waters.

The nutrient deficient and competitive stresses encountered in rainwater tanks are generally greater on enteric pathogens than environmental bacteria. Despite the limited range of environmental survival mechanisms exhibited by many pathogens, enteric bacteria typically lose cultivability and viability more rapidly than environmental species under harsh low-nutrient conditions. This is due to most pathogens having copiotrophic characteristics, i.e. capable of prolific growth under high-nutrient and high-temperature conditions such as encountered in gastrointestinal tracts, though are not capable of any growth in low-nutrient environments. In contrast, many environmental and aquatic bacteria could be considered oligotrophic, being capable of slow growth in low nutrient environments though not stimulated by high-nutrient zones (Kjelleberg *et al.*, 1982).

Byrd *et al.* (1991) found that the environmental bacteria *Pseudomonas* and *Bacillus* survive in aquatic environments for long periods without losing their culturability. The two tested species of *Pseudomonas* had retained their culturability after more than 95 days in a drinking water microcosm. Conversely, a range of other species including *Enterococcus faecalis*, *Micrococcus flavus*, *Enterobacter aerogenes*, *Klebsiella pneumoniae* and *Agrobacterium tumifaciens*, were found to have lost culturability in less than 5 days in the low-nutrient water microcosm (Byrd *et al.*, 1991).

The loss of cultivability does not necessarily mean a cell has lost its viability, but rather it has entered a viable-but-non-culturable (VBNC) state in which basic metabolic processes continue (e.g. synthesis of RNA and proteins) though cell division does not (Roszak & Colwell, 1987). The VBNC state is analogous to sporulation in spore-forming bacteria whereby cells become more resilient to total inactivation though are dependent upon significant alterations of environmental conditions or else progressive inactivation occurs. Hence, competition from the heterotrophic bacterial community can reduce nutrient availability, increase starvation pressure, and accelerate the adoption of the VBNC state in many enteric and pathogenic bacteria.

The second advantage for rainwater tanks containing a diverse microbial ecology is the development of biofilms. Biofilms are one of the major mechanisms used by microbial communities for nutrient utilisation in aquatic environments. Biofilms are heterogeneous communities of bacteria associated with surfaces which are encapsulated in a slimy extracellular polysaccharide (EPS) framework. This EPS framework allows a wide variety of contaminants from the water column to be adsorbed onto the biofilm and subsequently consumed or co-metabolically utilised by the heterogeneous range of organisms in the underlying microcolonies. Biofilms are particularly useful for their ability to biodegrade a range of recalcitrant substances through diverse co-metabolic processes, many of which remain unidentified. The utilisation of biofilms is a common practice in water and wastewater treatment plants, and the greater the range of species present within the biofilms the greater the range of substance capable of being removed and biodegraded.

Many of the species detected in the water columns of the four rainwater tanks have reportedly been detected in aquatic biofilms (e.g. Wijman *et al.*, 2007; Rice *et al.*, 2005; Bonaventura *et al.*, 2004; Tendolkar *et al.*, 2004; Donlan, 2002; Pratt & Kolter, 1998). Much of the previous research into aquatic biofilm development has focussed on *Pseudomonas* for their conspicuous phenotypic changes when attaching to biofilms. *Pseudomonas* are known to down-regulate the production of motility organelle in favour of EPS production when associating with a surface (Watnick & Kolter, 2000). Multiple species of *Pseudomonas* were detected in the water columns of the rainwater tanks and have been found to make up a moderate to substantial fraction of total bacteria counts. EPS-producing bacteria may also facilitate flocculation of particulate matter suspended in the water column. The slime produced by some bacteria attracts and binds compounds and cells into clumps that may develop a high enough specific gravity to be able to settle out of the water column. Biofilms will be the focus of investigation in the following chapter.

Finally, water quality and the surrounding environment can be improved by the presence of insecticidal bacteria. Some species of environmental bacteria have evolved a capacity to produce toxins lethal to other organisms, including mosquitoes and insects. Two bacteria possessing insecticidal properties are *Bacillus thuringiensis* and *Bacillus sphaericus* which were detected in water samples from the Newcastle rainwater tanks. Varying strains of *B. thuringiensis* and *B. sphaericus* are capable of destroying a range of insect species among the orders Lepidoptera (caterpillars), Diptera (mosquitoes, black flies), Coleoptera (beetles), Hymenoptera (ants, wasps, bees), Homoptera (cicadas, aphids), Orthoptera (grasshoppers, crickets, locusts) and Mallophaga (lice), and have shown toxicity against nematodes, mites and protozoa (Schnepf *et al.*, 1998). *B. thuringiensis* and *B. sphaericus* differ in their host range of mosquitoes, with *B. thuringiensis* more active against *Aedes* and *Culex* spp. while *B. sphaericus* is more active against *Culex* and *Anopheles* spp (Baumann *et al.*, 1991).

B. thuringiensis is the more exploited of the two bacteria and has been widely promoted as a commercial bio-insecticide due to its efficient control of mosquito populations and its non-pathogenicity to humans. *B. thuringiensis* is utilised in a range of commercially available insecticide and pesticide products including Able™, Biobit®, Cutlass™, Dipel®, Foray®, Javelin®, Thuricide® and Vectobac®. Its toxicity comes from a crystal toxin which, when ingested, effectively dissolves the midgut of the mosquito larvae (Bhalla et al., 2005; Schnepf *et al.*, 1998; Mesrati *et al.*, 2005). The toxicity of *B. thuringiensis* to mosquito larvae is largely dependent upon the presence of the pBtoxis plasmid, found primarily in *B. thuringiensis* subsp. *israelensis*, which genetically encodes six of the major toxins. However, conjugation of this plasmid into other *Bacillus* species (including *B. cereus*) has been observed, effectively allowing mosquito-toxicity to be passed between a limited range of bacteria that come into physical contact (Hu *et al.*, 2005).

The detection of *B. thuringiensis* and *B. sphaericus* in urban rainwater tanks was not surprising given their widespread distribution in the environment (Martin and Travers, 1989). Neither species pose health risks to humans due to the host specificity of the toxins and are ubiquitous in soil and in the environment. While the precise strains of the *B. thuringiensis* and *B. sphaericus* organisms detected in rainwater tanks were not determined, Martin and Travers (1989) found that over 60% (1,052 isolates) of the *B.*

thuringiensis isolate taken from soil samples from several countries were lethal to insects in the orders Diptera or Lepidoptera. The presence of these bacteria in rainwater tanks could therefore potentially act as a natural mosquito control mechanism, benefiting the quality of the system as a whole. Further research in this area should focus on elucidating the insecticidal capacities of the species present in rainwater tanks and determining concentrations and distributions within rainwater harvesting systems.

5.3.4 Addition of Chlorinated Mains Water

While the improvements in water quality in the days following rain events were probably significantly influenced by the action of biofilms (chapter 6), sedimentation (chapter 7), starvation and water column stratification, the addition of chlorinated municipal waters was also investigated for its impact on water quality. The design configuration of the rainwater harvesting systems meant that as the harvested rainwater in the tanks was used and water levels in the tanks fell, chlorinated mains water was used to maintain a minimum water level allowing tanks to be constantly used.

It was hypothesised that the mixing of chlorine-disinfected mains water with stored rainwater would provide additional benefits to rainwater quality further to the tanks' own incidental treatment mechanisms. Water quality in a selected field tank was examined during the two week period following two separate rain events. Improvements in water quality after the first rain event were attributed to incidental treatment mechanisms operating within the tank due to the absence of mains water input during this period. Improvements in water quality in the two week period following the second rain event were attributed the combination of mains water input plus incidental treatment mechanisms due to the immediate activation of mains water top-up.

The addition of mains water was found to have a relatively sudden impact on the tank water quality. As shown in Table 5.4, the concentrations of the four monitored bacterial groups were reduced significantly within two days of mains water addition. Although after two days mains water comprised only 22% of the total tank water volume resulting in a free chlorine residual of 0.08mg/L, populations of *E. coli* and total coliform bacteria had almost been eliminated. The pH of the tank water had also risen significantly (1.5 units) after two days of mains water addition indicating that the stored rainwater, despite its acidic nature, was only lightly buffered and easily neutralised. After three days of top-up, *Pseudomonas* and HPC had also been reduced by more than 2 orders of magnitude.

Table 5-4: Influence of mains water top up on tank water quality

Days since Top-up	Sample	pH	*E. coli* CFU/100mL	TC CFU/100mL	Ps CFU/100mL	HPC CFU/mL	Free Chl. mg/L	Rain Water
0	R	5.36	1110	1810	4080	6500	0	100 %
	M	-	-	-	-	-	0.42	
2	R	6.89	0	2	220	2750	0.08	78 %
	M	-	-	-	-	-	0.39	
3	R	7.42	0	0	2	50	0.03	52 %
	M	-	-	-	-	-	0.46	
4	R	7.58	0	0	3	56	0.17	37 %
	M	7.69	0	0	0	0	0.34	
5	R	7.68	0	1	0	12	0.14	26 %
	M	7.64	0	0	1	1	0.51	
6	R	7.74	0	0	1	7	0.20	20 %
	M	7.75	0	0	0	2	0.51	
9	R	7.71	0	1	1	3	0.23	11 %
	M	7.67	0	0	0	1	0.60	

R – Harvested Rainwater M – Chlorinated Mains water

The improvement in tank water quality was a result of the combination of the presence of low levels of free chlorine residual, the dilution of harvested rainwaters with 'cleaner' mains water, and the action of the tanks' incidental treatment mechanisms. In order to distinguish and compare the influence of the incidental treatment mechanisms and the input of mains water on microbial reduction, the decimal reduction times were evaluated for the two events. Table 5.5 presents these decimal reduction times, which are the number of days required to reduce the microbial population by 90% (1 log reduction).

The incidental treatment mechanisms within the tank required approximately 6 days to reduce the *E. coli* and total coliform concentrations by 90% in the absence of chlorine, shown in Table 5.5. *Pseudomonas* were seen to be the most persistent survivors in the tank water, requiring an estimated three weeks before concentrations decreased by an order of magnitude, while the total heterotrophic population required four days. The decimal reduction times were significantly shorter for all bacterial parameters in the presence of low levels of chlorine. The greatest increase in inactivation rate was seen for *Pseudomonas*, which were inactivated more than 20 times faster when tank water was mixed with mains water. Total heterotrophs displayed the least response to mains water input, being inactivated 3 times faster, while inactivation of *E. coli* and total coliforms increased around 12 and 9 times, respectively.

Table 5-5: Number of days required to reduce bacterial parameters by 90% in the water column with and without the presence of chlorine

	Decimal reduction time (days)	
	No chlorine	Chlorine
E. coli	6.2	0.5
Total coliform	6.1	0.7
Pseudomonas	21.1	0.9
HPC	3.9	1.4

These data indicate that the intermittent addition of disinfected municipal water to stored rainwater provides further treatment on top of that provided by the tanks intrinsic treatment mechanisms. Addition of mains water not only enhances the reduction of microbial constituents in the water column but rapidly increases the pH of rainwater to within accepted drinking water ranges.

5.4 Conclusions

It was hypothesised that the internal rainwater tank environment is capable of improving harvested rainwater quality and should be considered as part of the treatment train. Water quality in rainwater tanks was indeed found to be dynamic, varying both spatially and temporally. Some rainwater tanks displayed microbial stratification within the water columns, with higher concentrations of microbes observed in the surface layers of the water columns. Temporal variations were more clearly apparent, with concentrations of bacteria and metals often decreasing in the days following rain events.

Whether through passive or active remediation, it was apparent that incidental treatment mechanisms were operating in tanks to improve water quality.

The second hypothesis, that a diverse microbial ecology exists in the water columns of rainwater tanks, was also found to be true and was found to be comprised of a range of environmental and enteric bacteria. While a relatively small number of bacterial species are potentially pathogenic and unwanted in rainwater tanks, a large range of bacteria exist which may confer a benefit to the quality of the overall system. The presence of a diverse heterotrophic bacterial community within the water column of tanks can increase competitive pressures on pathogens by utilising nutrients, provide suitable inoculum species for the formation of biofilms which have the ability to remove toxic metals and compounds from the water column, and contain insecticidal bacteria, including *B. thuringiensis* and *B. sphaericus*, capable of destroying mosquito larvae.

Furthermore, the addition of chlorinated mains water to stored rainwaters was examined and found to cause considerable reductions in bacterial concentrations. Despite low free chlorine residual concentrations in the tank water columns for the first three days of mains water addition, concentrations of all bacterial groups were reduced by greater than two orders of magnitude, representing an increase above that achieved by the tanks' incidental treatment mechanisms alone.

Hence, the finding that harvested rainwater quality is not only dynamic but that variations in water quality typically result in improvements in quality confirms the internal tank environment as an important part of the overall rainwater harvesting treatment train. The following two chapters will discuss the roles of two key components of the tank treatment train, biofilms (chapter 6) and sedimentation (chapter 7). Chapter 8 will then discuss the impact of thermal stress from domestic hotwater systems on rainwater quality.

CHAPTER 6

Biofilms

6.1 Introduction

The discovery of biofilms and their ability to resist environmental stresses and remove contaminants from water columns have made them a popular subject of recent water-related research (e.g. Langmark *et al.*, 2005; Teitzel & Parsek, 2003; Brown & Lester, 1982). While drinking water suppliers are investigating ways to eliminate them from distribution pipelines, wastewater engineers are ever increasingly utilising their capacity for contaminant removal. However, the absence of any published data on rainwater tank biofilms means that their function and their ability to influence the quality of harvested rainwater is unknown and their presence in rainwater tanks is not confirmed.

The extensive and ubiquitous growth of biofilms on aquatic surfaces indicates that their presence on the inner surfaces of rainwater tanks would be likely. Furthermore, the physical conditions within rainwater tanks, namely low water-column shear forces and often corrugated tank walls means that the development and influence of biofilms on water quality may be significant. A number of broad hypotheses were developed to investigate rainwater tank biofilms, including that biofilms would develop on the walls of rainwater tanks and that the extent of development would be influenced by the surface material of the tank, that biofilm development would be influenced by the height within the tank that they were grown, that not all cells in the biofilms would have the same degree of attachment to the biofilm, that biofilms would be comprised of different species depending upon slide material and depth, and that biofilms would have the capacity to improve tank water quality by removing a variety of heavy metals from the water column.

The aims of this chapter were therefore to; Firstly, confirm the presence of biofilms on rainwater tank surfaces and demonstrate their capacity to form on various types of materials; Secondly, examine variations in cell distribution within biofilms, including comparing proportions of viable, culturable and dead cells in biofilms formed at different water depths and on different slide materials; Thirdly, identify some of the major bacterial species in rainwater tank biofilms, and; Finally, demonstrate the ability of rainwater tank biofilms to remove heavy metals from the water column.

6.2 Experimental Design

This investigation into the composition and function of rainwater tank biofilms involved a combination of field and laboratory experimentation. Biofilms were grown in an operational urban rainwater tank and subsequently analysed and subjected to additional examination within the laboratory.

6.2.1 Growing and Harvesting Rainwater Tank Biofilms

The central feature of the methodology employed here to grow rainwater tank biofilms was the use of a slide rack containing numerous microscope slides. In order for the desired range of variables to be examined, it was necessary to standardise the growth conditions of the biofilms to enable valid comparisons to be made. The adoption of the slide rack was particularly necessary for examination of the tank material variable.

6.2.1.1 Slide Rack

Biofilms were grown in a selected rainwater tank on glass, plastic (polyethylene) and metal (galvanised iron) microscope slides. The glass slides used were standard laboratory specimen slides, the plastic slides were food grade UV-hardened virgin polyethylene donated from Bushman's Tanks®, while the galvanised iron slides were cut to size at the University of Newcastle from a galvanised iron sheet. The plastic and metal slides represented two common rainwater tank construction materials while the glass slides acted as a control material. The dimensions of all slides were approximately 25mm x 80mm, with a final surface exposure area of 13.5cm^2. The slides were suspended in the water column in the designated position through attachment to the slide rack, shown in Figure 6.1. The frame was made of stainless steel with foam padding running along the inner edges of the clamps holding the slides. Six slides of each material were connected to the frame at three different heights. The bottom level joined at a height of 10–30cm from the base, the mid-level joined at 50–70cm and the top level joined at a height of 90–110cm. The tank was subjected to constant use and when the water level fell below 300mm the tank was topped-up with chlorinated mains water to maintain the depth of 300mm. The slides were placed in the rainwater tank in July 2003 and removed in May 2005 giving the cultured biofilms a total age of 22 months.

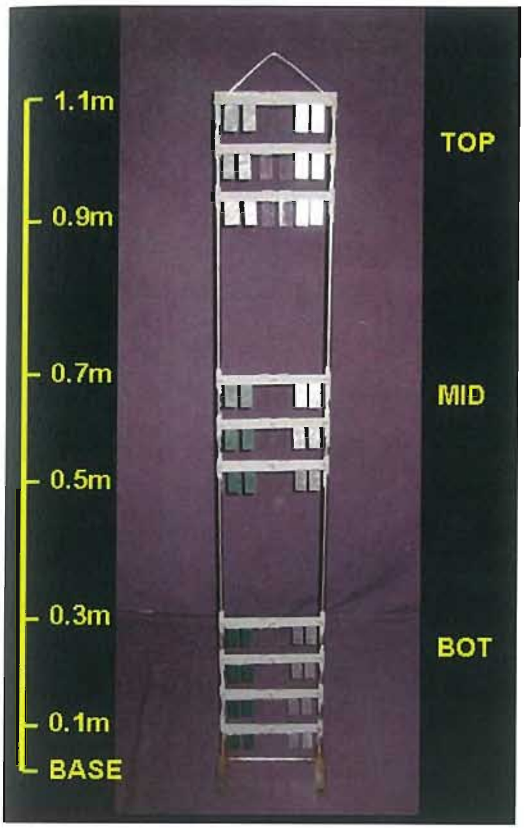


Figure 6-1: The slide rack used to suspended biofilm-slides at three depths within the water column of a rainwater tank.

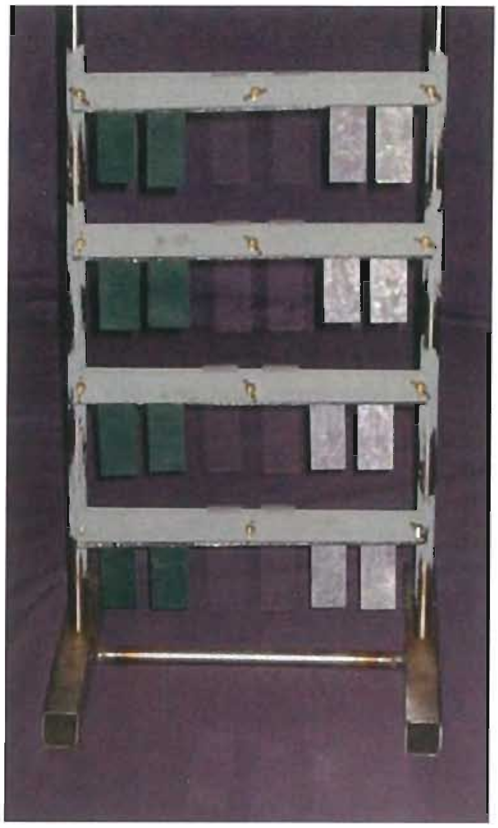


Figure 6-2: Polyethylene, glass and galvanised iron slides were clamped to the slide rack in multiple replicates.

The slides growing biofilms were harvested over a two-week period by carefully removing the drop-in rack through the man-hole in the top of the rainwater tank. Slides were unclamped and placed in sterile 80mL plastic containers with approximately 3mL of added sterile de-ionised water to prevent desiccation of the biofilms during transport. Biofilms were analysed for cell concentrations, cell distribution, microbial composition and heavy metal concentrations. Analysis of biofilms was initiated on the day of sampling with scrapings, spread plates and direct counts being conducted within 24hrs of sampling, while fluorescence microscopy examination was conducted within two days and Electron and Light Field microscopic examinations conducted within seven days.

6.2.1.2 Biofilm Removal from Slides - Scraping

Loosely attached organic and cellular material ('rinse material') was first removed from the biofilm by rinsing with 5mL of filter sterilised (0.22um) deionised water. This rinse material was subjected to identical analyses as that of the following scraped material in order to compare the content distribution and characteristics of each biofilm. The area of biofilm subjected to rinsing was approximately $13.5cm^2$, with precise areas recorded for each slide.

The material remaining attached to the slides after rinsing was defined as 'scraped biofilm'. To remove this material, an adaptation of the scrape/vortex method evaluated by Gagnon and Slawson (1999) was used. Briefly, this method employed a 14mm square-ended metal scraper which was used to scrape the biofilm vertically off the slides, using 5 to 10 strokes after being flame sterilised. The biofilms were scraped across the width of the slides (25mm) giving a total scraped area for each slide of $3.5cm^2$. The position of the scrape varied for each slide to avoid any spatial bias that may have resulted from the highly heterogeneous nature of biofilms. One drop of filter sterilised water was placed on the relevant area of biofilm immediately before each scrape to facilitate with the removal of the material into the underlying 10mL collection tube.

6.2.1.3 Re-suspension - Vortexing

After scraping, the volume of water in each collection tube was raised to 5mL and tubes were vortexed at high speed for 20mins. To reduce the delay period during vortexing, up to eight tubes were clamped to a retort stand and vortexed together. From these 5mL biofilm solutions, samples were taken for heterotrophic bacterial plate counts, direct bacterial counts and elemental analysis.

6.2.2 Enumeration of Bacteria

6.2.2.1 Direct Observations using the LIVE/DEAD® BacLight™ Bacterial Viability Kit

For direct total and viable cell counts of the rinsed and scraped biofilm portions, a relatively new commercially available technique, LIVE/DEAD® *Bac*Light™ (Kit L-7012), was used. This new technique includes the use of SYTO® 9 green-fluorescent nucleic acid stain and the red-fluorescent nucleic acid stain, propidium iodide. The use of these stains allows the differentiation of viable and non-viable cells due to their differing spectral characteristics and abilities to penetrate cell membranes. SYTO 9 is able to penetrate cell membranes, resulting in cells with fully intact membranes to fluoresce green. Conversely, propidium iodide is unable to penetrate cell membranes, and thus will only enter cells that have disrupted membranes. Upon the entry into ruptured cells, propidium iodide reduces the fluorescence of SYTO 9 causing the bacteria to fluoresce red. Hence, with the appropriate mixture of SYTO 9 and propidium iodide, viable cells fluoresce green and non-viable cells fluoresce red. The excitation/emission maxima for these dyes are about 480/500 nm for SYTO 9 stain and 490/635 nm for propidium iodide (Molecular Probes Product Information Catalogue MP07007, 2003).

The assumption with this technique is that cells that have at least a partly compromised membrane will allow entry of propidium iodide and hence appear non-viable, although recovery and regaining of the ability to reproduce may occur under some environmental conditions. Likewise, the integrity of a cell membrane does not exclusively indicate the ability to reproduce, though would be counted as viable using this technique.

The preparation of the BacLight stock solutions were prepared according to manufacturers instructions (Molecular Probes Product Information Catalogue MP07007, 2003) and the methods described by Boulos *et al.* (1999). The two BacLight stains, SYTO 9 and propidium iodide, were mixed together (300uL + 300uL) and dissolved in DMSO. This mixture was diluted 1:10 in a NaCl solution (0.085%) giving a 6 mL BacLight stock solution, which was stored as individual 1 mL stocks at –20°C and protected from light. When required, a 1 mL stock was removed from storage and melted by leaving to thaw at room temperature while wrapped in foil. 1 mL of the vortexed rinsed or scraped biofilm sample was placed in a 10mL dilution tube along with 30uL of the BacLight stock solution. Samples were incubated in the dark at room temperature for 15 min, followed by filtration through a 0.2-um Nuclepore black polycarbonate filter. The filter was mounted in BacLight mounting oil, as described by manufacturers instructions, and covered with glass microscope slide cover slips. Prepared filters were stored in the dark until analysed with fluorescence microscopy, as described below in section (6.2.4.1).

6.2.2.2 R2A Agar Plate Counts

Preliminary biofilm samples were grown on Horse Blood Agar, Nutrient Agar and the low-nutrient R2A Agar to determine the most suitable generic media for aerobic growth of rainwater tank biofilm organisms. Recovery concentrations varied between the Horse Blood Agar and the two Nutrient agars. Only one media was to be chosen due to the extremely large number of plates required for the subsequent subbing and isolation of bacterial colonies from the initial samples. The R2A agar was chosen as it was designed to maximise culturability of partially starved organisms acclimatised to low nutrient environments and also produced comparable concentrations to the Blood Agar. 1mL portions from the biofilm solution were serial diluted and spread plated onto low nutrient R2A agar (Oxoid) and incubated aerobically for 5 days at room temperature (22°C).

6.2.3 Identification of Bacteria

The various biofilm samples (rinsed, scraped, and detachment samples) were cultured on R2A nutrient agar plates as described above, resulting in a mixture of species on each nutrient plate. Bacterial colonies were categorised based on morphology characteristics and gram-stains and were subsequently counted and isolated onto new nutrient plates. Colonies were re-subbed two or three times until pure plates were achieved. In total, approximately 150 isolates were determined to be separate species based on the results of colony morphology, gram staining and basic metabolic reactions.

6.2.3.1 PCR and Gene Sequencing

Of the 150 biofilm isolates, 16 were selected for identification using Polymerase Chain Reaction (PCR) followed by gene sequencing. Isolates were selected on the basis of distribution and prevalence, with the most commonly occurring isolates and those of the highest concentrations preferentially selected for identification. The isolates were identified by PCR through amplification of the 16S rRNA gene and the sequences identified in GenBank using the BLAST 2.1 program, as described in section 5.2.

6.2.4 Heavy Metal analysis

6.2.4.1 ICP-MS

Elemental analysis was conducted using a Sector Field (High Resolution) Inductively Coupled Plasma – Mass Spectrometer, operated by technical staff at the University of Newcastle. Metals were recovered from the biofilms using nitric acid extraction, whereby 2–3mL of the biofilm solution was acidified in Nitric acid at pH 1.5 for 7 days. Samples were then filtered through a 0.45um filter to prevent any undissolved silicaceous material from blocking the ICP-MS extraction tube.

6.2.5 Microscopy

6.2.5.1 Epifluorescence Microscopy

Epifluorescence microscopy was used for direct observations of biofilms growing on glass slides and counts of scraped BacLight™ stained cells. A microscope with attached epifluorescence tube and Kodak camera were used for viewing and photographing the samples. Samples prepared with BacLight™ were observed immediately in a dark room using Filter Set 9 on the epifluorescence microscope, an effective combination of a 480μm and a 490μm laser. This combination of lasers allowed both live and dead cells to be observed together simultaneously. The working photographic field of view for the epifluorescence microscope was 200x400μm in diameter. For each slide, 10 photographs were taken and concentrations of live, dead and total cells were determined and expressed as 'direct counts' (DC/cm^2)

6.2.5.2 Electron Microscopy

Electron micrographs were taken of the surface topography of biofilms growing on each slide material at each depth and were qualitatively assessed for development. Samples were placed in a desiccator for 24 hrs prior to being coated with gold and were observed the following day. The coating of the samples with gold and the actual operation of the electron microscope were conducted under the supervision and assistance of technical staff of the University of Newcastle. The electron microscope allowed high magnification and high resolution of samples with the working field typically between 5-50μm in diameter.

6.2.5.3 Bright-Field Microscopy

Basic bright-field micrographs were also taken of biofilm samples grown on each of the different materials. This required no sample preparation and photographed the images as they are seen with the naked eye. The working field of view of the bright-field micrographs were approximately 20mm in diameter.

6.2.6 Comparing Biofilm and Water Column Concentrations

The major interest in biofilms in this project was related to their ability to remove contaminants from the water column in domestic rainwater tanks. In order to determine whether bacteria and heavy metals were being removed and concentrated in the biofilm, a common unit for comparison had to be established. The samples of biofilm were estimated to have a maximum thickness of 100μm, equating to a volume of $0.01cm^3$ from each $1cm^2$ sample. The comparative factors (CF) were then calculated, which indicated the number of times greater the concentrations of contaminants were in the biofilm compared to the water column on a volume:volume ratio. As the volume of biofilm taken from the $1cm^2$ samples were maximum estimates, the CF's were considered to be conservative estimates of the level of magnification of contaminants in biofilms.

6.3 *RESULTS & DISCUSSION*

On harvesting the biofilm slides from the rainwater tank after 22 months of growth it was clear that some rusting of the frame and of the galvanised iron slides had occurred. This was probably due to insufficient galvanising on the frame and the exposure of the ungalvanised cut edges of the iron slides. The deposition of rust was largely restricted to the lower level slides probably due to their longer overall submersion in water. However, the general appearance of the slides indicated that the experiment had successfully grown thin layered biofilms on all surfaces and, apart from the lower level slides, were mostly free of rust contamination and suitable for analysis.

6.3.1 Topography and Physical Structure

From the initial visual inspections of the slides, biofilms did appear to be present in the form of thin slimy layers with many small perturbations apparently comprised of settled particulate matter. From both the bright field and electron micrographs it appeared that the extent of biofilm development was least on the top level slides situated 1.5 m above the bottom of the tank, with greater development on the middle slides situated 0.6 m from the tank base and even greater on the lowest level slides situated 10-30cm from the tank base. This presence of surface-associated cells was also seen in the epifluorscence micrographs which also showed the top layer containing a greater number of fluorescing cells than the middle layer slides, though rust appeared to have significantly inhibited the fluorescence in the bottom layered slides which contained few fluorescing cells.

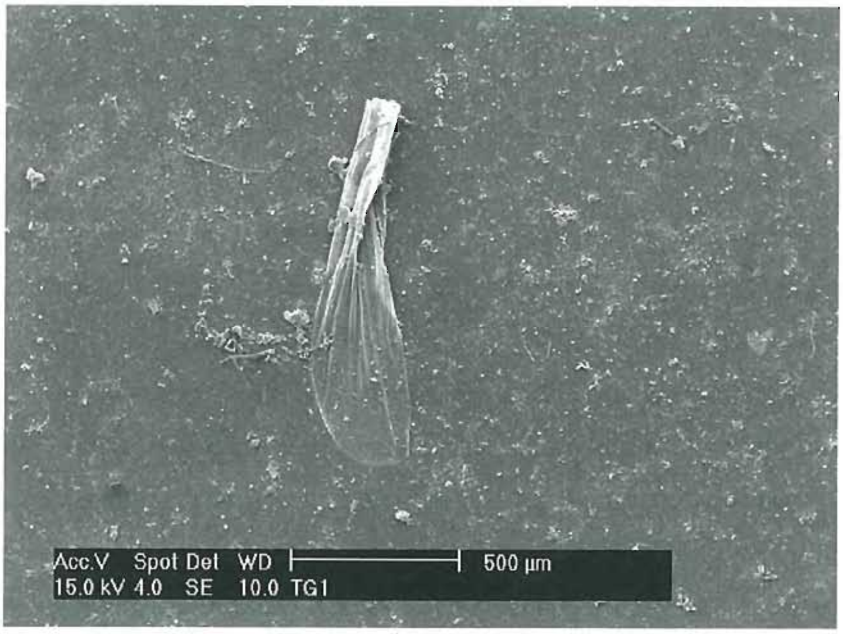


Figure 6-3: Electron micrograph showing an insect wing being incorporated into a relatively thin section of biofilm developing on a glass slide in the upper layer of a rainwater tank.

From the electron micrographs it could be seen that many of the small perturbations were parts of decaying insect bodies (such as Figure 6.3), settled organic matter, micro-organisms and matrix material. Figures 6.4 and 6.5 show small perturbations comprised of mixtures of microorganisms and corrosion products in biofilms grown on glass slides in the top and mid levels, respectively. The presence of simple-structured diatoms were also noted on a polyethylene slide grown at the top level, seen in Figure 6.6, indicating that a mixture of microorganisms may be involved in the formation of rainwater tank biofilms. Figure 6.7 shows the etching corrosion of zinc from the iron slide. The size of the etched corrosion grooves appear to be similar to the size of typical bacterial cells and were possibly associated with microbial action.

The epifluorescence micrographs supported the notion that the development of biofilm microcolonies in rainwater tanks were often based around organic debris or macro-invertebrate carcass remains. Figure 6.8 shows a perturbation on the biofilm under normal light conditions and the same perturbation again in Figure 6.9 under fluorescence which shows it is actually a microcolony packed with viable cells. Figures 6.10 and 6.11 show a piece of probable organic debris under normal light and fluorescence conditions, respectively. The green fluorescent material in Figure 6.11 indicates the presence of viable bacteria and suggests that the debris was subject to microbial colonisation. The development of biofilms on tank walls may not have been only due to the colonisation of the tank walls but also through active cell growth.

The presence of chains of viable bacteria, shown in Figure 6.12, does not confirm the occurrence of cellular division within the biofilms but does indicate it may have been occurring and contributing to the formation of microcolonies. Cellular division in biofilms is known to occur under low nutrient conditions (e.g. Camper et al 1996; LeChevallier, 1990), and the active growth of bacteria within rainwater tank biofilms would be consistent with this. It was also seen from the epifluorescence micrographs that the densities of dead cells varied significantly in different regions of biofilm. While many areas of biofilm contained almost exclusively live cells, some areas contained approximately equal numbers of viable and dead cells (Figure 6.13) and other areas of biofilm were found to be predominantly comprised of dead cells (Figure 6.14). Thus, the visual data from the micrographs provided strong supporting evidence for the presence of living microbial biofilms and demonstrated their ability to develop on a range of materials within a rainwater tank environment.

6.3.2 Microbial Structure: Composition & Distribution

The concentrations of bacterial cells and their distribution within the biofilms were also determined to provide quantified evidence of their presence and structure. The three categories of bacteria examined included viable cells (including both culturable and non-culturable organisms), culturable cells (R2A Agar plate counts) and dead cells (defined as having a ruptured cell membrane). Understanding the concentration differences between viable, dead and culturable bacteria in the water column and the biofilms provided insights into the significance of biofilms within the total tank micro-ecology as well as indicating the nature of cellular colonisation and migration activity within the biofilms. The following four sections discuss the variations in microbial concentrations between the biofilm and the water column, between the surface layers of the biofilm and the underlying basal layers of biofilm, between the biofilms grown on the various substrate materials, and between the biofilms grown at the top and middle layers of the water column.

6.3.2.1 Concentration comparisons of viable, dead and culturable bacteria between biofilms and the water column

Direct counts (DC) of viable and dead bacteria from the water column were conducted on three samples taken from the water column of the tank containing the biofilm rack during a period shortly after a rain event when the tank was nearly full. A 1mL portion of each of these samples was processed and analysed in an identical fashion to that of the scraped and rinsed biofilm samples after vortexing. The culturable cell count of the water column, presented in Table 6.1, was calculated from the average concentrations observed in the tank water column over the entire 22-month period of biofilm growth.

The concentrations of bacteria in the water column of the rainwater tank were found to be approximately 1000 DC/0.01mL for direct viable counts, 160 DC/0.01mL for dead cells and 10 CFU/0.01mL for culturable bacteria. The concentrations of bacteria in biofilms were found to be significantly higher than concentrations in the water column, as shown in Table 6.1. The comparative factors (CF) revealed that biofilm concentrations exceeded water column concentrations by two to four orders of magnitude across the three categories of cells measured (i.e. viable, dead and culturable). Concentrations were also significantly higher in biofilms across all slide materials tested (i.e. glass, metal and plastic). Hence, it was firmly established that biofilms do have the potential to establish on the walls of rainwater tanks and provide an important niche for bacteria within the microbial ecosystem of rainwater tanks.

Table 6-1: Concentrations (standard deviations) of viable, dead and culturable bacteria in biofilms compared to water column concentrations (presented as comparative factors - CF) from three replications

		Viable Counts		Dead Counts		Culturable Counts	
		DC/0.01mL	CF	DC/0.01mL	CF	CFU/0.01mL	CF
Water							
		1,010		160		11	
		(310)		(45)		(9.1)	
Biofilm							
Glass	Top	1.18 x10^5	120	6.03 x10^4	380	1.4 x10^4	1,280
		(4.3 x10^4)		(2.8 x10^4)		(5.9 x10^3)	
	Mid	1.13 x10^6	1,130	5.83 x10^5	3,640	1.35 x10^5	12,390
		(2.9 x10^5)		(3.2 x10^5)		(6.5 x10^4)	
Metal	Top	3.95 x10^5	390	1.46 x10^5	910	1.56 x10^4	1,430
		(1.5 x10^5)		(5.0 x10^4)		(1.3 x10^4)	
	Mid	1.33 x10^5	130	8.14 x10^5	510	7.3 x10^4	6,690
		(3.0 x10^4)		(4.9 x10^4)		(1.4 x10^4)	
Plastic	Top	2.3 x10^5	230	8.27 x10^4	520	2.32 x10^4	2,130
		(1.4 x10^5)		(4.5 x10^4)		(1.8 x10^4)	
	Mid	7.75 x10^5	770	3.09 x10^5	1,930	1.53 x10^5	14,060
		(2.4 x10^5)		(8.1 x10^4)		(2.3 x10^4)	

Table 6.2 summarises the results of the statistical analysis between biofilm and water column concentrations. The greater concentrations of viable and culturable bacteria in the majority of biofilms were found to be highly statistically significant (i.e. $p<0.01$). This was particularly so for culturable bacteria and for biofilms grown at the mid water level. Bacterial concentrations in all biofilms' samples from glass slides were also highly significantly ($p<0.01$) greater than concentrations in the water column for both viable and culturable bacteria at the top and mid levels.

The non-significant difference in concentrations of viable cells found between the plastic-slide biofilms and the water column appears to be due to the relatively high standard deviation of the biofilm counts rather than similar average concentrations between the biofilms and water column. The high standard deviation resulting from the biofilm replicates is not surprising given that biofilms are known to be highly heterogeneous structures with widely variable bacterial counts between relatively close regions (Lawrence *et al.*, 1991). Culturable organisms showed the highest relative difference between the biofilm and water column concentrations. The concentrations of culturable bacteria on the mid level glass and plastic biofilms were 12,000 – 14,000 times greater than concentrations in the water column, and were 1,200 – 2,100 times greater on biofilms from the top level, seen in Table 6.1.

Table 6-2: The statistical confidence of the differences between biofilm and water column concentrations of viable and culturable bacteria. In all cases, biofilm concentrations were greater than water column concentrations.

	Top (Viable)	Top (Culturable)	Mid (Viable)	Mid (Culturable)
Glass Slides	P<0.01	P<0.01	P<0.01	P<0.01
Metal Slides	P<0.05	P<0.01	P<0.01	P<0.01
Plastic Slides	–	P<0.01	P<0.01	P<0.01

One of the reasons for higher levels of culturable bacteria in biofilms may relate to the protection conferred to biofilm-associated cells during chlorination. Codony *et al.* (2005) demonstrated that biofilm cells may be susceptible to intermittent chlorination during initial exposures though develop an increased capacity for resistance after several exposures. The initial high susceptibility of environmental bacteria to chlorine is consistent with the findings of Chapter 5 where it was shown that top-up with chlorinated mains water can result in rapid reductions of bacterial populations in the water column even when free chlorine residual levels are low or undetectable. As was hypothesised in Chapter 5, this may be due to the lack of chlorine resistance amongst the rainwater bacterial community, a large proportion of which would never have encountered chlorination or similar chemical stress before. Conversely, the stationary nature of biofilm cells increased the likelihood of these cells encountering multiple exposures to chlorination and consequently developing a degree chlorine-resistance as a result of various biofilm-specific mechanisms. As such, the water column populations were probably significantly reduced each time the tank underwent mains water top-up while the biofilm populations steadily grew with the aid of increasing chlorine resistance.

Viable cells were also found in higher concentrations in the biofilms than the water column. While the water column concentrations of viable cells averaged 1.01×10^3 DC/0.01mL, the concentrations of viable cells in biofilms ranged between 1.18×10^5 DC/0.01cm^3 (CF 120) and 1.13×10^4 DC/cm^2 (CF 1,130). Interestingly, the proportions of dead cells to viable cells were far greater in biofilms, ranging up to 36 times higher than in the water column. The higher proportion of dead cells in the biofilms did not appear to be a result of cellular damage incurred by the scraping removal of cells from the biofilms, as Figure 6.15 clearly shows the lines of scraping and the lack of ruptured cells around the edges of scraped areas.

The higher concentrations of dead cells in biofilms may have been related to regions of the biofilm where the EPS was inadequate to protect the underlying cells against impending stress. In these instances, large numbers of biofilm cells in specific regions may have been destroyed by chemical or environmental stresses, such as free chlorine residual from mains water top-up or from heat and desiccation upon exposure to air. Slower decay rates of dead cells in biofilms may further accentuate their greater relative proportion through the additional physical protection conferred to cells by the EPS matrix.

6.3.2.2 Cell density in attached and surface layers

It was hypothesised that not all cells within the biofilms would have the same degree of attachment to the biofilm. During biofilm harvesting, bacteria were removed from the slides by rinsing followed by scraping to determine the proportions of loosely and firmly attached cells. As shown in Figure 6.16, the bacterial concentrations in the BASAL (scraped) layers were generally higher than in the SURFACE (rinsed) layers, indicating a higher proportion of cells were securely attached than loosely attached. The average contribution of BASAL cells to the total biofilm population was 69% (45–88%) for viable cells and 66% (14–88%) for culturable cells. The average concentrations of loosely attached SURFACE viable and culturable bacteria therefore averaged 31% and 34%, respectively.

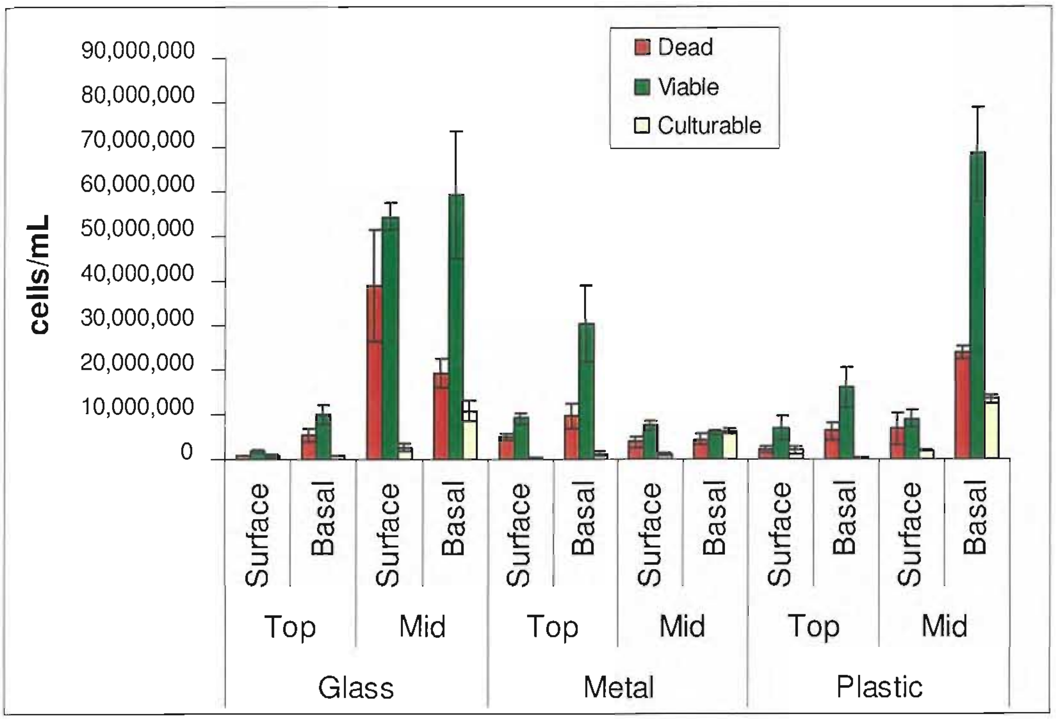


Figure 6-16: Concentrations of dead cells, viable cells and culturable cells in the (surface) rinsed and scraped (basal) portions of biofilms grown on glass, metal and plastic slides at high and mid water depths from three replicates.

The higher concentrations of bacteria in the basal layers were found to be statistically significant in a number of biofilm samples, as summarised in Table 6.3. The concentrations of live, dead and culturable bacteria in the basal layer of biofilms grown on glass at the top level were all found to be statistically greater than concentrations in the surface layer. This was similarly so for basal layer concentrations in biofilms grown on plastic slides at the mid level, and for culturable bacteria on metal slides at the mid level.

Table 6-3: The degree of statistical confidence that the concentrations of bacteria in the basal and surface layers of biofilms were significantly different. All significant results show the basal layer containing greater concentrations than the surface layer.

		Viable	Dead	Culturable
Glass	Top	$P<0.05$	$P<0.05$	$P<0.05$
	Mid	NS	NS	NS
Metal	Top	NS	NS	NS
	Mid	NS	NS	$P<0.01$
Plastic	Top	NS	NS	NS
	Mid	$P<0.01$	$P<0.05$	$P<0.01$

A relatively high proportion of non-viable bacteria were observed in both the SURFACE and BASAL layers of the biofilm on all materials. The dead cells accounted for 23-43% of total cell numbers, consistently exceeding the number of culturable bacteria in the biofilms. The reason for this has not been elucidated, though, as speculated above, is possibly related to the high proportion of dead cells in regions containing inadequate EPS protection and confounded by the likelihood of slow decay rates of bacteria in biofilms.

The SURFACE cells are likely to be in a transient state of either attachment or detachment and may be utilising some of the processes and metabolites of the underlying biofilm communities (Allison *et al.*, 1998). The concentration and composition of cells in these outer layers of the rainwater tank biofilms may have been related to the specific species within the biofilms and the ways in which they interacted and distributed themselves. In their studies of mixed *P. aeruginosa/K. pneumoniae* biofilms, Stewart *et al.* (1997) showed that while *P. aeruginosa* were able to rapidly colonise surfaces and maintain a long-term competitive advantage, *K. pneumoniae* were able to attach to *P. aeruginosa* biofilms and grow more rapidly in the surface layers. These surface layer cells may also be in a more active state of cell division due to their lack of spatial restrictions, or may contain daughter cells produced by the division of the underlying community (Hall-Stoodley & Stoodley, 2002). Surface cells may be more readily transformed into the planktonic phenotype due to the lower surface affinities of daughter cells to substrate surfaces and as part of the holistic integrated strategy of biofilms to enable their resident species to colonise other aquatic surfaces (Baty *et al.*, 2000).

The firmly attached BASAL portion of the bacterial community represented the more permanently attached cells which operate in a more spatially rigid framework. These cells reside closer to the surface of the substrate (tank wall) at a greater distance from the water column and were likely to have exhibited a zero-net growth rate (Watnick & Kolter, 2000). The attached cells may contain the ability to utilise components of the tank wall for nutrients, possess advantageous attachment organelle such as flagella or pili, and fulfil important roles in intercellular communication or co-metabolic processes (Donlan, 2002; Lazarova & Manem, 1995; Colbourne, 1985).

6.3.2.3 Influence of Substrate Material on Colonisation

It was hypothesised that biofilms would develop on materials used to construct rainwater tanks and that the extent of biofilm development may be influenced by the material used. The development of biofilm growth on two commonly used rainwater tank materials, galvanised iron and polyethylene, were compared along with growth on glass control slides. Glass was chosen as a control material because it contains a limited number of additives which effectively renders the material inert. Nutrient support provided by plumbing and piping materials for biofilm growth are not usually provided by the substrate materials themselves, but rather by simple short-chained carbon based additives within the substrate (Colbourne, 1985). Biofilm development on glass was therefore considered to be due to the provision of nutrients from the water column only, and not assisted by additives within the glass.

Both the polyethylene slides and the glass control slides showed very similar patterns of microbial growth, as seen in Figure 6.16. The similarities in BASAL cell growth between the glass and polyethylene slides were not limited to cell concentrations, but were also consistent for relative concentration differences between top and mid level slides, as well as for the ratios of viable, culturable and dead cells. Statistical comparison of total biofilm concentrations between glass and plastic-slide biofilms confirmed that there were no significant differences in biofilm concentrations between the two materials (Table 6.4).

Table 6-4: The degree of statistical confidence that the concentrations of bacteria in the biofilms cultured on glass, plastic and metal slides were significantly different.

	Top (Viable)	Top (Culturable)	Mid (Viable)	Mid (Culturable)
Glass v Plastie	NS	NS	NS	NS
Plastic v Metal	NS	NS	$P<0.05^{P}$	$P<0.01^{P}$
Metal v Glass	$P<0.05^{M}$	NS	$P<0.01^{G}$	NS

^{G} Glass ^{P} Plastic ^{M} Mctal (Material containing significantly higher baeterial concentration)

The colonisation of the glass and polyethylene slides was not surprising given that they are both hydrophobic substances, which are preferred for colonisation by most bacterial species. This is due to hydrophobic substances providing microbial cells with less direct competition from water molecules for absorption sites and to the similar levels of free surface energy between many hydrophobic substances and microbial cells (Pringle & Fletcher, 1983). From the similar patterns of bacterial colonisation of plastic and glass slides and the lack of statistical difference between the two materials, it appeared that, while cells were able to colonise the walls of polyethylene rainwater tanks, their growth was not supported by the leaching of additives from within the substrate but was limited to nutrient supplies from the bulk water phase. This is a significant finding which indicates that polyethylene rainwater tanks may not contribute to or provide additional support for the growth of bacteria in harvested rainwaters.

Bacterial colonisation of metal slides displayed a very different pattern to that of glass and plastic slides. As seen from Figure 6.16, concentrations of bacteria on metal slides at the top-level were generally as high as concentrations on the other two materials, with metal slides even containing statistically greater concentrations of viable cells than glass slides (Table 6.4). However, comparison of metal slides with glass and plastic slides at the mid-water level gave different results. At the mid-water level, biofilms grown on glass slides contained significantly more viable bacteria than metal slides. Furthermore, the concentrations of both viable and culturable bacteria in biofilms grown on plastic slides were significantly greater than concentrations on metal slides. This was most likely influenced by the presence of rust on the mid level metal slides. Corrosion of submerged metal surfaces has recently been shown to be related to microbial activity (Jeffrey & Melchers, 2003) and the rusting of the slides may have been directly related to the action of biofilms, though the establishment of this phenomenon was not an objective of this here.

Rust may have interfered with the enumeration of bacteria for two reasons. Firstly, the presence of significant amounts of corrosion product may have inhibited the growth of bacteria on the metal slides and restricted the level of biofilm development. Conversely, the true number of cells in the biofilm may not have been established due to the interference of rust with the enumeration methods. Rust was found to inhibit the fluorescence of the SYTO 9 and propidium iodide stains during direct counts, suggesting a high probability that direct counts underestimated the actual concentrations of cells on metal slides. This was confirmed with the scraped biofilm on the mid level metal slides where culturable concentrations were higher than direct viable counts. This suggests that while both explanations may be partly responsible, the latter is likely to be the major contributor.

6.3.2.4 Influence of Height within Rainwater Tank

A further hypothesis was that the height within the rainwater tank that the biofilms were grown would influence the extent of their development. Six slides of each material were connected to the biofilm rack at two different depths within the rainwater tank. The mid and top-levels were positioned at heights of 50–70 cm and 90–110 cm from the base of the tank, respectively. The general trend showed much greater biofilm development on the mid-level slides than the top level. From Figure 6.16, large differences in concentrations can be seen between the top and mid-levels for both basal and surface cells in the biofilms.

The higher bacterial concentrations in mid-level biofilms were confirmed through statistical analysis, where all top/mid comparisons were found to be significantly different (Table 6.5). This was consistent for both viable and culturable populations grown on glass and plastic slides, and for culturable bacteria grown on metal slides. However, the one exception to this trend was the lower concentration of viable bacteria on metal-slide biofilms grown at the mid-level, which was thought to be related to rust interference, as discussed above.

Table 6-5: The degree of statistical confidence that the concentrations of bacteria in the top and mid-level biofilms were significantly different. All significant results show the mid-level biofilms containing greater concentrations than the top level.

	Live	Culturable
Glass Slides	$P<0.01^{MID}$	$P<0.05^{MID}$
Plastic Slides	$P<0.05^{MID}$	$P<0.01^{MID}$
Metal Slides	$P<0.05^{TOP}$	$P<0.01^{MID}$

^{MID} mid-level ^{TOP} top-level (Level containing significantly higher bacterial concentration)

The reasons for the greater concentrations of bacteria in the mid-level biofilms were most probably related to the functions of biofilm/water phase contact time, effects of drying, and settlement of particle-associated bacteria. It was also possible that the concentrations of chlorine in the tank, introduced through mains water top-up, may have influenced biofilm development incrementally with depth.

6.3.3 Microbial Composition of Rainwater Tank Biofilms

Biofilms have been well documented throughout the literature as being heterogeneous communities of microbes comprising a range of bacterial species. While the above section examined a number of variables shown to influence the extent of biofilm development, it was also hypothesised that rainwater-grown biofilms would be dynamic in their composition of bacterial species and that differences in species composition would be related to slide material and depth within the tank. To examine this, samples of biofilm from each slide were cultured on R2A agar in triplicate and examined for bacterial composition, with differentiation based on colony morphology characteristics and Gram-staining. Representative samples of each colony type were identified using PCR, as described in section 6.2.3.1.

A variety of bacteria were isolated and identified as major components of the biofilm samples grown on the various surface matrices. Figure 6.17 shows the presence of each species on the different substrate materials from which they were isolated. A core group of bacteria (30% of identified species) were isolated from at least one of three samples from each of the three materials and appeared to be representative of the typical biofilm microflora in this rainwater tank. These species included *Bacillus* spp., *Bacillus cereus*, *Pedobacter suwonensis*, Beta proteobacterium (A1040) and *Stenotrophomonas maltophilia*. With the exception of the unidentified Beta proteobacterium and *P. suwonensis*, these species were also identified in the water columns of a variety of other rainwater tanks and as surviving bacteria in hotwater samples taken from a number of domestic hotwater systems (discussed in chapter 8).

While none of the identified species were isolated exclusively from the glass-slide biofilms, the plastic and metal-slide biofilms did contain species that were not cultivated on the other surfaces. One quarter of the identified species were found to occur exclusively in the plastic-slide biofilms, illustrated in Figure 6.17, indicating that surface material may influence species composition. These included the environmental bacteria *Herbaspirillum hiltneri, Pseudomonas putida, Delftia acidovorans* and *Gordonia polyisoprenivorans*. While the precise activity of these species in the rainwater tank biofilms was not measured, some of these species have demonstrated interesting waste processing capabilities. *D. acidovorans* has been shown to degrade a number of organic compounds (such as 2-4-[sulfophenyl]butyrate and linear alkylbenzenesulfonate) and is thought to be an important species for wastewater treatment (Shulz *et al.*, 2000), while *G. polyisoprenivorans* was discovered through its ability to degrade the rubber in automobile tyres (Linos *et al.*, 1999). The benefits of these bacterial capabilities for harvested rainwater quality is not clear, though potentially may prove to be a novel method of incidental rainwater treatment.

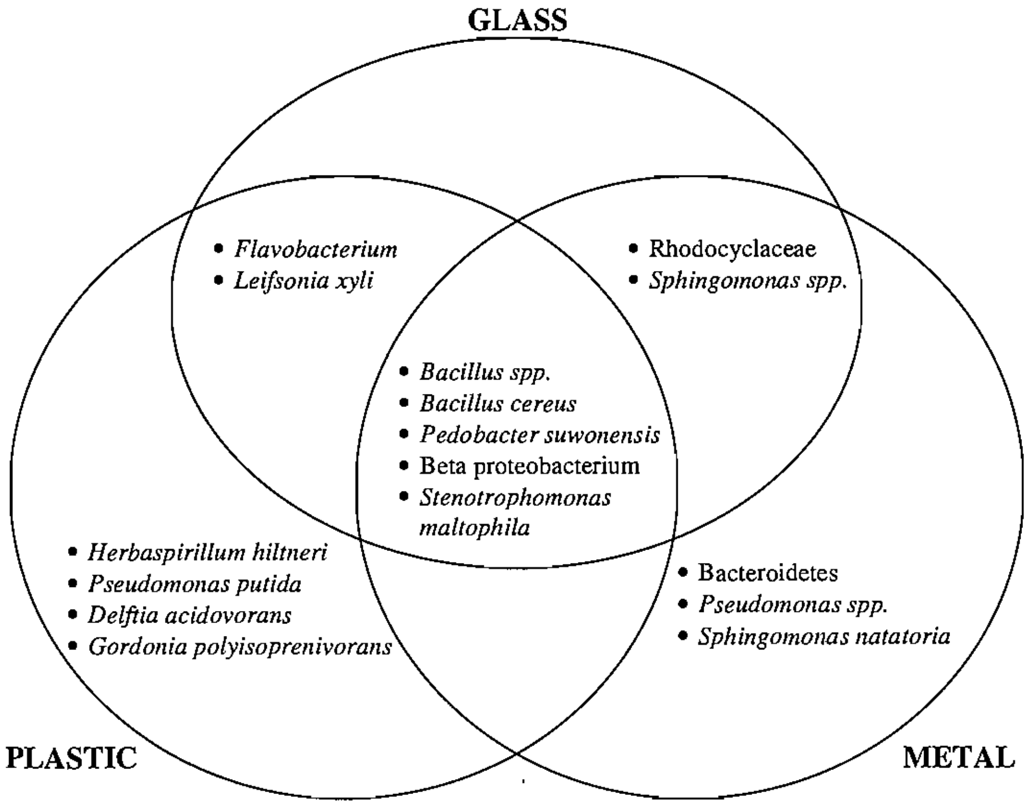


Figure 6-17: Bacterial species in biofilms grown on glass, plastic and metal slides

Three bacteria were identified exclusively in the metal-slide biofilms including *Pseudomonas* spp., *Sphingomonas natatoria* and a member of the Bacteroidetes phylum, providing further evidence that the surface attachment and proliferation of composite species is related to surface material. It is unclear whether these organisms were directly involved in the corrosion of the metal slides though the presence of these species amongst varying amounts of rust suggests they are probably not negatively impacted by high levels of oxidised iron. The detection of *Pseudomonas* spp. was not surprising given that these bacteria have been described extensively in the literature for their active role in biofilm development and extracellular polysaccharide production.

Pseudomonas sp. have often been recognised as the initial colonisers in aquatic biofilms and have been well investigated for their ability to facilitate the attachment of other species (e.g. Tolker-Nielsen *et al.*, 2000; Moller *et al.*, 1998; Stewart *et al.*, 1997; Davies *et al.*, 1993).

Two bacteria were isolated from both glass and plastic-slide biofilms though not from metal-slide biofilms. These included the environmental bacteria *Flavobacterium* spp. and *L. xyli.* A member of the Rhodocyclaceae family and a *Sphingomonas* spp. were also identified on glass and metal slides though not plastic. The species of bacteria identified in the rainwater tank biofilms offer potential water quality advantages, and despite rare clinical isolations of some species (e.g. *Pseudomonas* [Nordbring, 1982], *S. maltophila* [Koseoglu *et al.*, 2004], *D. acidovorans* [Oliver *et al.*, 2005]), these species pose minimal health risk to users.

While many of the widely distributed and heavily concentrated biofilm species were identified in this study, most of the minor constituent isolates were not identified. It is likely that a range of other environmental bacteria were also associated with the rainwater tank biofilms. It was also thought likely that a limited range of enteric species would be associated with the biofilms, given that populations of total coliform and *E. coli* were often present in the rainwater tank water column and that a range of enteric organisms have previously been identified in water system biofilms, such as *E. coli*, *Salmonella*, *Campylobacter*, *Klebsiella* and *Vibrio* (Tenorio *et al*, 2003; Armon *et al.*, 1997; Buswell *et al.*, 1998; Martino *et al.*, 2003; Rashid *et al.*, 2004). However, it was significant to report that no members of the virulent Enterobacteriaceae family were detected in any of the investigated rainwater tank biofilms.

Figure 6.18 shows the relative proportions of each species in biofilms grown on the three substrate materials. The culturable portions of the biofilms were generally dominated by a limited number of species, in particular *St. maltophilia* and *Sphingomonas* spp. These bacteria appeared to be capable of biofilm growth and seemed particularly suited to growth on glass and metal surfaces, indicated by the high proportions of these species in biofilms. The appearance that more than one species may be the dominant bacteria (comprising more than 50% of total population) on each material, for example *St. maltophila* and *Sphingomonas* on glass and metal slides, is explainable by the methodology used in which only the slides that the bacteria were detected on were included in the calculation of the average contribution of the species. Hence, it appears that there may be a degree of exclusivity between *St. maltophila* and *Sphingomonas* in rainwater tank biofilms, where the presence of one species typically excluded the presence of the other. This was clearly evident from Figure 6.19, which demonstrates the spatial distinction between populations of *St. maltophila* and *Sphingomonas*.

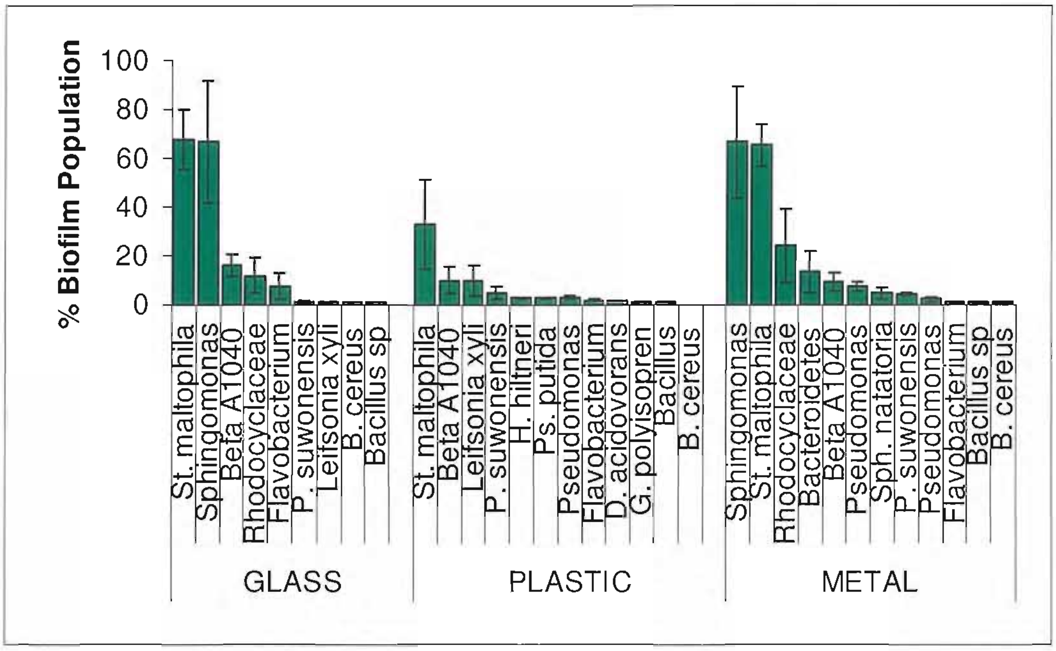


Figure 6-18: Proportions of individual species to total biofilm populations on glass, plastic and metal-slide biofilms.

There was a striking difference between the location of the *St. maltophilia* populations and *Sphingomonas* populations, shown in Figure 6.19. Concentrations of *St. maltophilia* were clearly residing in biofilms in the upper levels of the tank while being almost totally absent from biofilms in the mid-level of the tank. Conversely, *Sphingomonas* populations were minimal in the top-level biofilms but highly populous in the mid-level biofilms. This provided strong evidence that, at least for certain species, depth within the tank alters the microbial composition of biofilms. It is not clear why *St. maltophilia* would find it easier to flourish in the top-level biofilms given that these are subject to more regular desiccation than those of the mid-level biofilms. It seems plausible, therefore, that *St. maltophilia* are out-competed by *Sphingomonas* in the mid-level biofilms, though are more adapted to growth under conditions of intermittent desiccation than *Sphingomonas* giving them a competitive advantage in the top-level biofilms. Both of these species were found in higher concentrations in the scraped (S) fractions of the biofilms than the rinsed (R) fractions, indicating that they are indeed permanent and integral components of the biofilms.

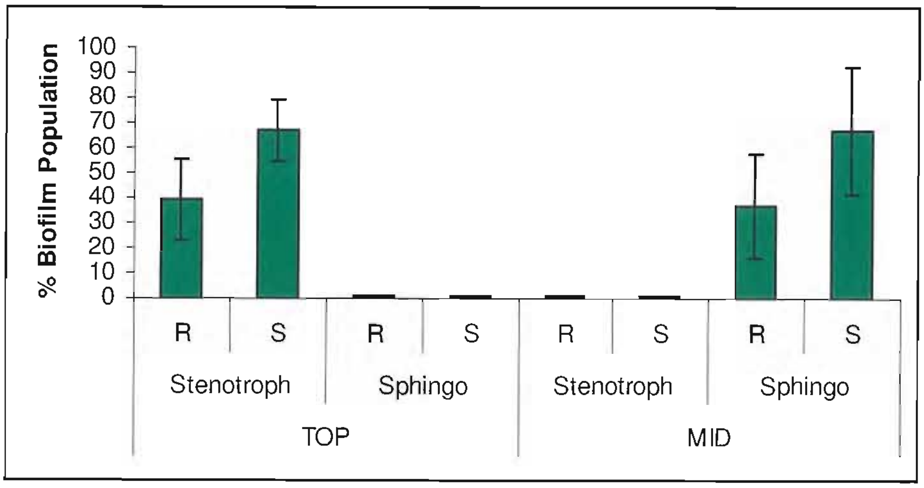


Figure 6-19: Distribution of *Stenotrophomonas maltophilia* and *Sphingomonas* sp. between top-level and mid-level biofilms and their presence in rinsed (R) and scraped (S) fractions.

The results of this study showed that biofilms cultured in rainwater on a variety of surface materials were indeed found to be heterogeneous in nature and comprised of a number of environmental bacteria. A limited number of dominant environmental species were identified in each biofilm along with a number of other minor constituent species. Although estimates of species diversity from agar-plate examinations indicated that the majority of species diversity was not identified, no enteric bacteria were identified in any of the biofilm samples. The findings of this study also demonstrated the dynamic nature of biofilms in relation to surface material and depth within rainwater tanks. Slide material was found to influence the species present in the biofilms, with biofilms cultured on both metal and plastic slides containing species that were not detected on any other surface material. Furthermore, depth within the rainwater tank was also found to be a variable influencing the species composition of biofilms.

6.3.4 Heavy Metal Accumulation in Biofilm

One of the major objectives of this study was to determine whether rainwater tank biofilms exhibited the capacity to remove heavy metals from the water column. The absorption of contaminant heavy metals from the water column into the biofilms would render the biofilms a novel incidental treatment mechanism within rainwater tanks. It was hypothesised that, similarly to bacterial concentrations, the accumulation of heavy metals would be influenced by surface material and depth within the rainwater tank. To asses these hypotheses, scraped portions of biofilm were analysed for a range of heavy metals and concentrations were compared to those in the water column.

6.3.4.1 Toxic Heavy Metals (Pb, Cd, Ni, Hg & Ag)

A number of potentially toxic heavy metals were detected in the water column of the rainwater tank housing the biofilm slide rack, though the majority of these metals were only detected in low or trace concentrations, summarised in Table 6.2. The majority of heavy metals in the water column were consistently below the ADWG limits. These concentrations were used as the baseiine against which the concentrations found in the biofilms were assessed.

Table 6-6: Metal concentrations in the water column of the tank housing the biofilm rack.

ng/mL	Pb	Cd	Ni	Hg	Ag	Fe	Zn	Cu
Average	6.02	<1	<1	0.45	<1	174	395	69
Range	3–12	<1	<1	0.45	<1	121–222	43–637	<1–313
ADWG	10	2	20	1	100	300	3,000	2,000

Figures 6.20 – 6.27 show the concentrations of potentially toxic heavy metals as well as metals used in the construction of rainwater tanks in the various *in situ* cultured biofilms. The metal concentrations are displayed on the left-hand axes of the figures while the magnification rates, derived from comparing metal concentrations in biofilms to concentrations in the water column, are displayed on the right-hand axes.

The heavy metal of most concern in urban rainwater harvesting systems due to its widespread distribution and high toxicity was found to be lead. Lead levels in some of the Newcastle and Brisbane tanks exceeded the ADWG limit of 10ug/L immediately after rain events and one tank suffered chronic lead contamination with no samples below the guideline limit (as discussed in Chapter 3). The average concentration of lead in the water column of the tank containing the biofilm slides was 6ng/mL, calculated from 11 samples taken over the experimental period. It was therefore a major finding to discover that rainwater tank biofilms have the capacity to accumulated lead in concentrations many times greater than in the water column. Lead concentrations in all biofilm samples were found to be significantly higher ($p<0.01$) than concentrations in the water column. Figure 6.20 shows the concentrations of lead in the biofilms grown on glass (G), metal (M) and plastic (P) slides at the top, mid and bottom water levels. From the biofilm scrapings, lead concentrations were found to range from 2,900ng/mL in the top-level glass slides to 72,700ng/mL in bottom level metal slides.

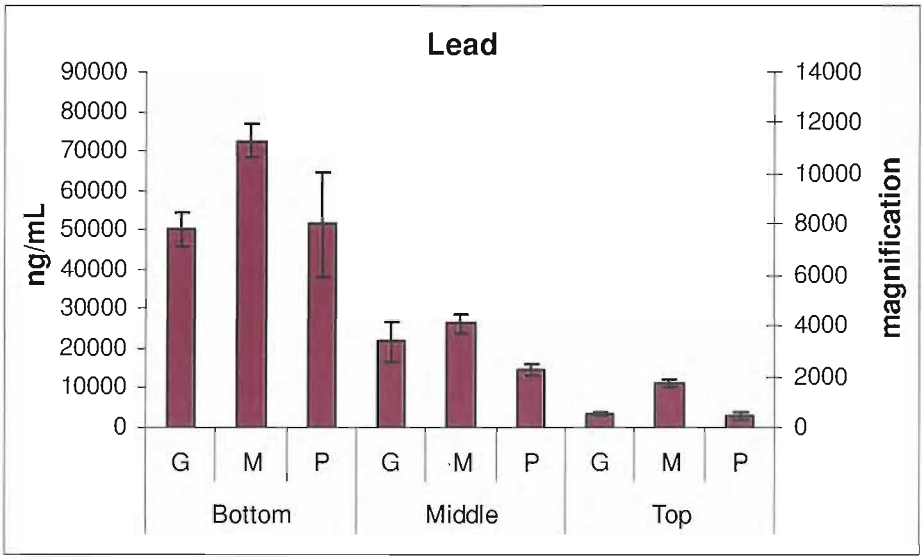


Figure 6-20: Lead accumulation in biofilms grown at varying water depths on glass (G), metal (M) and plastic (P) slides determined from 3 replicates.

The magnification rates of lead in the biofilms were found to be one of the highest of all metals. As shown graphically in Figure 6.20, the accumulation of lead in biofilms was strongly influenced by depth in the tank. Lead was found to accumulate in the top layer biofilms in concentrations 500 – 1,900 times greater than in the water column, while at the mid depth the magnification was 2,400 – 4,300 times higher within the biofilm. The magnification of lead in the bottom level biofilms was the most significant, approximately 10,000–fold greater than in the water column, making the lower level biofilms an important incidentally occurring treatment mechanism.

These differences in lead accumulation between the three depths were statistically significant for glass slides (BOT/MID $p<0.05$; MID/TOP $p<0.05$; BOT/TOP $p<0.01$), metal slides (MID/TOP $p<0.01$) and plastics slides (MID/TOP $p<0.01$; BOT/TOP $p<0.05$). Slide material was also found to be associated with differences in biofilm lead concentrations. At the top water level, biofilms cultured on metal slides contained significantly greater concentrations of lead than both glass slides ($p<0.01$) and plastic slides ($p<0.01$). At the mid depth, these differences were limited to metal and plastic ($p<0.05$), with no statistically significant differences at the bottom level.

The potentially toxic heavy metals cadmium, nickel, silver and mercury were also detected in higher concentrations in the biofilms than in the water column, shown in Figures 6.21 – 6.24. Similarly to lead, the extent of accumulation of these metals in the biofilms was clearly related to the depth in the tank that the biofilms were cultured, with bottom-level biofilms containing the greatest concentrations of these heavy metals. Slide material was again also strongly related to the concentration of metals in the biofilms. Biofilms grown on metal slides generally contained the highest concentrations of heavy metals, with the exception of mercury which was found to have a greater affinity for glass slides. Glass and plastic slide biofilms generally displayed a similar level of accumulation for the other heavy metals.

The total extent of bioaccumulation of cadmium and nickel could not be precisely quantified due to their undetectable concentrations in the water column samples. As a consequence, the most conservative estimates of cadmium and nickel magnifications have been presented, calculated from their detection limit concentration of 1μg/L. The relatively low magnification of mercury and silver probably relates to the trace concentrations of these elements in both the water column samples and the biofilm samples, exacerbated by the relatively large experimental errors resulting from the analysis of such low concentrations. The toxicity of these metals to microbial cells is not likely to be a major contributing factor for their relatively low magnification rates given that the magnification of copper, which is highly microbicidal, was relatively high.

Despite the fact that none of these metals were found to pose health threats in any of the rainwater tanks investigated in the Brisbane or Newcastle studies, this study has shown that the biofilms developed in the experimental tank system bioaccumulated cadmium, nickel, silver and mercury. Whether metal uptake was primarily due to active cellular uptake or to passive adsorption onto the biofilm EPS would make an interesting topic for further research.

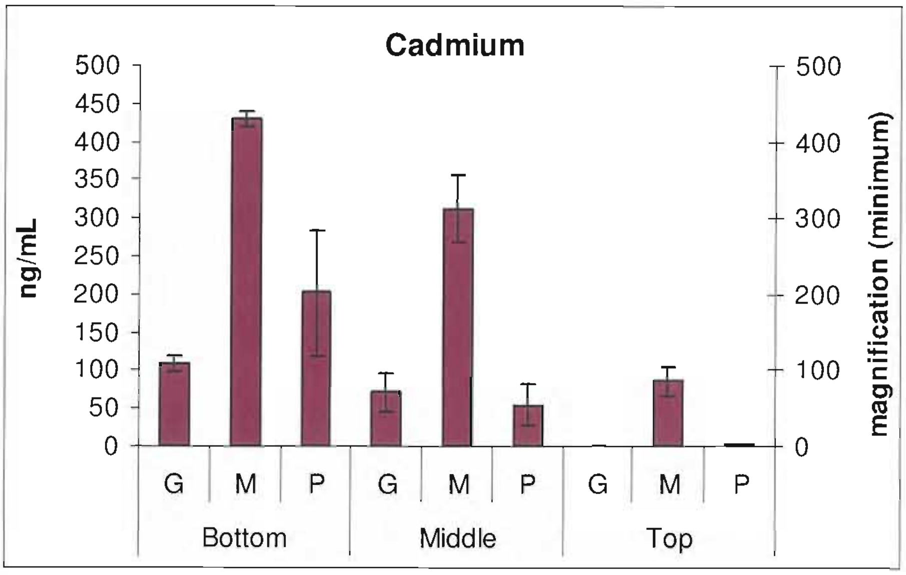


Figure 6-21: Cadmium in biofilms grown at varying water depths on glass (G), metal (M) and plastic (P) slides determined from 3 replicates

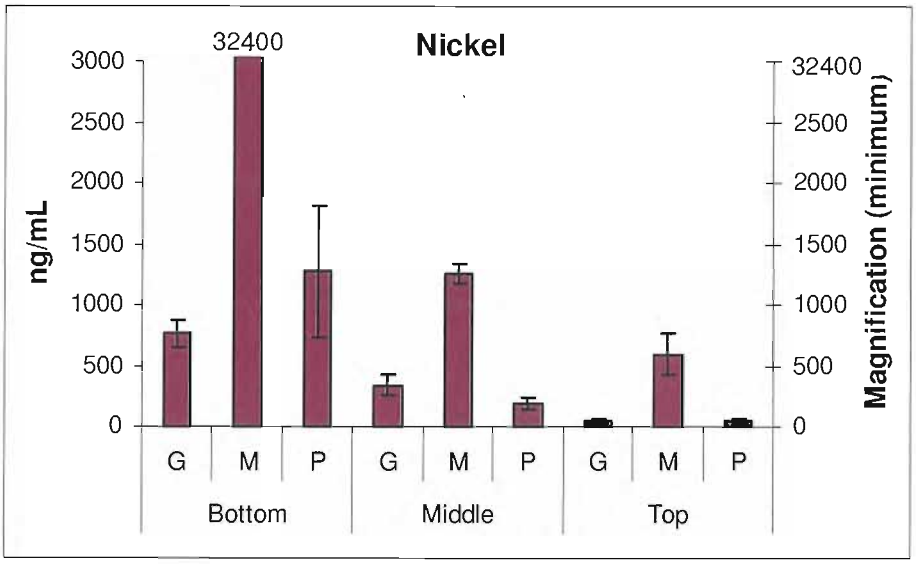


Figure 6-22: Nickel in biofilms grown at varying water depths on glass (G), metal (M) and plastic (P) slides determined from 3 replicates

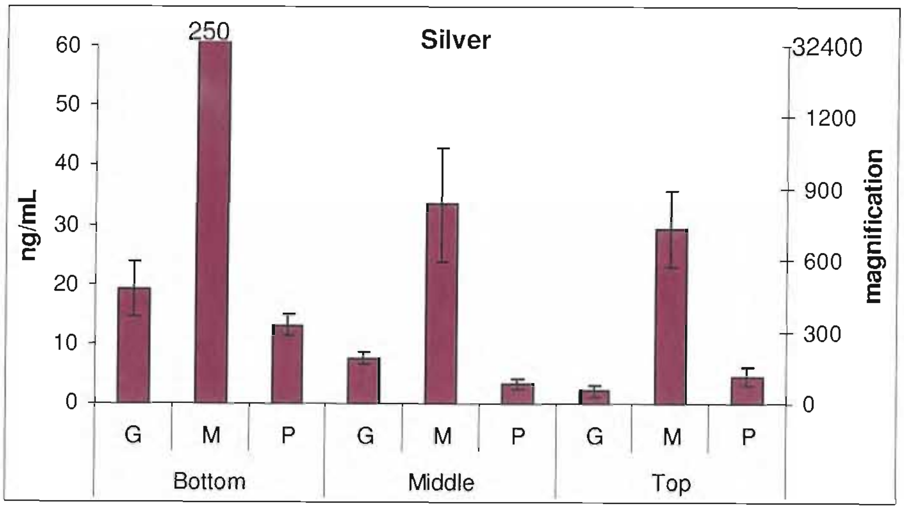


Figure 6-23: Silver in biofilms grown at varying water depths on glass (G), metal (M) and plastic (P) slides determined from 3 replicates

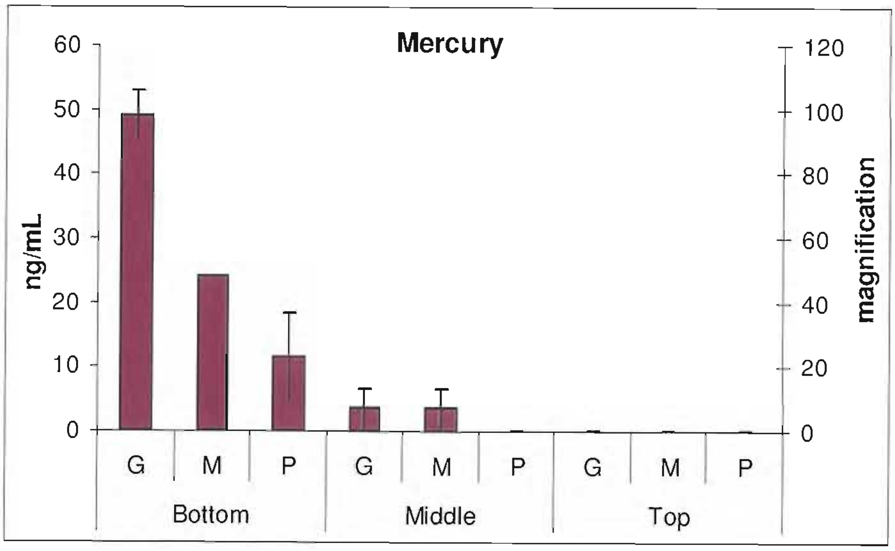


Figure 6-24: Mercury in biofilms grown at varying water depths on glass (G), metal (M) and plastic (P) slides determined from 3 replicates

6.3.4.2 Construction Material Metals (Fe, Zn & Cu)

Galvanised iron rainwater tanks are made predominantly from iron and contain a zinc coating designed to sacrificially corrode before the iron in the tank walls in order to prolong the lifespan of the tank. Corrosion (i.e. oxidation of metallic iron, Fe^{+2}, to rust, Fe^{+3}) was observed to have occurred, evident from the rust depositions on some of the metal slides. The extent of the microbial influence on rusting of the metal slides was not examined here, though may be worthy of further investigation. Iron and zinc levels on the surface of bottom level metal slides were 7,700 and 5,000 times higher than in the water column, respectively, shown in Figures 6.25 and 6.26. In comparison, levels on glass and plastic slides were only a factor of 500 – 700 higher for iron and 16 – 20 higher for zinc, respectively. Higher levels of iron were found on glass and plastic slides from the bottom level compared to the higher levels, although this may have been influenced by the deposition of rust products from the rusting metal microscope slides located in close proximity to the glass and plastic slides.

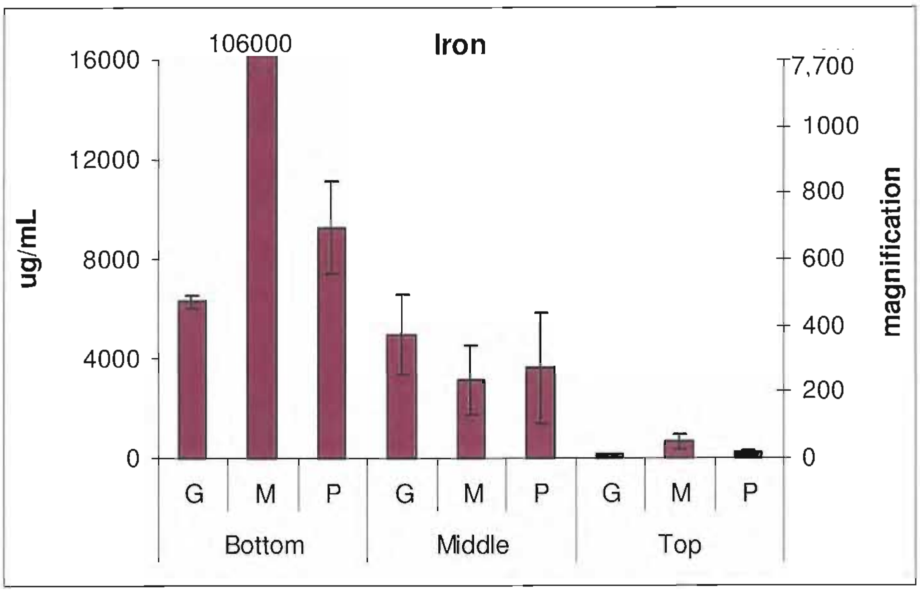


Figure 6-25: Iron in biofilms grown at varying water depths on glass (G), metal (M) and plastic (P) slides determined from 3 replicates

The concentrations of zinc in biofilms displayed a striking relationship with slide material. The negligible magnification rates of zinc on glass and plastic slides compared to metal slides strongly suggest that the source of zinc was the galvanised coating on the metal slides. The galvanised iron slides had a thickness of 1.5mm and contained a 45μm zinc layer, equating theoretically to a quantity of 3.2mg zinc per cm^2, as specified by Australian galvanising standards (AS/NZS 4680). The measured quantity of zinc in each cm^2 of biofilm (volume 0.01mL) was 0.75mg, 0.4mg and 0.35mg for the bottom, mid and top metal slides, respectively. This suggested that either not all of the zinc in the galvanising layer had been corroded or that a portion of the corroded zinc had passed through the biofilm and into the water column.

The significantly magnified iron concentrations in bottom level metal-biofilms suggest that corrosion of the underlying iron was commencing at the lower level but not at the middle or top levels. This is consistent with the iron and zinc concentration ratios between metal and glass/plastic slides. The smaller concentration differences between metal and glass/plastic slides for iron in comparison to zinc indicate that corrosion of the bottom level metal slides was beginning to reach the delta (90% Zn, 10% Fe) and gamma (75% Zn, 25% Fe) layers of the zinc galvanising where iron was present as a minority constituent. Whether the source of the iron and zinc was from rusting or from an external source, it was clear that biofilms were associated with significantly elevated levels of iron and zinc. The fate of these metals would presumably have otherwise been the water column or the sediment layer, with biofilms therefore contributing effectively to reducing the concentration of metals in the water column.

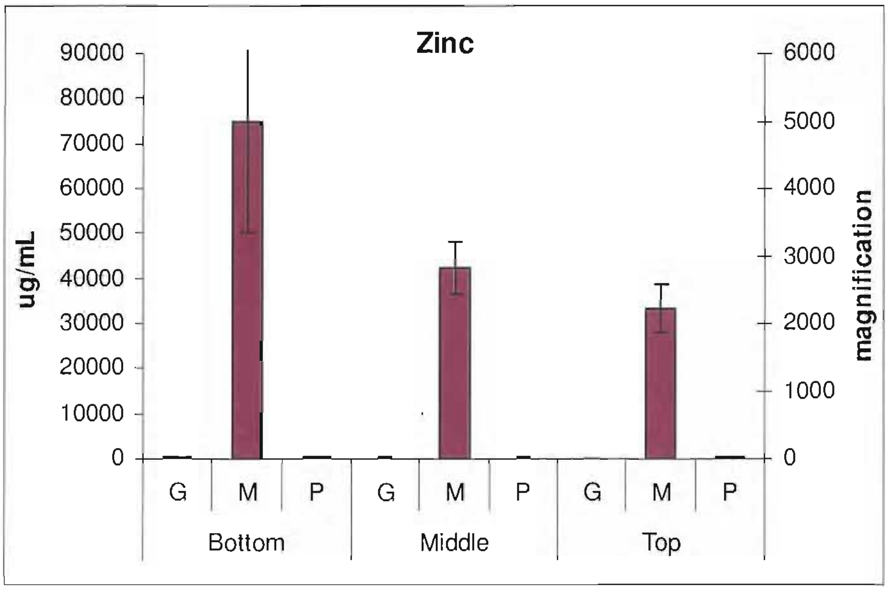


Figure 6-26: Zinc in biofilms grown at varying water depths on glass (G), metal (M) and plastic (P) slides determined from 3 replicates

Despite the low concentrations of copper in the water column and the high toxicity of copper to microbes, concentrations of copper in biofilms showed a significant level of magnification (Figure 6.27). Concentrations of copper in the water column of the rainwater tank averaged almost two orders of magnitude below the ADWG limit, as shown in Table 6.2, with the highest recorded value being only 15% of the ADWG limit. The pattern of copper removal was consistent with that seen for most other metals, with the greatest concentrations biased towards biofilms grown in the lower water levels and those grown on metal slides.

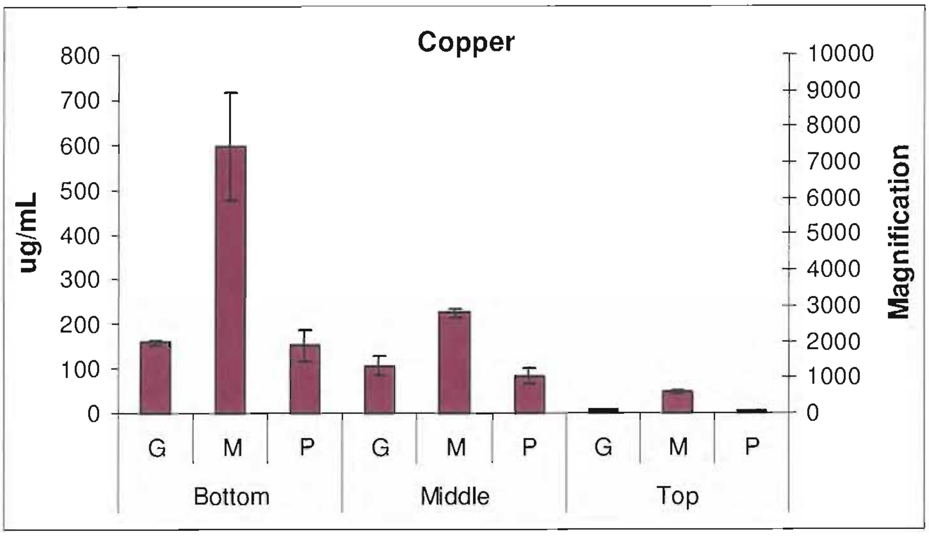


Figure 6-27: Copper in biofilms grown at varying water depths on glass (G), metal (M) and plastic (P) slides determined from 3 replicates

6.3.4.3 Discussion of Metal Uptake in Biofilms

The beneficial effects of biofilms for toxic metal remediation have previously been identified in a number of aquatic and terrestrial environments. The aquatic environments in which biofilms have bee found to provide a beneficial effect include streams and lakes contaminated by mines, dumps and other industrial and urban wastes (e.g. Scott & Karanjkar, 1998; Baldi, *et al.*, 2001), groundwater reservoirs (e.g. Langmark *et al.*, 2004), marine environments (e.g. Davis *et al.*, 2003), and sewage/sludge treatment facilities (e.g. Brown & Lester, 1982). The internal rainwater tank environment is now also known to be a suitable aquatic environment for the development of biofilms capable of heavy metal remediation. Rainwater tank biofilms in this study displayed an ability to improve and remediate tank water quality by removing significant amounts of metals from the water column of the test rainwater tank. The precise mechanisms by which the rainwater tank biofilms removed heavy metals from the water column would be a worthy topic for further research.

Within biofilms, there are numerous potential binding sites for metals and ionic compounds. Flemming (1995) identified five such regions within model biofilms capable of metal adsorption including:

(i) EPS with

a. Cationic groups in amino sugars and proteins (e.g. $–NH^+–$)

b. Anionic groups in uronic acids and proteins (e.g. $–COO^-$; $–HPO_4^-–$)

c. Apolar groups from proteins (such as in aromatic amino acids)

d. Groups with a high hydrogen bonding potential, such as polysaccharides;

(ii) Outer membrane and lipopolysaccharides of gram-negative cells with their lipid membrane, and the lipoteichoic acids in gram positive cells

(iii) Cell wall consisting of N-acetylglucosamine and N-acetylmuramic acid, offering cationic and anionic sites;

(iv) Cytoplasmic membrane, offering a lipophilic region;

(v) Cytoplasm, as a water phase separated from the surrounding water

There is no consensus in the literature as to which of these biofilm components contribute most to providing metal binding sites and it seems likely that metal uptake varies for different biofilms under differing environmental conditions. Spath *et al.* (1998) found that for organic pollutants (benzene, toluene, m-xylene) the EPS provided the majority of adsorption sites, while for heavy metals (cadmium) cellular uptake was responsible for the majority of cadmium removal. Pumpel *et al.* (2003) also found that the metabolising cellular fraction of biofilms was integral in the sorption of soluble nickel, while other studies have found metal binding to be more pronounced in the EPS (e.g. Scott & Karanjkar, 1998).

Many biofilms contain EPS components that have particularly high capacities for heavy metal sorption, such as the carboxyl- and hydroxyl- groups, enabling small amounts of EPS to potentially bind large amounts of metals. However, biofilms themselves are synonymous with heterogeneity and different environments produce biofilms with unique characteristics and varying metal sequestering capacities (Malik, 2004). Differences occur in microbial composition, thickness, structure and EPS composition, with the affinity of the EPS for metal complexation largely influenced by the specific microbial species residing in the biofilm (Scott & Karanjkar, 1998). However, the metal binding potential of a biofilm is not only influenced by the characteristics of the biofilm, but also by the surrounding environmental conditions.

Scott and Karanjkar (1998) found that the pH and temperature of the water phase affects adsorption capacity. As pH falls below 4–5, the cell surfaces become less negatively charged from changes to functional groups (such as –COOH and –OH) and subsequently have a diminished ability to attract positively charged metals. It was interesting that the tank housing the biofilm slides contained one of the lowest average pH levels (5.7) of tanks in the Newcastle study (overall average 6.7). It is likely that the metal removal rates by biofilms in this study were, therefore, a conservative estimate and may be higher in other tanks.

The water phase concentration of metals also influences complexation rates within biofilm, with decreasing rates of uptake corresponding with increasing initial metal concentrations (Jang *et al.*, 2001). The metal–biofilm complexation has also been shown to be strongly related to metal solubility, with the sequence of solubilities of metals generally found to be the reverse of the complexation sequence, i.e. with highly soluble metals accumulating at lower rates in biofilms than metals with low solubility (Brown & Lester, 1982). This is consistent with the finding in this thesis that lead, which has a very low solubility, was magnified in the rainwater tank biofilms more than all other examined metals. However, it must be noted that the loading concentrations of the various metals on the rainwater tank were not equal, as it was not an objective of this study to compare uptake rates between metals. It is clear that environmental conditions and inoculum bacteria in rainwater tanks will differ significantly from those in the more popularly studied wastewater and mains water systems. Consequently, the rainwater tank environment will produce unique biofilms with distinctive characteristics and metal-binding trends.

The general trend of metal uptake into rainwater tank biofilms showed the greatest concentrations of metals in the lower level biofilms and the lowest metal concentrations in the top level biofilms. This trend was similar to that observed for microbial concentrations in the top and middle levels, though was even more pronounced for metals. This was not a surprising finding and is logical given that the bottom level slides were located at a depth of 10-30 cm from the base of the tank which was below the top-up depth (30cm). The lower level biofilms were therefore permanently submerged and exposed to continuous chemical loading and would have, in all likelihood, received greater deposits of settling metal-bound particulate matter.

Lead, cadmium, nickel, silver, iron, zinc and copper concentrations were also consistently higher in metal-slide biofilms. It is possible that this phenomenon was due to metal-slide biofilms possessing greater metal absorption characteristics than the glass and plastic slide biofilms, though seems far more plausible that the higher concentrations were a result of leaching of impurities from the galvanised iron slides. The greater extent of rust on the lower level slides also supports the latter explanation.

The question that then arises is, have the plastic and glass-biofilms actually removed and bio-accumulated water column metals or have they simply been contaminated with rust from the lower level metal slides? By determining the concentration ratios of each of the rust elements (i.e. iron and zinc) between metal-slide and non-metal-slides, and comparing these to the concentration ratios of other metals (namely lead), it is clearly seen that rust contamination can not account for the magnification of lead and other 'non-rust' elements in the plastic and glass-biofilms. As seen in Figures 6.26 and 6.27, the iron concentrations in metal-biofilms were 11 and 17 times greater than in plastic and glass-biofilms respectively, while zinc concentrations were 310 and 247 times greater, respectively. These ratios suggest that the rust products are relatively immobile and essentially restricted to the metal slides. When observing the concentrations of lead in glass, metal and plastic-biofilms, it is clear that the distribution of lead is far more even between the slides, strongly indicating that the magnification of lead in the biofilms has occurred as a result of the biofilms taking up lead impurities from the water column rather than from the deposition of rust products.

6.4 CONCLUSIONS

Through this study it was confirmed that biofilms can develop within a rainwater tank environment on materials used for constructing rainwater tanks. A number of significant findings were made from this research relating to cell densities, species composition, and metal removal capacity of rainwater tank biofilms.

The hypotheses of this work were that biofilms would develop on the walls of rainwater tanks and that the extent of development would be influenced by the surface material of the tank and the depth within the tank that the biofilms were cultured. These hypotheses were indeed found to be true. Concentrations of culturable bacteria in biofilms were found to range between 1.4×10^4 and 1.53×10^5 CFU/0.01mL, representing a 1000 – 14,000 fold increase to concentrations measured in the water column, while direct viable counts ranged between 1.18×10^5 and 1.13×10^6 CFU/0.01mL, representing a 100 – 1,100 fold increase to concentrations found in the water column. These data, along with the epifluorescence microscopic observations, clearly showed the establishment of biofilms within a rainwater tank and indicate that bacteria were either removed from the water column by the biofilms and/or were actively multiplying within the biofilms.

The depth in the tank that biofilms were cultured was also found to influence the extent of biofilm development. It was consistently found that the mid-level biofilms had significantly greater concentrations of viable and culturable cells than biofilms grown higher in the tank. To a lesser extent, slide material also influenced the concentration of cells in the biofilms. At the top level, cell counts were relatively even in biofilms grown on the various slide materials, with the exception of higher viable cell counts found on metal slides than glass slides. At the mid-level, however, metal-slide biofilms contained significantly lower counts of viable and culturable cells than both glass and plastic-slide biofilms. The hypothesis that not all cells in the biofilms would have the same degree of attachment to the biofilm was also found to be true, with approximately two thirds of biofilm-associated cells firmly attached to the substrate, with the remaining one third loosely attached to the surface of the biofilm.

The bacterial species comprising the biofilm were also examined. It was hypothesised that biofilms would be comprised of a variety of species which, as with cell density, would be influenced by slide material and depth. Biofilms were indeed found to be comprised of a range of species, with a number of environmental bacteria being isolated from the biofilms. Of the 16 isolates subjected to PCR and gene sequencing, a core group of five bacteria were found to occur on all three substrate materials, including *Bacillus*, *Pedobacter* and *Stenotrophomonas*. However, both plastic and metal-slide biofilms contained species exclusive to those materials, indicating that slide material did have a bearing on the species present.

The influence of depth also had a striking influence on species composition in some biofilms. This was clearly seen in the case of *Stenotrophomonas* and *Sphingomonas* where an almost mutually exclusive relationship developed, with *Stenotrophomonas* dominating top-level biofilms and *Sphingomonas* dominating mid-level biofilms. A number of the species identified in the rainwater tank biofilms have been reported in the literature as having beneficial uses in organic waste and wastewater treatment, indicating that rainwater tank biofilms may be inoculated with suitable species to provide potential for improving tank water quality.

One of the most beneficial known functions of biofilms is their ability to remove contaminants from water bodies. It was therefore hypothesised that rainwater tank biofilms would have the capacity to improve tank water quality by removing a variety of heavy metals from the water column. One of the most significant outcomes of this research was the confirmation of this hypothesis. Rainwater tank biofilms did indeed display substantial potential to bioaccumulate several species of heavy metals, including lead, from the water column. All analysed metals showed signs of magnification within the biofilms, with concentrations found up to four orders of magnitude greater than in the water column.

As with species density and species composition, metal uptake rates in biofilms were found to be dynamic and varied with slide material and depth. Greater levels of bioaccumulation were observed in the middle and lower sections of the tank than in the upper level biofilms. Consistently higher concentrations of heavy metals were also associated with biofilms cultured on metal slides. A limited degree of corrosion of the underlying substrate is likely to have contributed to this, which was particularly clear for zinc. However, biofilms cultured on glass and plastic slides were also found to display consistently high magnification rates of heavy metals, demonstrating that substantial metal uptake rates were a common characteristic in all cultured biofilms. The consistently high rates of metal magnification in biofilms highlighted these phenomena as important natural components of the incidental rainwater treatment train.

The implications of this research bring into question the need for regular tank cleaning that has traditionally been part of tank maintenance recommendations.

This chapter has examined the development of biofilms in rainwater tanks and investigated their ability to improve water quality. The following chapter will examine the role of sedimentation in improving tank water quality and will investigate some of the characteristics of tank sludges.

CHAPTER 7

Sedimentation

7.1 INTRODUCTION

Simple observations of rainwater tanks indicate that one of the major mechanisms responsible for improving the quality of stored rainwater is sedimentation. Sedimentation, or the settlement of particulate matter, results in the formation of a layer of sludge at the base of rainwater tanks. Despite the potential significance of this process for reducing contaminant levels in rainwater tank water columns, only a limited amount of research has been conducted on the processes of sedimentation and sludge accumulation in rainwater tank systems. Scott and Waller (1987) examined a range of physicochemical parameters and total bacteria counts in a single sludge sample from one tank. The sludge sample in this study was shown to have significantly higher concentrations of heavy metals than samples from the water column and the authors consequently recommended the practice of de-sludging tanks every two years. Coombes *et al.* (1999) also noted elevated concentrations of bacteria and heavy metals in tank sludges at the Figtree Place Water-Sensitive-Urban-Design development. These important, though limited, investigations have been restricted to observing the occurrence of sedimentation in rainwater tanks and examining sludge composition.

While the process of sedimentation is undisputedly beneficial to water quality, the influence of a sludge layer at the bottom of the tank remains unknown. Sludges may contain a variety of biological, organic and inorganic impurities providing a potential source of contamination, though also potentially offer applications for improving water quality. Sludges are often used in wastewater treatment where the activated biomass is able to remove a great deal of the suspended and dissolved matter (Lester & Birkett, 1999). Aerobic and anaerobic biological activity in sludges can degrade a wide range of contaminants. The by-products of these may consequently be consumed by the biomass, released as a gas or be transformed into a stable end product incorporated into the sludge matrix. In municipal water and wastewater treatment facilities, the composition and activity of sludges are largely controlled and the distribution of sludges restricted to prevent the sludge from mobilising and becoming a source of contamination. However, no published data is available on the distribution, mobilisation and behavioural characteristics of rainwater tank sludges apart from that of Spinks *et al.* (2005). As rainwater tanks generally have only a single-point inlet it was hypothesised that tank sludges will not be distributed evenly across the base of rainwater tanks. It was also hypothesised that the cumulative effect of sedimentation would result in sludges containing elevated concentrations of heavy metals and bacteria. The final hypothesis was that the mobilisation and behavioural characteristics of rainwater tank sludges would influence water quality, including the settling rates of particulate matter, the potential of sludges for re-suspension, and the flocculant-forming potential of re-suspended sludge.

The aims of this chapter were therefore three-fold; Firstly, to determine the physical distribution and accumulation rates of sludges in a number of rainwater tanks; Secondly, to determine the microbiological and metal composition of these sludges, and; Finally, to investigate the physical behavioural characteristics of rainwater tank sludges, including determining settling rates, identifying potential factors relevant to sludge re-suspension, and assessing the potential of re-suspended sludge to enhance flocculation of suspended bacteria.

7.2 EXPERIMENTAL DESIGN

This study involved the examination of both sludge-composition variables along with behaviour-based characteristics. Consequently, field analysis was combined with a range of laboratory experiments to allow the full range of interested variables to be investigated.

7.2.1 Sludge Extraction

Sludges from six rainwater tanks located in urban areas of Newcastle, Australia, were sampled. Within each tank, sludge was sampled from three locations, including directly below the inlet and approximately 1–1.5m to the left and right of the inlet to form a triangle pattern. Sludge was extracted from the tanks by lowering a metal O-ring (75mm diameter) with sharpened bottom edge onto the sludge and siphoning the sludge from within the ring. The sample containers were transported immediately to the laboratory where they were left to sit in the dark at room temperature for seven days to allow complete settlement of sludge before being drained of excess water.

7.2.2 Sludge Analysis

Sludge volume was measured as saturated volume, defined here as the volume occupied by the sludge in water after seven days of settling. This was calculated by multiplying the average sludge height (measured from five points around the perimeter of the storage vessel) by the base area of the storage vessel. Dry weights of the sludges were also recorded after drying for 48 hrs at 40°C. The low-temperature drying procedure was employed to minimise the loss of volatile organic matter.

Total bacterial counts were made by transferring 250µL of undried sludge to a 10mL centrifuge tube and diluting 1:20 with 4.75mL of sterile deionised water. Samples were then vortexed for 20mins and 1mL was spread onto Nutrient Agar plates in triplicate and incubated either aerobically or anaerobically for 24hrs at 37°C followed by 48hrs at 22°C. Colonies growing anaerobically were tested for oxygen sensitivity to determine whether they were strictly or facultatively anaerobic. 1mL of the diluted samples was also filtered through a Millipore Membrane Filtration unit (0.45µm filters) and cultured on the chromogenic mColiBlue media for *Escherichia coli* and total coliform, *Pseudomonas* Selective Broth for *Pseudomonas* sp., and mEndo broth for Heterotrophic Plate Counts (HPC). Samples containing greater than 50µL of sludge could not be filtered through the unit without significant build up of sludge on the filter pad.

Dried sludge samples were analysed for heavy metals by Hunter Water Laboratories using atomic absorption spectroscopy. The Certified Reference Material PACS-2 was included in the analyses and laboratory results had <10% error for Pb, As, Cd and Ni. For a number of experiments in this study, the extent of sludge re-suspension in the water column was measured spectrophotometrically, with samples measured for light absorbance at 360nm wavelength.

7.2.3 Sludge Behavioural Characteristics

7.2.3.1 Settling Rates

Approximately 180mL of sludge was transferred into 4L plastic beakers creating a sludge depth of 10mm with an overlaying water column of 200mm. The 10mm sludge depth represented an average sludge depth, while the water column depth of 200mm represents the minimum height at which top-up devices are activated (i.e. the minimum possible water depth and therefore the water depth with the greatest potential to facilitate re-suspension). Sludges were vigorously stirred to achieve complete re-suspension. Samples were taken at predetermined times thereafter from a depth of 100mm (representing the height of outlets from rainwater tanks). Samples were analysed spectrophotometrically and for HPC bacteria.

7.2.3.2 Fractioning Sludge

As lead was found to be the most significant heavy metal contaminant in rainwater tanks, analysis of the distribution of lead within sludges was undertaken. Sludge from two of the six tanks, referred to from here as SLUDGE–S and SLUDGE–O, were partitioned into three fractions based on settling rate. The sludges were placed into 4L containers with 3.5L of water (water depth of 200mm) and completely resuspended into the water column through vigorous mechanical stirring. As SLUDGE–S and SLUDGE–O had differing particle size distributions which resulted in differing settling rates, the fractionation times were adjusted accordingly. For the first fraction, the sludges were left to settle for 1 min (SLUDGE–S) and 5 mins (SLUDGE–O) before siphoning off the water containing the residual suspended sludge into a second container. This process was repeated for the second fraction with siphoning occurring after 5 mins (SLUDGE–S) and 30 mins (SLUDGE–O). The third transfer was left to sit in the dark at room temperature for 7 days before excess water was drained. Sludge fractions were then dried and analysed for lead, as described above.

7.2.3.3 Flocculation Capacity

Coombes *et al.* (1999) postulated that resuspended sludge may act as a coagulant and accelerate the flocculation and settlement rates of bacterial cells suspended in the water column. Two marker bacteria, *E. coli* (a common index bacteria of faecal contamination) and *Pseudomonas aeruginosa* (a well known producer of extracellular polysaccharide), were used to examine whether bacteria suspended in the water column would be removed through the interactions with resuspended and settling sludge. To test this hypothesis, combinations of un-sterilised sludge, sterilised sludge and no sludge, together with injections of *E. coli*, *P. aeruginosa* and no bacteria, were added to a series of 4L beakers. The combinations were designed to show the extent to which the living and non-living parts of sludge contribute to flocculation, and indicated the extent to which HPC from the sludge could be resuspended.

Two controls were employed containing injections of *E. coli* or *P. aeruginosa* into water columns containing no sludge. Beakers were first filled with 10mm of sludge followed by 250mm of sterile de-ionised water, then injected with the appropriate bacterial culture. Bacterial cultures were injected at a height of 150mm using a 10mL glass pipette. The injection of the bacterial cultures into the water columns was sufficient to achieve uniform dispersal without the need for subsequent mixing. This was demonstrated in the control where five water column samples contained small variation in bacterial counts (0.1 log). Sludges were resuspended into the water column to a height of 150mm in a controlled fashion by rotating a sterile glass hook paddle. 1mL samples were taken at predetermined times thereafter from a height of 100mm within the water column and tested for HPC bacteria as described above.

7.3 RESULTS & DISCUSSION

7.3.1 Sludge distribution

The distribution and physical properties of the sludges in the six studied rainwater tanks are given in Table 7.1. It was found that tank sludge was not distributed evenly across the floor of the tanks and that the greatest deposition occurred directly below the inlets (refer to Appendix E). Sludge deposits sampled 1–1.5m to the left and right of the inlets varied in depth between 87% and 12% of the sludge depth at the inlet, ranging in depth from <1mm to over 15mm. Two distinct types of sludge were observed differentiated on the basis of physical appearance and texture. The more common type, categorised here as loamy, was found in five of the six tanks and contained a very dark humus composition appearing rich in organic matter. The second type, categorised as clay, was a lighter brown finely grained clay-based sludge. Both types of sludge resembled the texture and composition of soil in the surrounding environment.

The greater deposition of sediment directly under the inlet was not surprising given that the majority of heavy particles would be expected to settle out of the water column quickly. Given the relatively large volume of static water in tanks, tank waters would effectively act as sediment traps, dissipating the energy from the inflowing water allowing particulate matter to settle. However, accumulation of sludge 1–1.5m away from the inlet suggests that some transport of particulates is occurring and that a degree of shear is being established within the receiving tank water body.

Table 7-1: Physical distribution of sludge over the base of six rainwater tanks. Sludge samples were taken from three locations across the base of the tanks (directly below the inlet, 1.5m from the inlet on a 45° angle to the left, and 45° to the right) as shown in Appendix E

Tank	Sample Position	Inlet Trajectory	Sludge Type	Sludge Depth (mm)	*Dry Weight (g)	*Wet Volume (mL)	Sludge Density Saturated
Tank-O	Inlet	Horizontal,	Clay	15.3	8.35	67	1.125
	Left	Right,		7	3.83	30.8	1.124
	Right	Unscreened		1.9	0.92	8.4	1.11
Tank-B	Inlet	Horizontal	Loamy	14	9.6	61.6	1.156
	Left	Centre,		10.2	5.67	44.8	1.127
	Right	Screened		5.7	2.12	25.2	1.084
Tank-W	Inlet	Horizontal,	Loamy	7	1.78	30.8	1.058
	Left	Centre,		1.9	0.19	8.4	1.023
	Right	Screened		2.6	0.55	11.2	1.049
Tank-L	Inlet	45° Angle,	Loamy	1.3	0.54	5.6	1.097
	Left	Centre,		0.3	0.10	0.13	1.758
	Right	Screened		0.3	0.07	0.13	1.53
Tank-C	Inlet	45° Angle,	Loamy	5.1	0.96	22.4	1.043
	Left	Centre,		4.5	0.68	19.6	1.035
	Right	Screened		1.3	0.34	5.6	1.061
Tank-S	Inlet	Horizontal,	Loamy	9.6	4.01	42.0	1.095
	Left	Right,		2.6	0.83	11.2	1.074
	Right	Screened		6.4	2.05	28.0	1.073

*Dry weights and wet volumes are given as totals of the sludge collected with the 47em^2 metal O-ring.

As shown in Table 7.1, many tanks accumulated sludge more predominantly on one side of the inlet. This may have been due to hydraulic conditions created by the trajectory of the inlets or from the drawing of water from the outlet. However, no single explanation could account for the variations in sludge depth for all tanks when the data was compared to the positions of the inlets and outlets, illustrated in Appendix E. Four of the six tanks contained inlets that projected water to the centre of the water column and therefore could not explain the differences in sludge depth between the left and right sides. The inlets on two of the tanks (Tank-O and Tank-S) were aligned to project water to the right side of the tank. However, in only one of these tanks (Tank-S) the right-sided trajectory of the inlet matched a greater depth of sludge on the right side of the tank. Tank-O, also with a right-sided inlet trajectory, had a larger accumulation of sludge on the left side of the inlet.

The same two rainwater tanks (Tank-O and Tank-S) were the only tanks for which the outlet was located to the side of the tank (i.e. greater than 45° arc between the inlet and outlet). In both of these tanks, sludge depths were found to be significantly lower on the side of the outlet. Although the remaining tanks contained large variations in sludge depths that could not logically be explained by the same mechanism (due to the inlet and outlet being closely aligned), it is possible that the subtle flow created by drawing water through the outlet could suspend small amounts of sludge. Over time this may have resulted in the lowering of the sludge depth around the outlet. In tanks where the outlet was in close alignment with the inlet (i.e. Tank–B, Tank–W, Tank–L & Tank–C) this effect may have been hidden by the greater deposition loads of particulate matter directly under the inlet. Consequently, the location of the outlet relative to the inlet may be a more significant design variable for water quality than the trajectory of the inlet. Michaelides and Young (1984) have suggested the use of baffles to restrict re-suspended sediment from being drawn out of the tank. Positioning the outlet at the opposite side of the tank to the inlet may be a simple alternative design capable of reducing the level of sludge uptake by the outlet and hence, improving the quality of water at the point of use.

A relationship was also found in some tanks between sludge depth and sludge density. Table 7.1 shows that the two tanks with the greatest quantity of sludge accumulation were Tank-B and Tank-O. In both of these tanks correlations were found between sludge depth and sludge density within the three samples taken from each tank. As shown in Figure 7.1, increases in the depth of sludge were associated with increases in the density of the sludge. The slope of the line of best fit was much steeper for Tank-B sludge than Tank-O sludge, indicating that for Tank-B sludge, greater relative increases in density occurred as a function of depth. In the cases of Tank-B and Tank-O, a converse relationship was seen between sludge density and distribution. Tank-O sludge was found to have greater uniformity of density (flatter slope in Figure 7.1) though was found to be predominantly distributed around the inlet (Table 7.1). Tank-B sludge on the other hand was found to have a greater range of densities (Figure 7.1) though was more uniformly distributed across the floor of the tank than Tank-O sludge.

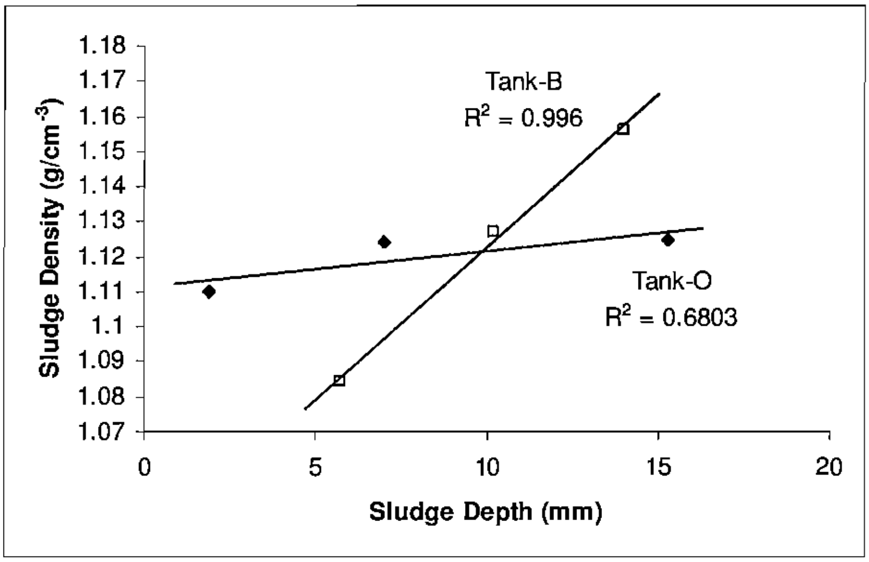


Figure 7-1: The Scatter Plot correlations between sludge depth and sludge density of the two tanks with greatest sludge accumulation, Tank-O and Tank-B

The influence of inlet screens (typically 1mm mesh) was not found to reduce the dispersal of sediment across the floor of tanks. It was thought that screens may disperse the energy of inflowing water and disrupt the formation of a jet stream within the tanks, consequently facilitating more rapid settling of particulate matter. However, it can be seen in Table 7.1 that tanks with screens generally had a more even distribution of sludge across the tank base than the single tank that had no screen. Other variables may have confounded this result, such as tank size. The five screened tanks were each less than 9KL, while the unscreened tank was 17KL. The greater hydraulic force presumably imparted by the inflowing water on the unscreened tank may have been partly negated by the larger body of water through the greater absorption of the kinetic energy in the surface layers of the water column.

7.3.2 Sludge Accumulation Rates

The sludge accumulation rates (SAR) for each tank are given in Table 7.2. It was hypothesised that the SAR would be the same for each tank. The measurement of SAR as L/yr showed that there were in fact significant differences in sludge loadings for each tank, varying between 4.2 and 28.4 L/year. While the volumes of sludge loading varied, these volumes have different significance depending on the size of the tank base and the relative saturated density of the different sludges. The most rapid vertical build up of sludge occurred in Tank-B which averaged 7.8mm of sludge accumulation per year, equating to a dry weight of 2.59kg.

Table 7-2: Sludge Accumulation Rates (SAR)

	Tank-O	Tank-B	Tank-W	Tank-L	Tank-C	Tank-S
Age (years)	14	2	1.7	1.7	2	1.7
Tank Area m^2	13.1	2.72	2.78	2.44	1.5	5.07
Depth (mm)[a]	12.6	15.6	6	1	5.6	9.6
SAR – mm/yr	0.9	7.8	3.5	0.6	2.8	5.6
SAR – L/yr	11.8	21.2	9.7	1.5	4.2	28.4
SAR – Kg/yr[b]	1.41	2.59	0.42	0.68	0.19	2.29

[a]Depth mm = average depth of the three samples, [b]dry weight

With the exception of Tank-O, all tanks contained wire mesh screens on the inlets. Inlet screens were observed to restrict the entry of larger particles (>1mm) into tanks. However, a lack of regular maintenance was also observed and screens were often found to be filling with sediment and organic matter. The presence of screens in such cases simply delayed the entry of larger particles into the tank by holding them until they had decayed sufficiently to pass through the screen pores. Therefore, self-cleaning screening mechanisms which prevent the build up debris within the system would be a more effective solution in reducing total sludge loads.

Un-maintained screens may also have restricted the level of inflow into the tank, causing run-off during heavy rain events. However, the screens do successfully prevent small insects, mammals, birds and reptiles from entering the tanks. This can prove important in the restriction of disease transmission. During inspection of the sediment in the un-screened tank, two large Blue Tongue lizard carcases were found decaying in the sludge. While the users reported no illness from the consumption of this water, inoculation of rainwater with animal carcases greatly increases the risk of exposure to pathogenic organisms.

The sludge itself originates in the catchment, from the catchment surface and surrounding environment. The sources of the inorganic fraction may include the catchment materials themselves, atmospheric deposition and airborne soil dust. The organic fraction is likely dominated by overhanging vegetation and airborne particulates. A small part of the sludge may also be faecal contamination from animal and bird droppings. The sludge loading rates on tanks varies depending on the vulnerability of the catchment to the local environment, such as the extent of trees overhanging the roofs/gutters and access to the roof or tank for small animals and reptiles, some of which, as described above, may actually enter and perish within the tank.

None of the catchments were free of trees or tall shrubs (>2m height) that could potential be a source of organic matter. However, the number, species and proximity of vegetation did vary between houses. Tank-B was found to have the greatest sludge loading rates (by dry weight). For the first 12 months of the study, the catchment supplying Tank-B was overhung by a large silky oak tree. As described in the first case study in chapter 4, this tree appeared to be the source of microbial contamination as well as odour and colouration, and it seems logical that the high levels of dropped foliage by the tree would have contributed significantly to the high SAR. The SAR for this tank was determined from the first two years of the study. Consequently, the SAR may have been higher in the first year (with silky oak) and lower in the second year (no silky oak). The felling of this tree was found to improve water quality and probably substantially reduced the organic load on the tank. Similarly, Tank-S and Tank-O also contained several trees and shrubs which directly overhung the catchment or the tank inlet. From field observations, directly overhanging vegetation appeared to contribute the greatest volume of organic debris to the catchment systems.

Despite the absence of first flush devices, Tank-C, Tank-W and Tank-L were found to have the lowest SAR of the six systems. While vegetation was present in the local vicinity of these three systems, the extent that vegetation directly overhung the roofs was minimal. These three systems also had self-cleaning leaf diverters located on the down pipes, which from regular visual inspections appeared to be working effectively. Of the three systems with the highest SAR, only one had a leaf diverter installed.

Hence, well design self-cleaning leaf diverters may play an important role in minimising sludge loading on tanks, particularly for houses with overhanging vegetation.

In determining the SAR for sludge depth in this study, the total depth of the sludge was divided by the age of the tank (years) to give an annual loading rate (mm/yr). This methodology assumed that sludge depth increased linearly over time. Although determining the linearity of sludge accumulation was not an objective of this study, it was possible that sludge depth accumulation was not linear due to microbial degradation of organic and particulate matter in the sludge. As the microbial ecology in the sludge develops, it is likely that some of the organic matter will be utilised for microbial metabolism. The decay of sludge material may significantly reduce the effective accumulation rates in tanks.

7.3.3 Sludge Composition: Bacteria & Heavy Metals

It was hypothesised that the cumulative effect of sedimentation would result in sludges containing elevated concentrations of bacteria and heavy metals. The sludges from the six tanks were therefore analysed for a number of bacterial groups and heavy metals. The concentration ranges for these parameters are presented in Table 7.3. Concentrations of bacteria and heavy metals were found to be significantly higher in the sludge than in the corresponding water columns, demonstrating that a significant amount of sedimentation had occurred and that the sludge acted as a sink for the majority of both bacteria and heavy metals entering the tanks.

Concentrations of total aerobic bacteria in the sludge ranged up to almost 1,000,000 CFU/mL, approximately an order of magnitude greater than the concentrations of anaerobic bacteria. Concentrations of total coliform, *E. coli* and *Pseudomonas* were also higher in the sludge than in the water column by approximately one to three orders of magnitude. It was interesting to note that total coliform concentrations were generally higher than *Pseudomonas* concentrations in the sludge, which was the opposite trend to that observed in the water columns. As with the microbial populations in biofilms on the walls of rainwater tanks, bacterial cells enter the sludge ecosystem through either the process of sedimentation or through cellular growth and division.

Table 7-3: Bacteria and heavy metal concentrations in sludge and water column samples and the comparison factor

Parameter	Sludge – Range of Averages	Water Column – Range of Averages	Comparative Factor
BACTERIA (CFU/mL)			
HPC Aerobes	$2.7x10^4 – 9.45x10^5$	582 – 4 355	10 – 426
Anaerobes	$<1x10^3 – 8.7x10^4$	NT	–
Total coliform	$20 – 1.1x10^4$	3 – 43	21 – 1349
E. coli	20 – 80	<1 – 6	3 – 133
Pseudomonas	$20 – 5x10^3$	18 – 238	5 – 261
METALS (μg/L)			
Lead	$7.2x10^5 – 6.6x10^6$	4 – 98	66 860 – 343 000
Nickel	$1.3x10^4 – 4.5x10^4$	<1	>13000 – >45000
Cadmium	$<1x10^3 – 1.5x10^4$	<1	1000 – >15 000
Arsenic	$4.8x10^3 – 3.1x10^4$	<0.5	>9 600 – >61 200
Copper	$1.1x10^5 – 5.6x10^6$	5 – 119	16000 – 203 000

NT Not tested

More than 30 unique colony types were observed on the nutrient agar plates used to culture the aerobic sludge bacteria, shown in Figure 7.2. A variety of macro-invertebrate organisms were also observed, including Cyclops and worms, shown in Figures 7.3, 7.4 and 7.5. Many of the bacterial colonies appeared to grow easily on the crowded mixed-species plates. Interestingly, the subsequent isolation of colonies onto pure growth plates proved difficult for a number of colonies which did not seem to grow in the absence of other species. A limited number of isolates were selected for PCR/gene sequencing identification, chosen on the basis of high colony prevalence and culturability. The identified species, along with the number of tanks they were identified in, included *Bacillus* sp. (six), *B. cereus* (six), *B. sphaericus* (three), *Pseudomonas* sp. (three) and an estrogen-degrading *Brevundimonas* sp. (one).

The extent of heavy metal accumulation in the sludges was found to be far greater than bacterial accumulation. As shown in Table 7.3, the concentrations of lead, nickel, cadmium, copper and arsenic in the sludges ranged from three to over five orders of magnitude greater than concentrations in the water columns. The large magnification of these metals in the sludges indicates that the process of sedimentation is an important incidental treatment mechanism in rainwater tanks. The effectiveness of the sedimentation process in removing these metals from the water column was probably aided by the low solubility of these metals. The concentrations of the heavy metals in the sludge of each individual rainwater tank are presented in Appendix B.

Of the heavy metals examined, lead was the most extensive magnified in the sludge, ranging between 67 000 and 343 000 times. This revealed that the fate of almost all lead in rainwater harvesting systems was the basal sludge layer. While Tank-C had the greatest magnification rates of lead, the highest concentrations of lead were found in Tank-B, which was the subject of a more intensive investigation in chapter 4. Interestingly, while the deposition of sludge was found to be greatest in the region directly below the inlet, concentrations of lead were lowest in the inlet sludge samples in four of the five[a] tanks (Appendix B). This was thought to be due to a greater association of lead with the finer, more easily suspended fraction of the sludge. This hypothesis was further investigated in the following section.

7.3.4 Lead Concentrations in Sludge Fractions

The hypothesis that lead was bound more extensively with the finer particulate matter was further investigated by examining sludge fractions. The sludges from two tanks were fractioned into three samples and analysed for lead. The fractioning process was based on the differential settling rates of the sludge fractions, with different times employed for the two sludges due to their different physical characteristics. The results are summarised in Table 7.4, which shows the settling times used to separate the fractions, as well as the sludge weights and lead concentrations in each fraction. It was found that the concentrations of lead in the sludge fractions increased as settling time increased. This trend was observed consistently for both sets of sludges. For Tank-S sludge (loamy), the concentration of lead in the fraction requiring >5mins to settle was 29% higher than that in the fraction requiring <1min to settle. For Tank-O sludge (clay), the difference between the first and final fractions was even greater at 66%.

Table 7-4: Concentrations of lead in each of the three sludge fractions from Tank-S and Tank-O. Sludge fractions were separated based on settling times. For both sludges, the slower settling fractions contained higher concentrations of lead.

Tank	Fraction	Weight (g)	% of Total Weight	Pb (μg/g)	% Conc. Increase
S	<1 min	2.44	83.3	2320	
	1-5 min	0.31	10.6	2620	+ 13%
	>5 min	0.18	6.1	3000	+ 29%
O	<5 min	2.65	77.5	830	
	5-30 min	0.56	16.4	1130	+ 36%
	>30 min	0.21	6.1	1380	+ 66%

Hence, it appears that lead is predominantly associated with the finer particulate matter in the sludge. This is consistent with the observations that the lowest concentrations of lead were found in the areas with the greatest sludge depth. However, on the basis of total lead quantities, the majority of lead was still seen to settle out in the first sludge fraction due to the significantly greater absolute volume of sludge. Therefore, within rainwater tanks, while the concentration of lead in the sludge around the inlet may be lower than that at other points in the tank, this is still likely to be the region of greatest lead deposition.

7.3.5 Sludge Settling Rates

Along with sludge composition, one of the most important aspects of sludge in relation to its effect on water quality is how long it stays suspended in the water column. Sediment may enter the water column with fresh rainfall or through resuspension of the sludge layer, during which time it is available to be withdrawn and potentially available for human exposure. Sludge settling rates were therefore investigated, applying the hypothesis that sludges would settle at the same rate. Settling rates were determined in the laboratory using two sludges found to have significantly different physical properties (i.e. loamy sludge from Tank-S & clay sludge from Tank-O). The sludges were completely resuspended into a 200mm water column by vigorous mixing and then left to settle, with settling rates determined by bacterial counts and light absorbance. As the sludges were completely re-suspended into the water column, the time required to achieve total re-settlement was taken as a maximum.

Sludge from Tank-S was defined as loamy, being rich in organic matter and containing a humus texture. Figure 7.6 shows the rapid settling rates of this sludge. Within 30 mins, the suspended sludge could no longer be detected in the water column by the naked eye or by diminished light absorbance in the spectrophotometer (0.01 absorbance at 360nm). This was consistent with significant linear reductions in HPC concentrations which had decreased by greater than 90% within 30mins from approximately 22,000 CFU/mL to 2,000 CFU/mL.

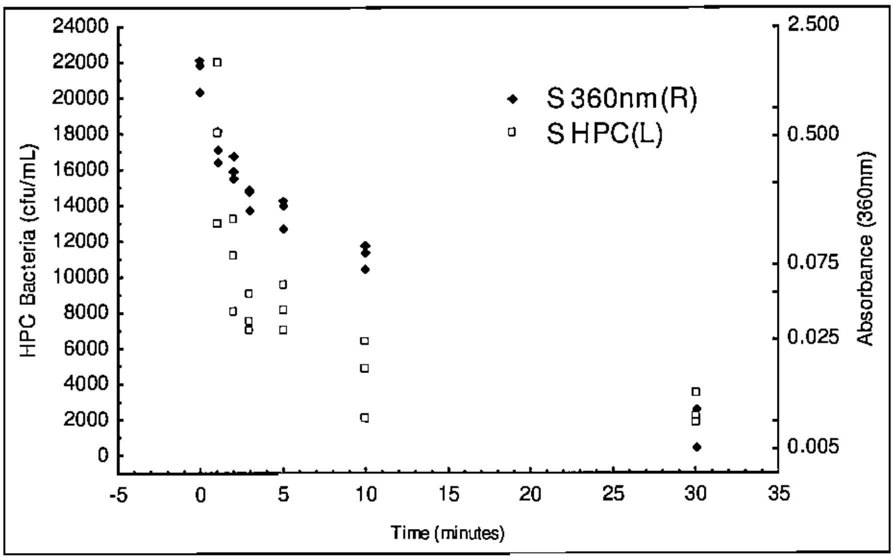


Figure 7-6: Loamy-type sludge from Tank-S was found to settle out of the water column at a consistent rate over a 30 minute period as measured by HPC concentrations and spectrophotometric readings.

In contrast, clay sludge from Tank-O required seven days before light absorbance decreased to 0.01. Figure 7.7 shows the light absorbance readings of the water column over the initial 840 minutes of the experiment, indicating the decreasing settlement rate of the sludge. HPC concentrations fell rapidly over the initial 30minute period and by 75% within the first 180 mins until stabilising with a residual HPC concentration persisting in the water column for the duration of the experiment. The high proportion of fine clay particles containing a specific gravity only slightly above 1 resulted in long settling times and the persistence of a slight cloudiness to the water.

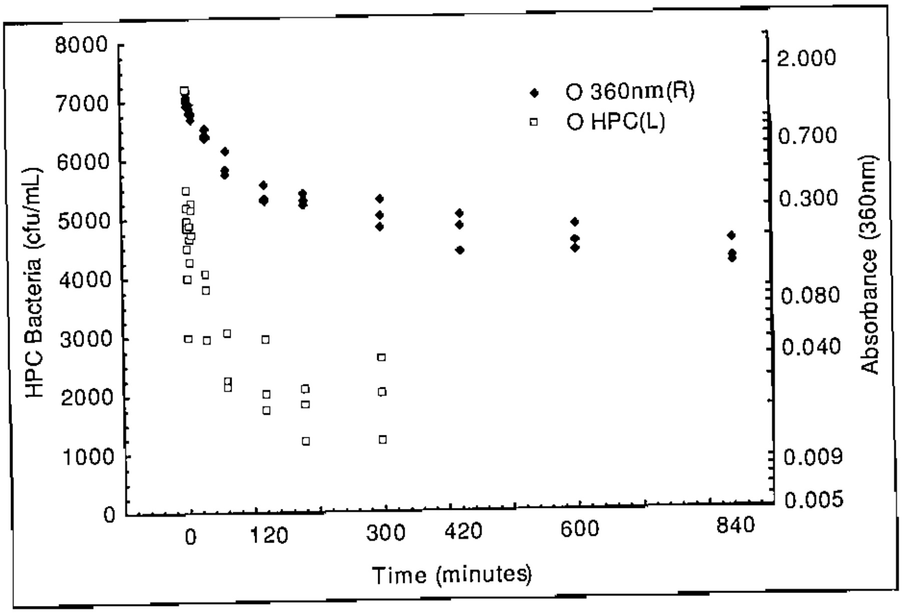


Figure 7-7: Clay-type sludge from Tank-O was found to settle out of the water column at a decreasing rate over the initial 840 minute period as measured by HPC concentrations and spectrophotometric readings.

As the settling rates of sediments are closely related to particle size distribution, analysis of the distribution of particle sizes was conducted on the sludges. In this analysis, particles were categorised into a series of sizes ranging from 0.01μm to 477μm, with each size category presented as a proportion of the total sample. The particle size distribution of sludge from Tank-S is shown in Figure 7.8, where particle size categories are presented in micrometres and by particle size classification. The majority of particles in Tank-S sludge were found to be sand-sized particles, with clay and silt comprising only a minor proportion of the total sludge composition. The relative contribution of each particle size category increased as the particle sizes increased, reaching a maximum contribution between approximately 150–350μm. A sudden reduction was then seen in the contribution of particles greater than approximately 350μm.

The sludge from Tank-O, similarly to that from Tank-S, was dominated by particles in the larger size ranges, as shown in Figure 7.9. However, the particle size distribution was slightly less skewed toward the larger particles with the peak contribution coming from particles ranging from approximately 100–350μm. A secondary peak was also seen in the clay size range (0.2–0.3μm) which was probably responsible for the longer times required for complete settlement to occur.

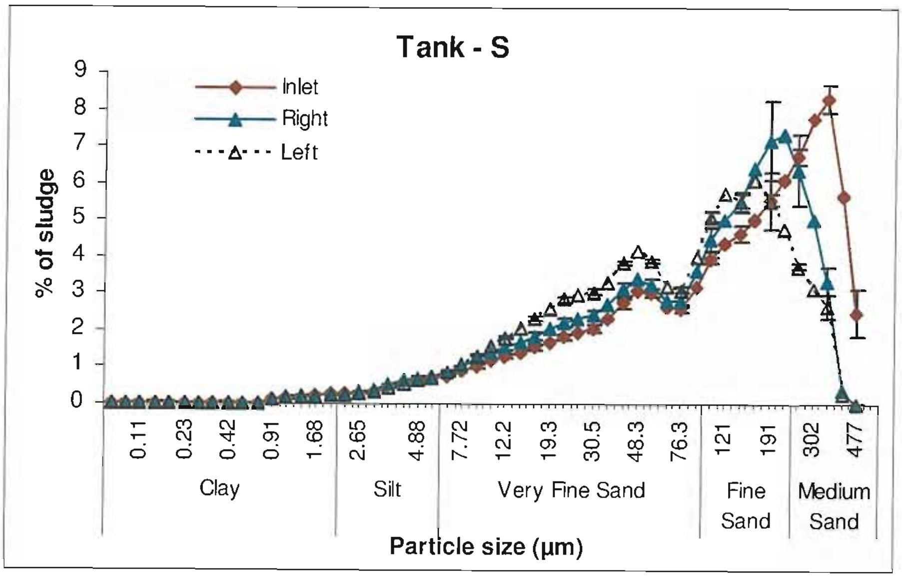


Figure 7-8: Particle size distribution of Tank-S sludge. The percentage of sludge (by volume) in each category is presented across a series of particle size categories ranging from 0.01μm to 477μm.

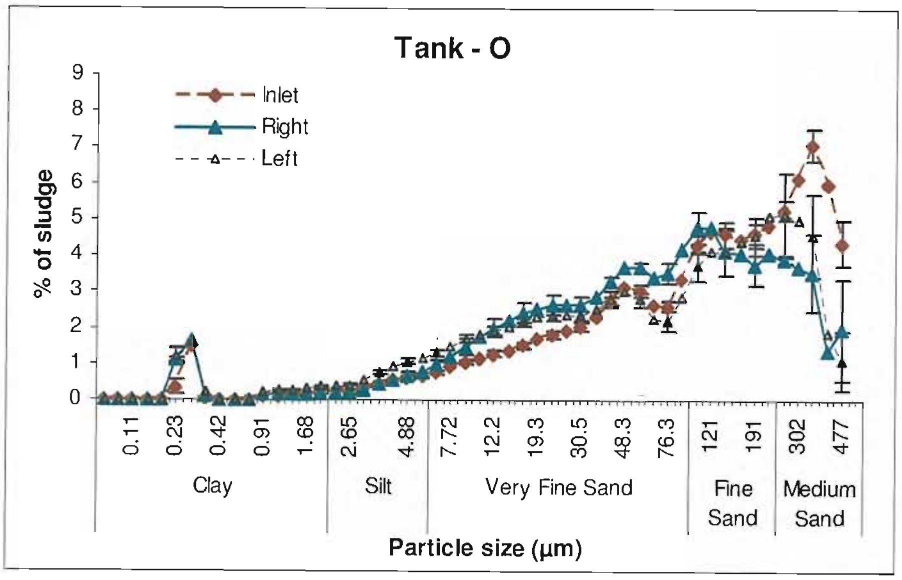


Figure 7-9: Particle size distribution of Tank-O sludge. The percentage of sludge (by volume) in each category is presented across a series of particle size categories ranging from 0.01μm to 477μm.

The total proportion of each sludge sample belonging to the major descriptive sediment classifications (e.g. clay, silt, sand etc) was then calculated to allow simple comparisons of the sediment sizes in the two sludges. These results are summarised in Table 7.5, where the major differences in particle size distribution between the two sludges can be easily highlighted. While the contributions of the SILT, VERY FINE SAND and MEDIUM

SAND were similar between the two sludges (<1% difference), larger differences were seen for the CLAY (7%) and FINE SAND (8%) fractions. The contribution of the CLAY fraction in Tank-O sludge was four and a half times greater than that in Tank-S sludge, while the FINE SAND fraction was equivalently lower. It is thought that the differences in these two categories accounts for a large degree of variation in settling rates between the two sludges.

Table 7-5: Distribution of Tank-S and Tank-O sludges based on particles size.

Particle	Size	Tank-S	Tank-O
CLAY	< 2 μm	2 %	9 %
SILT	2 – 6 μm	3 %	4 %
VERY FINE SAND	6 – 100 μm	41 %	41 %
FINE SAND	100 – 250 μm	34 %	26 %
MEDIUM SAND	250 – 500 μm	20 %	20 %

The settling characteristics of the sludges seemed to be related to the origin of the sludge material. The loamy sludge, which settled quickly, was composed primarily of rotting organic matter probably originating from overhanging vegetation, and did not contain a large proportion of fine particles. The clay sludge, on the other hand, closely resembled the soil in the surrounding environment and contained a high proportion of fine clays, and subsequently displays long settling times. Within rainwater tanks, the dynamics of sludge settling rates relate to both characteristics of the sludge particles and the water column. The physical properties of the particles, such as size, shape and specific gravity, will govern settling rates as well as the chemical nature of the particles, such as surface charge and their potential to form floccs. The hydraulic conditions of the tank will also dictate settlement rates, with the lower the kinetic energy in the water column (flow) the faster the settling rate. It was clearly shown that the two sludges examined in this study had dramatically different settling rates thought to be a result of differing particle size compositions. While the settling rates of sediments influence the length of time that a rainwater tank may contain elevated contamination levels, the vulnerability of the sludge layer to re-suspend into the water column is a highly important related factor.

7.3.6 Factors Influencing Sludge Re-suspension

Assessing potential re-suspension of tank sludge requires an understanding of the hydrodynamic forces interacting with the physical tank structure along with knowledge of sludge properties. The large number of variables involved in this process means that determining the probability and extent of sludge re-suspension in any given rain event is difficult. The large range of potential scenarios that exist within rainwater tanks differ both over time and between tanks. However, a number of factors are involved which are common to all tanks.

Five factors were identified which would influence the likelihood and extent of sludge re-suspension, listed in Table 7.6. The first was the water level in the rainwater tank during rain events. Water bodies that are static would be able to absorb and dissipate energy from inflowing water, and the larger the water body the greater its capacity for dissipating this energy. Consequently, tanks with low water levels may be more vulnerable to the risk of sludge re-suspension than tanks filled closer to capacity. The second factor was the intensity of rainfall. Obviously, rainfall of higher intensity would result in greater kinetic energy being transferred into the tank, which would increase the risk of re-suspension. The ability of the water column to disperse this energy is therefore related to first factor.

The design and orientation of the inlet was the third factor influencing the probability of sludge re-suspension. Captured rainwater encounters resistance as it flows through the collection system and the tank inlet. One of the major hindrances to inflowing water is the presence of meshed screens on the tank inlets. Screens physically divide the flow of water and reduce the chance of a concentrated jet entering the water column. The height and angle of the inlet would also influence the velocity of inflowing water. During laboratory experimentation with tank sludges, it was observed that the proximity of the inlet to the tank wall also influenced the level of sludge disruption. The re-suspension of sludge in a Perspex laboratory-scale tank was visually observed to be far greater when the inlet was located along the tank wall as opposed to draining water to the centre of the water column. This was thought to be due to the effective reduction of water in the water column available to dissipate the energy of the inflowing water. Inflow directed to the centre of the tank was absorbed by the 360° of water surrounding the inflow, as opposed to only 180° of water near the tank walls. The inflow of water along the wall of the tank also appeared to more readily stimulate the production of a current in the water column.

Table 7-6: Design and operational factors that may influence the extent of sludge re-suspension and water quality contamination

- Re-suspension factors
    - Water Level
    - Rainfall Intensity
    - Trajectory of inflowing water
        - Un/screened
        - Height of inlet
        - Vertical angle of inlet
        - Proximity to tank wall
    - Size and shape of tank
    - Type of sludge
- Water quality factors
    - Location of outlet
    - Height of outlet

The physical dimensions of the tank were the fourth factor influencing re-suspension potential. Larger tanks may hold a greater volume of water resulting in an increased capacity for absorbing inflowing energy, though if this volume was spread over a larger area the water levels may be lower. Several manufacturers now make rainwater tanks in a variety of shapes, including circular, square, and thin rectangular 'slimline' tanks for placing along fence lines. These shapes would each have a unique influence on the hydraulic conditions created in the water columns and hence, on the potential for sludge re-suspension. The final factor that would have bearing on the risk and extent of sludge re-suspension was the type of sludge present in the tanks. As seen in the above sections, the physical properties of sludges do vary between tanks. Clay and silt-based sludges would be more sensitive to shear forces imparted within the water column and would ultimately be re-suspended more readily and for greater periods of time than those consisting of sand-based sediments.

While these five points would influence the risk of sludge re-suspension, two further factors were proposed to determine the extent that re-suspended sludge would be drawn out of the tank and exposed to the user. The first of these is the location of the outlet. While each tank may produce unique hydraulic conditions resulting in differing patterns of sludge re-suspension, as a generalisation the closer the proximity of the outlet tap to the inlet the greater the risk of drawing re-suspended sediment into the extracted parcel of water. There may be an exception to this when the inlet discharges water along the wall of the tank. This increases the likelihood of creating vertical circling currents which may increase the risk of sludge re-suspension on the opposite wall of the tank. In such tanks, the risk of drawing off suspended sediment may be reduced by locating the outlet at a right-angle to the inlet. The second factor was the height of the outlet. Outlets drawing water from the base of the tank would be far more vulnerable to the risk of sludge contamination than outlets located above the base of the tank. While an outlet located at the base of the tank increases the volume of the effective supply, this marginal water-savings benefit is not as crucial for urban rainwater tanks where a supplementary mains water supply is available.

The withdrawal of water from the tank was not identified as a significant factor in sludge re-suspension. The relative contributions of water input and water withdrawal to sludge re-suspension were compared by empirical observations in laboratory experiments and in field tanks. It was clearly apparent through visual observations that sludge in laboratory experiments was much more sensitive to the input of water rather than the withdrawal of water. The observation was made in Tank-O (which had the outlet at the base of the tank and which contained a high proportion of easily suspended particles) of a small cone of approximately 50mm radius around the outlet which was devoid of sludge. This anecdotally suggested that the shear force created from water withdrawal that was required to move sludge horizontally was limited to around 50mm. Observations from the other tanks, which contained outlets at an elevation of 50-150mm above the tank bases, did not indicate any obvious pattern of disturbance to tank sludge associated to the withdrawal of water.

7.3.7 Flocculation Capacity

While the re-suspension of sludge has never been encouraged in rainwater tanks, Coombes *et al.* (2000) hypothesised that re-suspended sludge may hold properties of a coagulant and act to enhance the rates of flocculation of suspended bacteria and particulate matter. Coagulation-flocculation is a commonly used and well-known form of water and wastewater treatment in municipal facilities. This process accelerates the binding of small suspended particles into the formation of larger heavier clumps, or floccs, resulting in enhanced levels of sedimentation. It was therefore hypothesised that, if the re-suspension of sludge was to occur, water quality may be improved through the enhanced precipitation of previously suspended bacteria. To test the ability of tank sludges to enhance flocculation of suspended bacteria, sterilised and un-sterilised sludges (loamy and clay) were re-suspended into 200mm water columns containing either *E. coli* or *Pseudomonas aeruginosa*.

No apparent flocculation process occurred for loamy sludge with *E. coli* or *P. aeruginosa*, or for clay sludge with *E. coli*. Concentrations of suspended bacteria in these experiments remained stable through the process of sludge suspension and re-settlement, as shown in Appendix C. However, there was an enhancement of settlement rates for *P. aeruginosa* when subjected to mixing with resuspended clay sludge. Figure 7.10 shows concentrations of *P. aeruginosa* suspended in the water column before and after clay sludge re-suspension and settlement. The average concentration of *P. aeruginosa* in the water column after injection was 1.3×10^7 CFU/mL, which remained stable in each beaker over the 24 hours preceding sludge re-suspension. Distribution of the injected culture in the water column was shown to be uniform by the small standard deviation (11.7%) of the five samples taken at different locations in the water column immediately after injection. The control consisted of a *P. aeruginosa* suspension injected into the water column of a beaker not containing sludge. The concentration of *P. aeruginosa* in the water column of the control did not decline over the experimental period, whereas the two beakers containing sterile and non-sterile resuspended sludge showed clear reductions. The contribution of HPC bacteria to the water column by the non-sterile resuspended sludge was negligible (0.08% of *Pseudomonas* injection concentration).

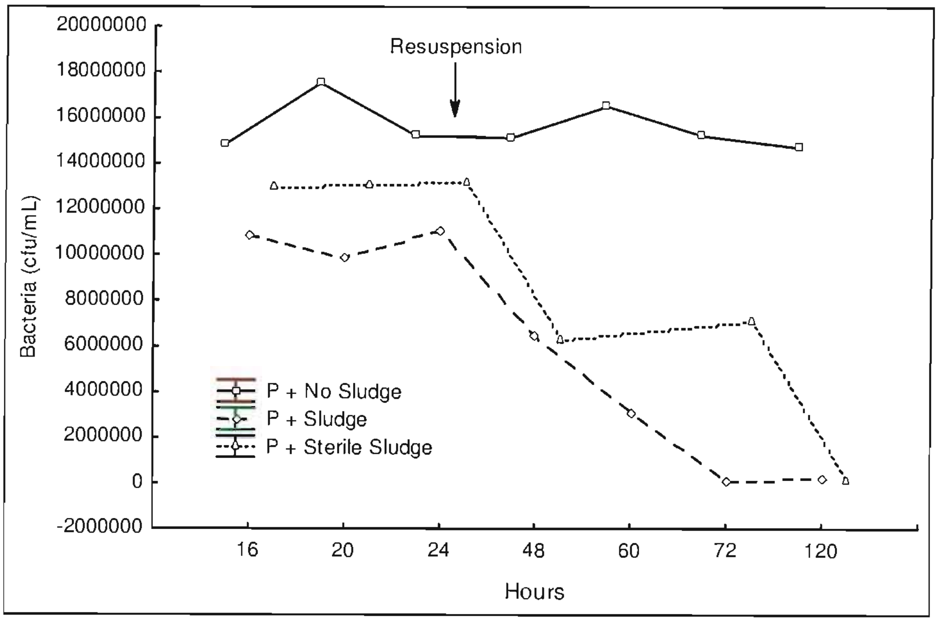


Figure 7-10: Tank-O sludge enhancing settlement of *P. aeruginosa*

Currently, there are two recognised phenomena that result in microbial adsorption to particulate matter. Weak adsorption occurs when the attractive van der Waals forces exceed the repulsive forces between the bacteria and the particle, while strong adsorption occurs as a result of extracellular polymers or cellular appendages binding to the particle (Jamieson *et al.*, 2005). The increased sedimentation of bacteria by the clay-based Tank-O sludge may have been the result of the greater number of potential adsorption sites (particles) and the lower repulsive forces of the smaller particles. The increased cellular attachment to clay particles observed in this study was consistent with the findings of Ling *et al.* (2002) who found greater attachment of indicator bacteria with clay particles.

However, it was somewhat surprising that only the concentrations of *Pseudomonas* were found to decline due to interaction with settling particulate matter. Jeng *et al.* (2005) observed higher rates of attachment to suspended solids by *E. coli* than by enterococci and faecal coliform bacteria in an estuarine water body. Large variations in the proportions of faecal coliform bacteria attached to particulate matter in surface water systems have been reported in the literature, ranging from 15% (Schillinger & Gannon, 1985) to 90% (Auber & Niehaus, 1993). The high rates of cellular attachment reported by Auber and Niehaus (1993) were associated with the smaller particulate matter (0.45–10μm). Muirhead *et al.* (2006) also found that when unattached *E. coli* cells were inoculated into a model overland-flow system the cells predominantly attached to particles <2μm. It is possible that *E. coli* were attaching to the smaller sized particles in this study, though the particles were not sufficiently able to form floccs of adequate size to facilitate settlement out of the water column. The presence of these particles in the water column at the end of the experiment would have gone undetected if the light absorbance of these particles was lower than the detection limits of the spectrophotometer.

The greater settlement of *P. aeruginosa* suggests that these bacteria are either capable of attaching to larger particles and/or can facilitate the formation of clumps of finer particles sufficient in weight to settle from the water column. *P. aeruginosa* is a well-known producer of extracellular polysaccharide (slime) which may have facilitated their strong adsorption to particles. The capacity of re-suspended sludges to reduce concentrations of suspended bacteria therefore appears to be a species specific phenomenon. The observation that the clay-based sludge has at least some potential for enhancing flocculation and settlement of bacteria is interesting, though is largely overshadowed by the length of time (>2 days) required for this to occur. Until the sludge has completely re-settled, water quality is significantly poorer for having the sludge in it, which in reality negates the benefits conferred by enhanced microbial settlement.

7.3.8 Maintenance and Human Health Risks

The significance of sludge in rainwater tanks relates to its ability to either improve or degrade water quality and hence affect the health of the user. While the concentrations of bacteria were higher in the sludges than the water columns, the most significant accumulations were of heavy metals, particularly lead. The lead levels in some tanks could be measured in g/kg which is comparable to levels at contaminated land sites. Furthermore, higher concentrations of lead were associated with slower-settling sludge fractions which would be more susceptible to re-suspension. However, the significance of these high concentrations of contaminants in the sludge is dependent upon the ability of sludges to re-suspend and become entrained in the drawn water supply.

The fact that the magnification of contaminants were so high in the sludges and the concentrations in the water columns were generally low, suggested that lead and other heavy metals were not readily resuspended or dissolved into the water column. The lead concentrations in the water columns did not fluctuate widely over time, including before and after rain events, suggesting re-suspension of sludge may be minimal. The low solubility of lead and many of the other heavy metals probably contributes strongly to their low concentrations in the water columns. Bacterial concentrations were also higher in the sludges than in the water columns, although it was not established whether this was due to the sludge environment being able to sustain cell growth or whether it was due simply to settlement, as was the case with heavy metals. It was demonstrated that in laboratory experiments that some re-suspended sludges are capable of increasingly bacterial removal from the water column, providing some benefit to water quality. However, this would really only be of health benefit if it occurred for sludge that settled quickly, and this was not observed.

Speculation has been made over the issue of whether it is best to de-sludge the tank or not. For example, in Australia the advice is given by enHealth (2004) to remove accumulated tank sludge every two to three years. Within the rainwater tanks in this study, the process of re-suspension did not seem to be occurring, indicating that the capacity of screens and the water body to dissipate the energy of the inflowing water was sufficient to protect the sludge from agitation. As such, this thesis does not provide strong evidence that the practice of de-sludging is necessary for maintaining water quality. This is a positive outcome given that the practice of de-sludging rainwater tanks is not widely practiced in Australia or many other parts of the world (REFS). A more effective solution for protecting water quality and human health may be the use of baffles around either the inlet or the outlet to restrict the potential risk of agitation and re-suspension of tank sludge.

7.4 CONCLUSIONS

Settlement of particulate matter to the bottom of the tank is probably the single most beneficial process harvested rainwater undergoes. It was found that sludge accumulation rates vary widely between tanks ranging from less than 1mm to almost 8mm per year, with the majority of deposition occurring directly below the inlets. One of the major findings of this study was the extent to which heavy metals accumulate in the sludge. Sludge contains highly magnified concentrations of a number of heavy metals, and to a lesser extent, bacteria. The magnification of lead in the sludges ranged from 65 000 times to almost 350 000 times that in the water columns, while bacterial counts were approximately 10–1000 times greater in the sludge.

Large differences were also noted in settling rates between the two different types of sludges. The most commonly identified type of sludge (loamy) required just 30 mins to settle out of a 200mm water column, while the clay sludge remained suspended for 7 days. The slower settling rates of the clay sludge were related to the composition of the sludge and specifically to the higher proportion of clay particles. The potential for settled sludge to subsequently re-suspend was thought to be due to five main factors. These included the depth of the water column, rainfall intensity, inlet trajectory, tank dimensions and the type of sludge. The position and height of the inlet will also influence the extent that re-suspended sludge may become entrained in the drawn supply.

Re-suspended clay sludge was found to possess the capacity to enhance the flocculation and settlement of *P. aeruginosa.* However, the benefit provided by enhanced flocculation by the smaller clay particles was not evident until a number of days after re-suspension, during which time water quality was worse for having the suspended sludge in it. The additional heavy metal contamination from the re-suspended sludge outweighs the benefits achieved by the increased settlement of suspended bacteria. This chapter evaluated the role of sedimentation and the behaviour of tank sludges as components of the incidental treatment train. In the following the influence of domestic hotwater systems on water quality will be investigated.

CHAPTER 8

Hotwater Quality

8.1 Introduction

The final potential treatment mechanism within the rainwater harvesting incidental treatment train is the hotwater system. It has been established that the widespread installation of rainwater tanks to supplement mains water supplies can have the benefits of reducing stormwater volumes and reducing urban water demand while simultaneously providing economic benefits, though the degree to which these benefits are realised is dependent upon the level of utilisation of the harvested rainwater resource (Coombes *et al.*, 2000, 2003). As hotwater usage represents a substantial fraction of indoor water demand, the use of rainwater for hotwater applications has been shown to be a significant factor in maximising the benefits to urban water-cycle sustainability (Coombes *et al.* 2002). The constant drawing down of the rainwater tank through hotwater use provides larger available tank capacity for rainwater harvesting during rain events and provides substantial reductions to mains water demand and peak demand.

Figure 8.1 shows the projected mains water demand in Sydney over the next century for a variety of possible demand management options, as predicted by the regional demand model developed by Coombes *et al.* (2003). The BASE case (no demand management intervention) and a number demand management options, including the installation of 'AAA-rated' water efficient appliances with a 2% per annum uptake rate (AAA_2%), the installation of rainwater tanks supplying toilet and outdoor uses with a 2% per annum uptake rate (T_TO_2%), and a variety of combinations of demand management strategies, each result in the continued increase in water demand on Sydney's supplies. However, when hotwater uses along with outdoor, laundry and toilet flushing are included in the suite of uses of harvested rainwater, demand is almost stabilised with a 1% per annum uptake rate (T_HTLO_1%) and is reduced with a 2% per annum uptake rate until the market is saturated in the mid 2040's (T_HTLO_2%).

However, domestic hotwater systems must demonstrate the ability to produce water of sufficiently high quality for showering and bathing purposes before the widespread recommendation to incorporate rainwater into hotwater systems can be made. As was seen in Chapter 3, harvested (unheated) rainwater, although not used for bathing or showering purposes, was in almost total compliance with bathing water (primary contact) guidelines. Despite this, the inclusion of hotwater in the suite of recommended uses of tank waters has received very little support from local councils and health departments. This is primarily due to the fact that drinking water guidelines are currently used to assess tank water quality and it is widely acknowledged that harvested rainwater regularly fails drinking water guidelines due to the presence of thermotolerant coliforms. Very few studies have examined the ability of domestic hotwater systems to destroy indicator bacteria, and none could be found that had evaluated thermal inactivation rates of pathogenic bacteria.

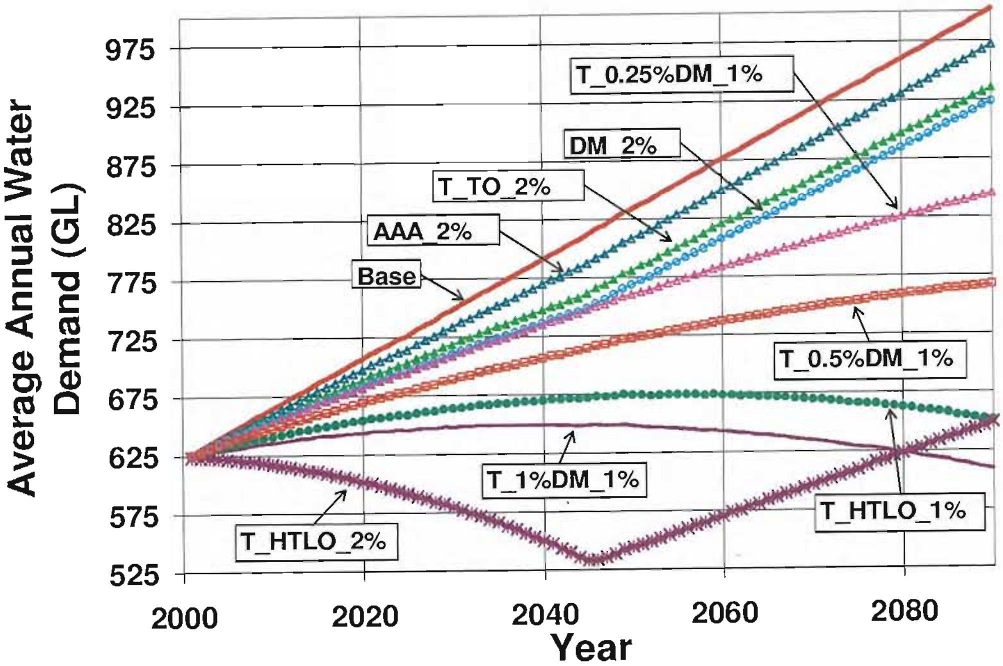


Figure 8-1: Projected mains water demand for Sydney produced the regional demand model developed by Coombes *et al.* (2003). Graph shows the significant reductions when hotwater is included in the suit of uses of tankwater with take-up rates of only 1–2%.

Limited field studies have revealed that domestic hot water systems can greatly reduce bacterial concentrations in rainwater supplies (Coombes *et al.*, 1999, Coombes *et al.*, 2003, Lye, 1991). Monitoring results from demonstration projects have revealed that rainwaters treated via storage and instantaneous hot water systems can produce water compliant with Australian Drinking Water Guidelines for *E. coli* and coliform organisms (Coombes *et al.*, 2002, Coombes *et al.*, 2003). However, the relevance of hotwater quality results based on these index organisms for specific pathogens is unknown due to a lack of data comparing thermal inactivation rates of indicator and pathogenic organisms in a freshwater medium. Studies from several countries have identified a variety of faecal, pathogenic, and opportunistically pathogenic bacteria within rainwater tanks, including, *Salmonella, Aeromonas, Pseudomonas, Shigella, Klebsiella* and *Vibrio* (Fujioka *et al.* 1991, 2002; Uba & Aghogho 2000), and the ability of these organisms to survive domestic hotwater systems has not been investigated.

Thermal inactivation data for a wide range of other pathogens have been developed within foods and perishable goods (e.g. Blackburn *et al.*, 1997; Juneja *et al.*, 1999; Oteiza *et al.*, 2003) and within liquid nutrient and saline broths (ICMSF, 1996). However, it has been clearly demonstrated that environmental variables, such as pH, water activity, and fat content of the medium, can greatly influence thermal inactivation rates (Humphry & Lanning, 1987; Goepfert *et al.*, 1970; Gibson, 1973; Oteiza *et al.*, 2003), making it difficult to extrapolate these results to heat inactivation in a fresh water medium. Growth phase and nutrient availability are also known to have pronounced influences on heat resistance with stationary-phase cells demonstrating maximum resistance, due at least partly to the translation of the global regulator rpoS gene (Fujita *et al.*, 1994; Loewen & Hengge-Aronis, 1994; Miksch & Dobrowloski, 1995; Yildiz & Schoolnik, 1998). The heat resistance of bacteria within rainwater tanks may also be influenced by water temperature, as stored rainwater is generally significantly lower in temperature, typically 12-25°C, than temperatures within the intestinal tract of warm blooded animals.

The regulation of water quality in hotwater systems is complicated somewhat by the availability of a number of different types of hotwater systems including instantaneous, solar-powered, and conventional electric and gas storage hotwater tanks. Domestic HWS operate in a range of sub-boiling temperatures, which is a critical factor when trying to distinguish between systems that will destroy bacteria and those that will promote bacterial growth. It was hypothesised that the temperature range in which domestic hotwater systems operate would significantly reduce bacterial numbers in harvested rainwaters, and that higher temperatures would inactivate bacteria more rapidly than lower temperatures. It was also hypothesised that different species of bacteria would have unique thermal resistance capacities which would be influenced by growth conditions. It was further hypothesised that the physicochemical quality of harvested rainwaters would not be altered by passage through domestic hotwater systems and that harvested rainwater would not cause accelerated corrosion of the hotwater systems.

The aims of this research were therefore seven–fold: Firstly, to determine the extent of inactivation of commonly used index and indictor bacteria in rainwaters passing through domestic solar-powered and electric-storage hotwater systems; Secondly, to determine which species have the greatest heat tolerances at various temperatures and which species are present in hotwater systems; Thirdly, to determine D-values in a water medium for eight non-spore-forming pathogenic and indicator bacteria used for assessing drinking water quality; Fourthly, to determine whether bacteria that had been multi-nutrient starved were more heat resistant than non-starved cells; Fifthly, to assess the effects of two growth temperatures on heat susceptibility of pathogenic and indicator bacteria; Sixthly, to determine whether obvious signs of accelerated corrosion is occurring in HWS supplied by mixtures of rainwater and mains water; And finally, to evaluate the suitability of using harvested rainwater for domestic hotwater applications.

8.2 Experimental Design

In order to fulfil these objectives, various types of research were conducted to provide sufficiently comprehensive data and understanding to be developed to allow robust conclusions to be made in regard to hotwater systems. Field sampling of hotwater systems was conducted in Brisbane and Newcastle, the two sites described in Chapter 3. A series of laboratory experiments were also conducted to assess thermal inactivation rates of specific species of pathogens and to assess heterogeneous communities of rainwater tank bacteria.

8.2.1 Field Sampling

8.2.1.1 Brisbane City Field Study Area

The two field sites in this study containing rainwater tanks and associated hotwater water systems were located in two urban regions on the east coast of Australia. The first region, located around Brisbane City, included 21 rainwater tanks and associated solar-powered hotwater systems, covering a range of commercially available brands. These included Edwards-solar (five), Beasley-solar (four), Rheem-solar (two), Saxon-solar (two), Solarhart-solar (three), Solco-solar (one), Quantum-heatpump (one), Rheem-heatpump (one), Elgas-LPG booster Rheem solar (one) and Dux-solar (one) hotwater systems. All rainwater tanks in both regions were sealed with screens on inlets and outlets and some had first flush devices to discard the initial portion of water during a rain event. Tanks were also connected to the local chlorinated mains water supply for topping-up when tank water levels fell below a designated threshold.

Sampling was conducted over a 24 month period, with samples taken on a monthly basis irrespective of rain events. Cold and hotwater samples were taken from indoor and outdoor taps, or from the relief valve on the hotwater tank, stored at 4°C in the dark during transport to the laboratory and analysed within 6 hours. Water samples from Brisbane were analysed at the Scientific Analytical Services laboratory for total coliforms using membrane filtration onto LES-Endo agar, thermotolerant coliforms using membrane filtration onto M-FC agar with *E. coli* colonies confirmed with the fluorescent protein MUG, and heterotrophic plate counts (HPC) using Plate Count Agar incubated at 35°C for 48 hrs.

8.2.1.2 Newcastle/Central Coast Field Study Area

The second region was located in Newcastle and included five rainwater tanks connected to storage hotwater systems. The rainwater tanks included Aquaplate®, polyethylene, concrete, stainless steel, galvanised iron and ranged from 2KL to 18KL in volume (average 4.5KL), while the storage HWS comprised a range of commercially available brands.

Sampling was conducted over a 24 month period, with sampling conducted in accordance with rain events, with samples taken one and seven days after rainfall. Cold and hotwater samples were taken from indoor or outdoor taps, or from the relief valve on the hotwater tank, stored at 4°C in the dark during transport to the laboratory and analysed within 6 hours. Samples from the Newcastle study region were analysed at the University of Newcastle using Membrane Filtration (Millipore) with enumeration of *E. coli* and total coliform bacteria on m-ColiBlue24® broth, *Pseudomonas spp.* with *Pseudomonas* Selective broth, and HPC on m-Heterotrophic Plate Count media at 35°C for 72 hrs.

8.2.2 Thermal Destruction Analyses

Two sets of thermal destruction analyses (TDA) experiments were conducted. One set of experiments were conducted on heterogeneous bacterial populations from rainwater tank samples after being enriched, while the second set of TDA experiments were conducted on single pathogenic and indicator organisms. The experimental design in both sets of TDA experiments were intended to simulate the thermal stress conditions likely to be encountered within domestic hotwater systems.

8.2.2.1 TDA: Heterogeneous Rainwater Bacterial Communities

8.2.2.1.1 Sampling

Bacterial populations from four rainwater tanks in Newcastle were examined for their response to thermal stress with the aim of identifying species which may contain both significant heat resistance capacity and pose public health risk. To competently assess thermal inactivation characteristics of bacterial populations, inactivation curves must be analysed over several log reductions. It was therefore necessary to enrich the heterogeneous rainwater bacterial populations to achieve initial populations large enough for several log reductions to occur.

8.2.2.1.2 Enrichment

Enrichment was achieved by splitting each of the four tankwater samples into two 100mL volumes and adding 100mL Brain Heart Infusion (BHI) broth and incubating one volume at 24°C and the other at 37°C aerobically for 24hrs. The split samples were then re-combined and incubated at 24°C for a further 24hrs. This method allowed growth of both environmental and enteric bacteria and resulted in the final population being acclimatised to 24°C, which was considered important as it has been shown that growth temperature can affect thermal inactivation rates of some species (Spinks *et al.* 2006, Manas *et al.*, 2003). After 48 hours of growth, samples were removed and 10mL portions were centrifuged (3373×g, 10 mins) at 4°C. The pellets were resuspended in 1.5mL of autoclaved deionised water, to give approximate concentrations of 5.4×10^6 cells mL^{-1}. Inoculum concentrations were determined by serial dilution and spread plating on nutrient agar.

8.2.2.2 TDA: Indicator & Pathogenic Organisms

Thermal destruction experiments were also conducted on a range of indicator and pathogenic organisms at temperatures relevant to HWS. This was necessary as the inoculation of a rainwater tank with a given pathogen is essentially a random occurrence, related to local environmental conditions and the activities of inhabitant birds, reptiles and small mammals, and can therefore easily go undetected during monitoring. The examination of non-pathogenic indicator bacteria *E. coli* and *E. faecalis*, along with the examination of a number of pathogenic bacteria also enabled the suitability of *E. coli* and *E. faecalis* as indicators of hotwater quality to be assessed.

8.2.2.2.1 Selection of Bacterial Species and Strains

The species of bacteria used in these investigations included two strains of *Enterococcus faecalis* (non-haemolytic and haemolytic), two strains of *Escherichia coli* (pathogenic EHEC O157:H7 ATCC 43895 and non-pathogenic O3:H6), *Pseudomonas aeruginosa*, *Salmonella typhimurium* (LT2), *Serratia marcescens*, *Klebsiella pneumoniae* (ATCC 13883), *Aeromonas hydrophila*, and *Shigella sonnei* (biotype A) which were obtained from TAFE NSW Hunter Institute or isolated from rainwater tanks. The eight species studied were common food and waterborne pathogens and are defined by the WHO and the ADWG as organisms of health concern or as key indicator and index organisms, and have been isolated from a number of rainwater catchment systems across the world (e.g. Simmons *et al.*, 2001; Uba & Aghogho, 2000; Ariyananda, 1999; Fujioka *et al.*, 1991; Waller *et al.*, 1984). The selected species have been implicated in a range of clinical illnesses including gastroenteritis, bacterial pneumonia, urinary tract infections, and opportunistic infections of the skin, ears and eyes. Two strains of *E. faecalis* and *E. coli* were used to assess the differences in heat resistance between pathogenic/haemolytic and non-pathogenic/haemolytic strains as these species are currently the two most commonly used indicator organisms for water quality monitoring. One important requirement of an indicator organism was that it exhibits a comparable or less susceptible response to environmental stress than the pathogens they represent. It was therefore necessary to evaluate a range of relevant pathogenic organisms along with the indicator organisms.

8.2.2.2.2 Growth Conditions

Cultures were maintained on Blood Agar (all broths and agars purchased from Oxoid) and periodically subcultured. Each strain was cultured in 100 ml Brain Heart Infusion (BHI) broth in 250 ml flasks incubated aerobically in a shaking incubator (180rpm) at 37°C. Two consecutive 12 h (*E. coli, S. typhimurium, S. sonnei*) or 22 h (*K. pneumoniae, P. aeruginosa*) transfers were made using 100 µL inocula. 10mL portions of the final stationary phase cultures (OD_{480} = 1.4) were centrifuged (3373×g, 10 mins) at 4°C, and the pellets resuspended in 1.5mL of sterile (autoclaved) deionised water, to give approximate concentrations of $2x10^{10}$ cells mL^{-1}. Inoculum concentrations were determined by serial dilution and spread plating on nutrient agar.

To determine the influence of starvation on the heat resistance of stationary phase cells, centrifuged pellets were resuspended in sterile de-ionised water (multi-nutrient deficient) and left at room temperature (24°C) for 24 h. For determining the influence of growth temperature on heat resistance, a relatively heat susceptible bacterium (*S. typhimurium* LT2) and the relatively heat resistant bacteria (*S. sonnei* biotype A and *E. coli* O157:H7) were also cultured aerobically in BHI at 20°C for 48 hours to stationary phase in an otherwise identical fashion to that described above for non-starved cells.

8.2.2.3 Experimental Configuration and Heat Application

A retort stand was used to suspend the Erlenmeyer flask containing the sample slightly above a Thermolyne Cimarec 2® heating block with magnetic stirrer. The configuration was designed to ensure a temperature fluctuation range of <±0.15°C as recommended by

Pflug and Holcomb (1991). A sterile magnetic stirrer and two Resistance Temperature Detectors (RTDs) were placed into the sample inside the Erlenmeyer flask. The magnetic stirrer was set to a low speed to create a slight vortex, with adequate mixing achieved within 3 seconds as determined from the controls. The RTDs logged directly into a Dataker DT50 data logger which was connected to a laptop computer programmed to display temperature profiles at one-second intervals using the DeTransfer™ software.

A fixed volume of sterile deionised water (199mL,) was placed into an Erlenmeyer flask held above the hot plate and heated by convection to the appropriate lethal temperature (55°C, 60°C, 65°C) prior to inoculation. After temperature stabilisation, 1mL of resuspended culture was injected into the water medium and timing was immediately initiated. Rapid mixing was visually observed and confirmed by multi-point sampling of the control, with uniform concentrations (<0.2 log variation) achieved within three seconds. 1mL samples were taken at pre-determined times thereafter and immediately quenched by diluting in room-temperature deionised water.

8.2.2.4 Enumeration of Survivors & Data Analysis

Bacteria surviving the heterogeneous TDA were enumerated by spread-plating appropriate dilutions onto nutrient agar and incubating at 24°C for 48hrs followed by 37°C for a further 24hrs. Bacteria surviving the homogeneous TDA were enumerated by spread-plating appropriate dilutions onto nutrient agar (non-starved cells) or R2A agar (starved cells) and incubating at 37°C for 48hrs. The type of agar used was not thought to bias the results as preliminary experiments showed no difference between plate counts of non-starved heat-shocked cells on nutrient and R2A agar. The D-value is defined as the time required to reduce a bacterial population by 90% or 1 log reduction (Prescott *et al.*, 1993). A minimum of three replicates were conducted for each experiment, and D-values were derived from the formula:

$$D_x = (T_2 - T_1) / (\text{Log}C_1 - \text{Log}C_2)$$

where D_x is the D-value in seconds for temperature x, T_2 is the number of elapsed seconds at the final sample point since time zero, T_1 is the number of elapsed seconds at the initial sample point since time zero, C_1 is the concentration of bacteria at T_1, and C_2 is the concentration of bacteria at T_2. Data were analysed using Microsoft Excel to calculate D-values and to perform Analysis of Variance (ANOVA).

The controls consisted of cell suspensions being inoculated into water at room temperature. The same experimental configuration was used including the insertion of the two RTDs and magnetic stirrer. Cell suspension samples used for controls were taken from the initial cultures used in the experiments after the centrifugation step.

8.2.3 Identification

The surviving bacteria from the hotwater samples and from the heterogeneous thermal destruction experiments were identified using Polymerase Chain Reaction (PCR) of the 16S rRNA gene using a universal primer (Wilson *et al.*, 1990). The amplified product was then sequenced by Hunter Sequencing Laboratories and species identified in GenBank using the BLAST 2.1 program (http://www.ncbi.nlm.nih.gov/BLAST/).

8.3 Field Results: Hotwater Quality

8.3.1 Brisbane Field Study

The focus of the Brisbane study was to determine the microbial and physicochemical quality of harvested rainwater and the degree to which water quality is influenced when passed through domestic hotwater systems. It was hypothesised that the microbial quality of the harvested rainwaters would improve as it passed through the domestic hotwater systems. The hotwater samples were compared to the unheated rainwater tank samples and were assessed using published Australian drinking and bathing water quality standards. The hotwater samples were also compared to the local mains water supply in order to gauge the relative quality differences between chlorine-treated municipal waters and heat-treated rain waters.

8.3.1.1 Hotwater Quality & Compliance with ADWG

8.3.1.1.1 Microbial Quality

The rainwater tank samples in the Brisbane study, which often contained *E. coli* and total coliform bacteria, were of significantly lower microbial quality than the chlorine-disinfected municipal water supply (Chapter 3). However, hotwater samples collected from the 21 solar-powered domestic HWS showed that significant reductions occurred in all bacterial parameters. Table 8.1 summarises the aggregated data from all hotwater samples and shows total coliform, thermotolerant coliform, and *E. coli* counts complying with ADWG in greater than 95% of samples. Almost all hotwater systems in this study appeared capable of disinfecting harvested rainwater. Of 458 hotwater samples, only eight samples (containing 1-5 CFU/100mL) were positive for *E. coli* from five systems (one system was positive three times, another was positive twice), and all hotwater samples positive for *E. coli* were below 48°C.

Table 8-1: Microbial concentrations in hotwater samples from the Brisbane study compared to samples from the rainwater tanks. Hotwater samples were evaluated against ADWG standards and found to have a high degree of compliance.

Parameter	N	RWT Av.	HWS Av.	HWS Max.	HWS Min.	HWS Compliance	ADWG
E. coli (CFU/100mL)	458	9	0.048	5	0	98.3%	0 (95%)
Th. coliforms (CFU/100mL)	458	14	0.076	11	0	97.8%	0 (95%)
Total coliform (CFU/100mL)	456	80	0.77	80	0	95.8%	0 (95%)
HPC (CFU/mL)	455	18520	957	26000	0	–	–
Temperature (°C)	441	24	51.8	71	31	–	–

The hotwater samples from the solar-powered HWS in the Brisbane study region were categorised into five temperature ranges based on hotwater temperature during sampling, as shown in Table 8.2. There was an apparent difference in hotwater quality between samples below 50°C and those above 50°C. Hotwater systems operating above 50°C achieved on average four-log reductions or 100% inactivation of *E. coli* and total coliform bacteria, and at least 98% inactivation of HPC bacteria. A significant finding from this study was that hotwater samples taken from systems operating above 60°C were of equal or higher bacterial quality than those taken from the chlorine-disinfected municipal supply.

Table 8-2: Average bacterial concentrations in water samples from the Brisbane municipal water supply, rainwater tauks, aud solar-powered hotwater systems, with hotwater samples categorised into five groupings based on temperature.

Parameter	Mains Water N=328 (SD)	RWT N=328 (SD)	Hotwater Temp.	N of HWS Samples	Hotwater	Reduction from RWT
E. coli						
(CFU/100mL)	0	9	< 50°C	111	0.1	98.44 %
	(0)	(66)	50 – 54°C	120	0	100 %
			55 – 59°C	69	0	100 %
			60 – 65°C	16	0	100 %
			> 65°C	12	0	100 %
Total coliform						
(CFU/100mL)	0.15	80	< 50°C	111	3.3	95.9 %
	(1.4)	(189)	50 – 54°C	120	0	100 %
			55 – 59°C	69	0	99.99 %
			60 – 65°C	16	0	100 %
			> 65°C	12	0	100 %
Heterotrophic						
Plate Counts	138	18 520	< 50°C	111	2153	88.37 %
(CFU/mL)	(466)	(21 018)	50 – 54°C	120	226	98.78 %
			55 – 59°C	69	328	98.22 %
			60 – 65°C	16	5	99.97 %
			> 65°C	12	9	99.95 %

HWS were also evaluated on an individual system basis, as was conducted on RWH systems in chapter 3, to determine the proportion of HWS that were 0%, >50%, >75%, >90% and >95% compliant with ADWG. Each of the 21 HWS undergoing water quality monitoring was assessed for the percentage of samples for which they contained 0 coliform or *E. coli* organisms per 100mL. Compliance with ADWG was improved for all houses for each bacterial parameter in hotwater samples. All hotwater systems were compliant in more than 50% of samples for bacterial parameters and 19 of the 21 systems were compliant in greater than 95% of hotwater samples for thermotolerant coliforms and *E. coli* (Table 8.3). Only a small number of the hotwater systems could not achieve 0 CFU/100mL in more than 95% of samples for total coliform (three systems), thermotolerant coliform (two systems) and *E. coli* (two systems). The two HWS containing total coliform, thermotolerant coliform and *E. coli* organisms consistently operated in the lowest temperature range (<50°C).

Table 8-3: Hotwater Compliance (0 CFU/100mL) per Household Basis

Compliance 0 CFU/100mL	0%	>50%	>75%	>90%	>95%
Total coliform	0%	100%	95.2%	85.7%	**85.7%**
Thermotolerant coliform	0%	100%	100%	95.2%	**90.5%**
E. coli	0%	100%	100%	95.2%	**90.5%**

8.3.1.1.2 Physicochemical Quality

The physical and chemical quality of harvested rainwater did not generally improve as it passed through HWSs and in some systems compliance for heavy metals and pH was reduced for hotwater samples. Table 8.4 summarises the aggregated physicochemical data from the hotwater systems in the Brisbane study. The percentage compliance of nickel and lead in hotwater samples increased over the monitoring period. The compliance of nickel with ADWG increased from 94.8% after 12 months, to 96% after 18 months and after completion of the two-year study had a compliance rate of 97.3%. Lead compliance also improved from 87% after 18 months to 88% after 2 years. However, the concentrations of lead and nickel in hotwater samples were still higher than in the associated rainwater tanks, suggesting some hotwater systems might be a potential source of lead and nickel contamination. Figure 8.2 shows the concentrations of lead in hotwater samples as well as rainwater tank and mains water samples (graphical presentation of other metals is given in section 8.8). The majority of lead samples complied with ADWG, indicated by the solid straight line, and followed the clear season trend exhibited by lead concentrations in tank waters.

Table 8-4: Physicochemical water quality of heated rainwater at the hotwater tap

(mg/L)	N	Average	Max	Min	Compliance	ADWG
pH	458	8.0	9.3	6.4	89.5%	6.5-8.5
Sodium	150	23.2	52.6	<1	100%	180*
Aluminium	150	0.078	0.14	0.007	100%	0.2*
Barium	150	0.0192	0.04	<0.005	100%	0.7
Cadmium	150	0.0	0.0	<0.001	100%	0.002
Chromium	150	0.0002	0.01	<0.002	100%	0.05
Copper	150	0.2586	1.63	0.047	100%	2
Iron	150	0.0256	0.29	<0.005	100%	0.3*
Manganese	150	0.0066	0.04	<0.001	100%	0.5
Nickel	150	0.0019	0.11	<0.002	97.3%	0.02
Lead	150	0.0041	0.03	<0.005	88%	0.01
Zinc	150	0.1739	1.29	0.013	100%	3*

*Aesthetic guideline only (no health guideline set)

The hypothesis that HWSs would not influence physicochemical water quality was also found to be false for iron and pH. Percentage compliance of iron in the hotwater systems was higher (100%) than in the rainwater tank samples (73.9%). The lower concentrations of iron in the hotwater samples than in rainwater samples suggests that some form of precipitation or adsorption of iron may be occurring within the hotwater systems resulting in an improvement of the aesthetic quality of the hotwater. The reduction in iron levels may have been related to increases in pH in hotwater systems. Greater than 10% of hotwater samples fell outside ADWG pH levels, the majority of these exceeding the upper band rather than the lower band. This shift of pH may have reduced the solubility of iron allowing it to settle out. This same effect would be expected to also reduce lead and nickel concentrations in hotwater samples, although if impurities or soldering within the hotwater systems were an additional source of contamination, then this would explain the increase in these heavy metal concentrations despite an increased pH. The majority of heavy metals were less significantly affected by passage through the HWS. Further discussion of metal concentrations within the context of corrosion is given in section 8.8 – Hotwater System Corrosion.

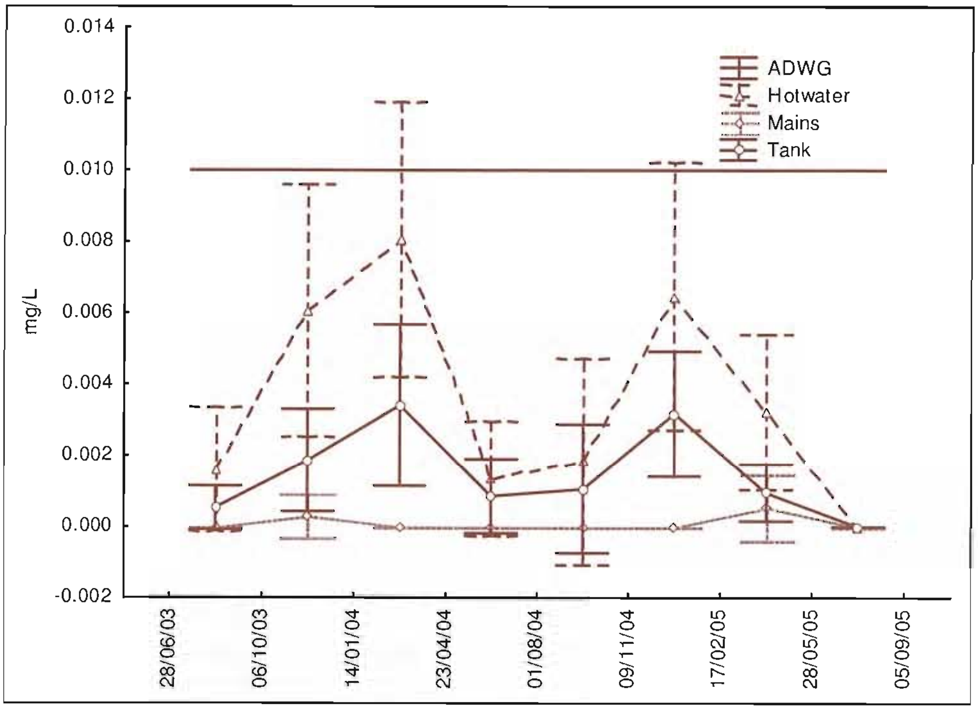


Figure 8-2: Lead concentrations in water samples from Brisbane's mains supply, rainwater tanks and corresponding hotwater systems. The ADWG limit for lead is denoted by the straight line at 0.01mg/L.

As with microbial parameters, evaluation of physicochemical parameters was also conducted on the 21 HWS on an individual system basis. From the results summarised in Table 8.5, it can be seen that in one HWS (Saxon–Solar) lead was found to exceed ADWG standards in every hotwater sample (N=7, Range=0.011–0.032mg/L), while 66.7% of HWS complied with ADWG lead levels in more than 95% of samples. In 66.7% of hotwater systems nickel levels were below ADWG in greater than 95% of samples, and all other heavy metals were found to be below the guidelines in more than 95% of samples in all houses. The source of the lead contamination in the single HWS suffering chronic lead contamination may have been the construction materials used in the HWS, though this could not be confirmed. However, this system did not contain high concentrations of any other metals, including nickel, in any hotwater samples, suggesting that the lead contamination may have been a result of inappropriately used lead soldering rather than any design or construction material impurities within the Saxon–Solar HWS itself.

Table 8-5: Hotwater Compliance (ADWG) per Household Basis

Compliance with ADWG	0%	>50%	>75%	>90%	>95%
Sodium	0%	100%	100%	100%	100%
Aluminium	0%	100%	100%	100%	100%
Barium	0%	100%	100%	100%	100%
Cadmium	0%	100%	100%	100%	100%
Chromium	0%	100%	100%	100%	100%
Copper	0%	100%	100%	100%	100%
Iron	0%	100%	100%	100%	100%
Manganese	0%	100%	100%	100%	100%
Nickel	0%	90.5%	85.7%	66.7%	66.7%
Lead	4.8%	95.2%	76.2%	66.7%	66.7%
Zinc	0%	100%	100%	100%	100%

8.3.1.2 Hotwater Quality and Compliance with GRWQA

One of the primary uses of domestic hotwater systems is for showering, which involves a high degree of direct human contact. The Australia/New Zealand Guidelines for Recreational Water Quality and Aesthetics cover the water quality requirements for water bodies that are subject to some degree of human contact. The highest standard of water quality within these guidelines applies to water bodies used for 'primary contact'. Primary contact includes water used for swimming, bathing and other direct water-contact sports where water may enter body cavities including ears and eyes and may be accidental swallowed. The bathing water guidelines specify that the median value of thermotolerant coliforms from at least five samples be below 150 CFU/100mL with more than 80% of all samples containing less than 600 CFU/100mL.

As discussed in chapter 3, harvested rainwater samples were in compliance with GRWQA. The quality of hotwater was also found to be in total compliance with the GRWQA for thermotolerant coliform limits, as seen in Table 8.6. The increase in pH of rainwater as it passed through HWS resulted in 4% of samples being above the pH limit of 9, while 0.7% of samples exceeded the limits for nickel. From the health perspective, the marginal exceedances of pH and nickel posed little direct danger to humans using the heated rainwater for showering and bathing purposes.

Table 8-6: Compliance of hotwater samples with the Australian bathing water guidelines (GRWQA).

Parameter	Compliance	GRWQA
Thermotolerant coliform	YES (med. 0 CFU/100mL)	Median <150 CFU/100mL &
	YES (100%)	>80% of samples <600CFU/100mL
pH	96%	5.0 – 9.0
Lead	100%	(<50μg/L)
Cadmium	100%	(<5μg/L)
Nickel	99.3%	(<100μg/L)
Chromium	100%	(<50μg/L)
Aluminium	100%	(<200μg/L)

8.3.2 Newcastle/Central Coast Field Study

8.3.2.1 Hotwater Quality and Compliance with ADWG

Hotwater samples were taken from five storage-tank HWS in the Newcastle region over the study period, with the results summarised in Table 8.7. The water temperature in the storage hotwater tanks averaged over 10°C higher than in the solar HWS from the Brisbane region. Despite this, higher residual total coliform concentrations were detected in the hotwater samples on the chromogenic m-ColiBlue24® media (Millipore). The identification of these surviving 'coliform' bacteria which cultured red on the chromogenic media were regularly confirmed through PCR and gene sequencing to be *Bacillus* sp. (predominantly *B. licheniformis*), which are spore-forming environmental bacteria and not part of the coliform group. The contribution of false positive *Bacillus* counts to the total coliform counts in cold water samples was likely to be negligible due to higher numbers of other actual coliform bacteria. However, as *Bacillus* species are not generally inactivated at the temperatures of the HWS under investigation, they proved to be a major influence on the perceived final water quality outcome of the storage HWS. Consequently, the microbial quality of the hotwater samples in the Newcastle study was higher than that indicated by the coliform counts with storage HWS achieving greater reduction rates than presented.

Table 8-7: Microbial concentrations in hotwater samples from the Newcastle study compared to samples from the rainwater tanks and evaluated against ADWG standards.

(CFU/100mL)	N	RWT Av.	HWS Av.	HWS Max.	HWS Min.	Compliance	ADWG
E. coli (CFU/100mL)	55	204	0.36	7	0	87.3%	0 (95%)
Total coliform (CFU/100mL)	55	1617	6.25	90	0	34.5%	0 (95%)
Pseudomonas (CFU/100mL)	55	5860	3.98	150	0	–	–
HPC (CFU/mL)	53	2030	62.8	3000	0	–	–
Temperature (°C)	48	18.2	60.9	47	70.5	–	–
pH	21	6.6	7.2	8.6	6.4	90.5%	6.5 – 8.5

Harvested rainwaters passing through the storage HWS in the Newcastle region were significantly improved. The storage HWS produced significant reductions in bacterial loads with 87% of samples containing no *E. coli,* and therefore compliant with ADWG, and with 95% of hotwater samples containing 1 or less *E. coli* organisms. The compliance of hotwater samples with ADWG for total coliform bacteria in tanks from the Brisbane study region (95.8%) appeared higher than for those in the Newcastle study region (34.5%), however, as explained this was likely influenced by the presence of *Bacillus* sp. in the total coliform counts. Despite this, 95% of hotwater samples in the Newcastle region contained 12 or fewer total coliform (including counts of *B. licheniformis*) bacteria.

As with the Brisbane study, the hypothesis that higher temperatures will result in greater microbial inactivation was applied to the Newcastle hotwater sample data. The hotwater samples from HWSs in the Newcastle region were categorised into three temperature ranges to examine the influence of temperature on microbial reduction, summarised in Table 8.8. Hotwater systems operating below 55°C demonstrated the capacity for efficient removal of *E. coli*, total coliform, and *Pseudomonas* bacteria, although reductions in HPC concentrations were less than one order of magnitude. HWS operating above 55°C achieved greater than 2-log reductions for all bacterial parameters. As with Brisbane, the microbial quality of hotwater from systems in Newcastle operating above 60°C was comparable to the quality of the local municipal water supply.

Table 8-8: Average concentrations of bacteria in disinfected municipal water supply, harvested rainwater, and electric-storage HWS

Parameter	Mains Water N=9 (SD)	RWT N=89 (SD)	Hotwater Temp.	N of HWS Samples	Hotwater	Reduction from RWT
E. coli						
(CFU/100mL)	0	204	< 55°C	11	0.7	99.66 %
	(0)	(793)	55 – 59°C	5	0	100 %
			> 60°C	34	0.09	99.96 %
Total coliform						
(CFU/100mL)	0	1617	< 55°C	11	22.3	98.62 %
	(0)	(3527)	55 – 59°C	5	2.4	99.85 %
			> 60°C	34	1.7	99.89 %
Pseudomonas						
(CFU/100mL)	0.4	5860	< 55°C	11	15.4	99.67 %
	(0.5)	(12 684)	55 – 59°C	5	3	99.95 %
			> 60°C	34	1.0	99.98 %
Heterotrophic						
Plate Counts	1	2030	< 55°C	10	317	84.38 %
(CFU/mL)	(1.1)	(5233)	55 – 59°C	5	12	99.41 %
			> 60°C	33	1.9	99.91 %

Water quality variations could also be seen within a single system when operating at different temperatures. One electric-storage HWS in the Newcastle region operated in a 'warm' temperature range for the first year of the study before increasing the operating temperature to a 'hot' temperature range for the second year. Table 8.9 summarises the water quality results during the periods before and after the change to operating temperature was made. The increase in temperature was approximately 10°C and hotwater was clearly of higher quality after the increase of temperature. While no *E. coli* organisms were detected in any hotwater samples, total coliform, *Pseudomonas* and HPC concentrations were approximately 1–2 orders of magnitude lower in the 'hot' water samples than in the 'warm' water samples.

Table 8-9: Microbial quality of hotwater samples taken from a single HWS in Newcastle before and after an increase in HWS temperature

	No. of Samples	Temperature (°C)	*E. coli*	TC	Pseudo	HPC
Before						
	9	47 – 53	0	16.6	18.8	353
After						
	9	57 – 64	0	0.9	0.1	2

8.3.2.2 Hotwater Quality & Compliance with GRWQA

When the hotwater samples from Newcastle were evaluated against bathing water guidelines they were found to be in total compliance for the microbial and pH parameters, shown in Table 8.10. Heavy metals were not analysed in hotwater systems taken from the Newcastle region. The median *E. coli* concentrations decreased from 12 CFU/100mL in the rainwater samples to 0 CFU/100mL in the hotwater samples, and exceedance of the 600 CFU/100mL upper band (permissible exceedance <20%) was reduced from 7% in the rainwater samples to 0% in hotwater samples.

Table 8-10: Compliance of hotwater samples with the Australian bathing water guidelines (GRWQA)

Parameter	Compliance	GRWQA
Thermotolerant coliform	YES (med. 0 CFU/100mL) YES (100%)	Median <150 CFU/100mL & >80% of samples <600/100mL
pH	100%	5.0 – 9.0

8.3.2.3 Identification of Surviving Bacteria

While hotwater systems were found to substantially reduce bacterial numbers in the harvested rainwater samples, low numbers of bacteria were regularly found to be surviving even in the higher-temperature hotwater systems. Random hotwater samples from the Newcastle study were chosen for further analysis. Identification of surviving bacteria was conducted by selecting colonies that appeared morphologically similar to *E. coli* from the mColiBlue® plates and selecting random colonies from the mEndo nutrient plates and sequencing the PCR products of the isolated colonies. Table 8.11 shows the predominant species identified in hotwater samples was *Bacillus cereus*, with a limited range of other *Bacillus* and non-*Bacillus* species also occasionally isolated.

Table 8-11: Identified bacterial species surviving in hotwater samples taken from 5 hotwater systems in the Newcastle study area.

HWS	1	2	3	4	5
Surviving Species	66°C	51°C	65°C	60°C	69°C
Bacillus cereus	+	+	+	+	+
Stenotrophomonas sp.	+	+	+	+	
Bacillus licheniformis	+	+	+		+
Bacillus fusiformis	+		+	+	
Escherichia coli		+	+		
Staphylococcus aureus				+	

B. cereus was noted as the dominant survivor of hotwater systems not only due to its isolation from all five of the hotwater systems under monitoring in the Newcastle area, but also due to the regularity of its isolation as well as it comprising the major proportion of the surviving bacterial communities in each of the hotwater samples. From a survey of 13 tankwater samples and 14 hotwater samples, *B. cereus* comprised on average 21% of the bacterial population in harvested rainwater samples and 92% of the surviving bacterial population in heated rainwater samples and was present in all but one of the hotwater samples. All species belonging to the genera *Bacillus* are spore-forming bacteria and are well known for their thermal resistance capacities, having been the subject of much research from the food industry (discussed in section 8.5).

Stenotrophomonas sp. and *Bacillus* non-*cereus* species were also detected in the majority of HWS although their isolation was far less frequent than that of *B. cereus* and typically constituted only a minor proportion of the surviving populations. Despite the low rates of *E. coli* identification in hotwater samples, the confirmation of a single surviving *E. coli* colony from a hotwater system operating at 65°C was surprising and not consistent with other findings relating to *E. coli* thermal inactivation characteristics. However, these results were largely biased by the fact that *E. coli* was the only non-randomly targeted species in the colony selection process, due to the significance they hold as an indicator of hygienic water quality. The survival of this *E. coli* organism through the HWS may have been as a result of cellular protection conferred by clumping with other cells or particulate matter, rapid passage of the cell through the hotwater system with minimal mixing with hotter water, additional heat resistance capacity of that particular strain, or possibly as a result of bacteria other than *E. coli* culturing blue on the chromogenic media giving a false positive detection of *E. coli*. Within the wider context of water quality and health risk, the isolated and extremely uncommon survival of single *E. coli* cells through hotwater systems does little to increase public health risk from using the supply for drinking or non-drinking purposes.

8.3.2.4 Microbial Quality produced by Field HWS

The heating of harvested rainwater in both solar-powered and storage hotwater systems clearly achieved massive reductions in bacterial loads. The four primary factors determining the concentration of bacteria surviving hotwater systems include the operating temperature of the HWS, the length of time water is held at this temperature, the inherent heat resistance capacities of the bacterial species, and the initial concentrations of each species in the rainwater. Typical plumbing standards for hotwater storage systems commonly state that domestic HWS should be maintained at a minimum of 60°C, irrespective of water source. At this temperature, hotwater samples taken from both solar-powered and electric-storage HWS were of comparable bacterial quality to the chlorine disinfected municipal supplies, suggesting that the practice of heating water to above 60°C is also a legitimate method of disinfection.

8.4 Heterogeneous Thermal Destruction Analyses

8.4.1 Heterogeneous TDA

While the index and indicator organisms presented in the field studies were useful for comparing measured water quality to recognised national and international water quality guidelines, these bacteria themselves are rarely pathogenic and, as such, merely act as representatives for the potential presence of pathogenic bacteria. Furthermore, the appropriateness of these organisms as indicators of pathogenic bacteria after undergoing heat stress has not been thoroughly investigated. Therefore, the bacterial communities in four rainwater tanks from the Newcastle study area were profiled to determine the actual range of organisms present.

As previously discussed in chapter 5 (section 5.3.3), a diverse bacterial community was found to exist in the water columns of rainwater tanks. Figure 5.3 showed the combined results of the bacterial community profiles from the four Newcastle tanks. Cold water samples were enriched and isolates were chosen based on unique colony morphology for identification. A diversity of environmental, enteric and spore forming bacteria were identified within the enriched rainwater samples. A variety of *Bacillus sp.* were identified in a number of tanks and appear to be common inhabitants of rainwater tanks, along with *E. coli*, *Stenotrophomonas sp.* and *Enterococcus sp.* These species were also detected in at least two of five hotwater samples taken from the associated storage HWS.

The enriched samples also provided an inoculum for examining the heat resistance capacities of relevant rainwater tank bacterial species. Thermal destruction experiments were conducted on the bacterial populations from the four enriched rainwater tank samples to determine the presence of any organisms containing significant heat resistance capacity and which may pose a public health concern. Figures 8.3a, 8.4a and 8.5a show the concentration reductions of the bacterial populations at the stress temperatures of 55°C (30mins), 60°C (90secs) and 65°C (60secs), respectively. The graphs are plotted as the log-percentage of surviving bacteria against time. Figures 8.3b, 8.4b and 8.5b show the species that were able to survive the respective temperatures stresses for the experimental period.

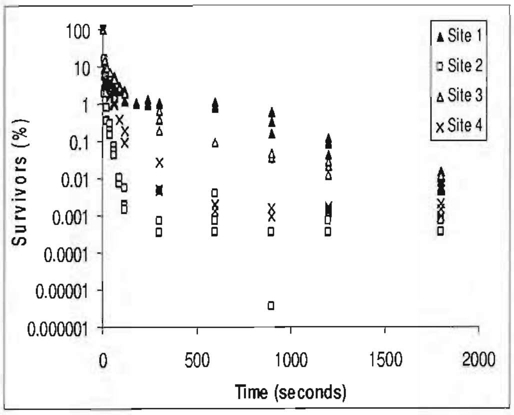


Figure 8-3a: Thermal inactivation curves (55°C) for four heterogeneous bacterial populations cultured from four rainwater tanks. Surviving concentrations are shown logarithmically as percentages of the initial concentration (100%).

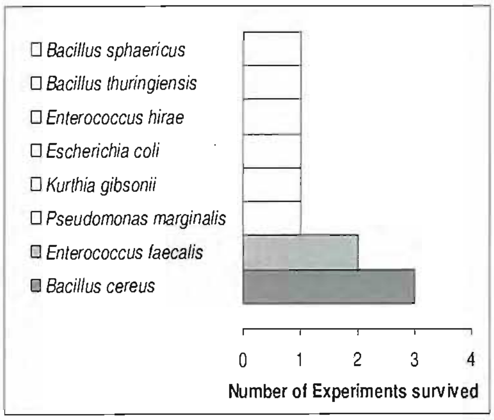


Figure 8-3b: Bacterial species surviving the duration of the 30min thermal inactivation experiments (55°C) on the four heterogenous bacterial populations.

At a thermal stress of 55°C, an initial rapid reduction occurred with all populations being reduced by 99% within 120 seconds, as shown in Figure 8.3a. Following this initial rapid decline, the reduction rate varied significantly between populations for a further 60 secs, with populations from tanks 2 and 4 decreasing by a further two to three orders of magnitude. By the duration of the experiment (30mins), concentrations of the four populations were within two orders of magnitude, having undergone a total of four to six log reductions. Figure 8.3b shows that spore-forming *Bacillus sp.* and gram positive *Enterococcus sp.* were the most common survivors of exposure to 55°C for 30 mins with limited survival of *E. coli*, *Kurthia gibsonii*, and *Pseudomonas marginalis*.

Exposure to 60°C resulted in a more immediate concentration separation between populations, with populations from tanks 2 and 4 being reduced by four log cycles within 10 seconds compared to populations from tanks 1 and 3 being reduced by less than 2 log cycles during the same initial exposure (Figure 8.4a). Concentrations stabilised in all populations after approximately 30 seconds and remained as such for the duration of the 90 second experimental period. Figure 8.4b shows the common surviving bacteria again included predominantly *Bacillus* and *Enterococcus* species.

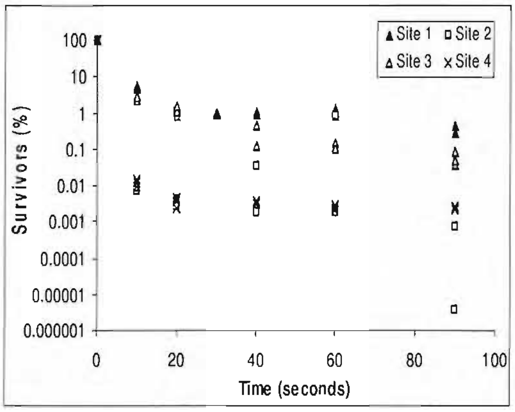


Figure 8-4a: Thermal inactivation curves (60°C) for four heterogeneous bacterial populations cultured from four rainwater tanks. Surviving concentrations are shown logarithmically as percentages of the initial concentration (100%).

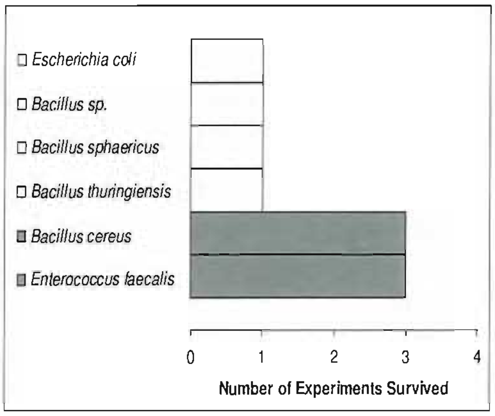


Figure 8.4b: Bacterial species surviving the duration of the 90 second thermal inactivation experiments (60°C) on the four heterogenous bacterial populations.

The initial reduction was more pronounced in all populations when exposed to 65°C, as shown in Figure 8.5a. As with exposure to 60°C, population reductions stabilised after 30 seconds, although at 2 orders of magnitude lower, and remained so for the duration of the experimental period. Again *Bacillus* and *Enterococcus* species were the predominant survivors along with *Stenotrophomonas maltophilia*, shown in Figure 8.5b.

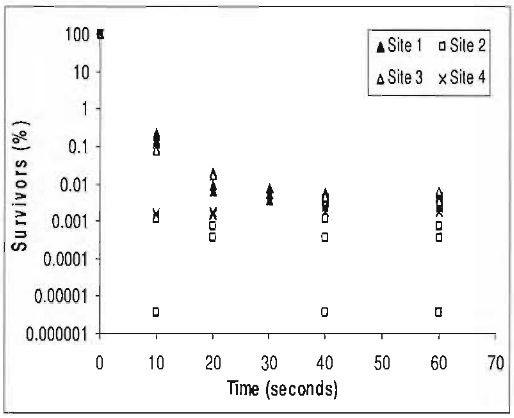


Figure 8-5a: Thermal inactivation curves (65°C) for four heterogeneous bacterial populations cultured from four rainwater tanks. Surviving concentrations are shown logarithmically as percentages of the initial concentration (100%).

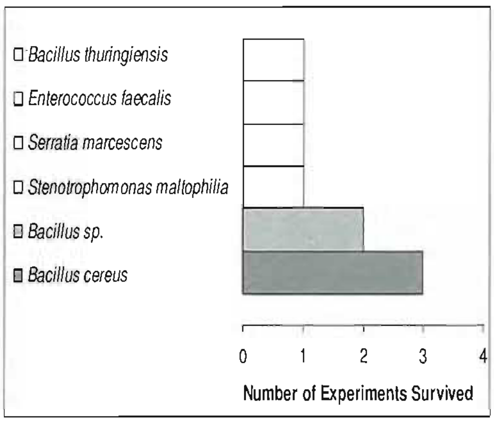


Figure 8.5b: Bacterial species surviving the duration of the 60 second thermal inactivation experiments (65°C) on the four heterogenous bacterial populations.

The bacteria detected in hotwater systems (section 8.3.2.3) were generally similar to those surviving the thermal destruction experiments (Figures 8.3b-8.5b), with the most commonly isolated bacteria being *Bacillus cereus* and other *Bacillus sp*. Only *Bacillus* and *Stenotrophomonas* were isolated from hotwater systems operating above 55°C, although *E. faecalis* was a prominent survivor of the thermal destruction experiments, suggesting that it is probably only present in rainwater tanks in low concentrations. The thermal inactivation experiments demonstrated the loss of efficiency of heat over time to inactivate heterogeneous bacterial communities at the three experimental temperature. Although the enrichment process would have altered the relative abundance of each species, it seems logical that declining inactivation rates observed in bacterial populations from environmental samples would be the result of the inactivation transition from the more heat sensitive environmental species, to the slightly more heat-tolerant enteric species, followed by the eventual inactivation of the highly resistant spore-forming bacteria.

8.5 Bacillus cereus

The consistent isolation of *B. cereus* from a number of hotwater samples, together with it's ubiquitous presence in the environment and previous reported links to clinical illness, made this bacteria the single most significant species in relation to domestic hotwater systems and worthy of brief discussion. *Bacillus* is a genus of gram-positive spore-forming bacteria ubiquitous in the environment, found in soil, water and on decaying vegetation. During unfavourable conditions *Bacillus* are capable of producing endospores, which are highly resistant to a range of environmental and chemical stresses. Of the 45 species of *Bacillus*, only *B. anthracis* and *B. cereus*, and to a lesser extent *B. subtilis* and *B. megaterium*, have demonstrated human pathogenicity.

B. cereus produces two types of toxins that have been associated with food poisoning. The (heat-labile) diarrhoeagenic enterotoxin has been associated with un-refrigerated meats and vegetable dishes and acts in a similar way to the Cholera Toxin by activating adenylate cyclase and producing watery diarrhoea. The production of the (heat-stable) emetic toxin is associated with sporulation and causes nausea and vomiting but little diarrhoea (Singleton

& Sainsbury, 2001). The emetic toxin is most commonly associated with un-refrigerated cooked rice and has been the more prevalent cause of *B. cereus* food poisoning, typically associated with oriental restaurants (Olsen *et al.*, 2000; Gilbert *et al.*, 1974). The infective doses from several cases of fried rice food poisoning have ranged between 3 x 10^5 and 2 x 10^9 *B. cereus* organisms per gram of contaminated food, with the majority of cases involving doses of greater than 10^6 CFU/gram (Khodr *et al.*, 1994; Gilbert *et al.*, 1974).

8.5.1 Occurrence & Heat Resistance

The prevalence of *B. cereus* in rainwater tanks and hotwater systems is not surprising given the ubiquity of its presence in the environment and the high heat resistance capacity of its endospores. As revealed in subsections 8.2 and 8.3, *B. cereus* was found to be the predominant survivor both in field hotwater systems and in the heterogeneous thermal inactivation experiments conducted on enriched rainwater samples. It was consistently detected in all five of the hotwater systems under monitoring, which were separated by a distance of 60kms, and comprised the majority of surviving HPC.

Table 8-12: Thermal inactivation rates (D-values) of *Bacillus cereus* in water reported in the literature. Spores of *B. cereus* have the capacity to survive for several minutes at boiling temperatures.

REFERENCE	MEDIUM	85°C	90°C	95°C	100°C
[a]Gilbert	Water		21–137	5–36	6.7–8.3
[b]Johnston	Phosphate Water	34–106			

([a]Gilbert et al., 1974; [b]Johnston et al., 1982)

The heat resistance capacity of *B. cereus* endospores has been reported within a number of perishable and liquid media, though due to its high resistance capacity investigations have focussed on temperature ranges above those relevant to domestic hotwater systems. Within a distilled water medium, Parry and Gilbert (1980) found the $D_{95°C}$ values to be 1.5–36mins. Gilbert *et al.* (1974) reported $D_{90°C}$ values of 21–137mins within an aqueous solution and Johnston *et al.* (1982) reported $D_{85°C}$ values of 34–106mins within a 0.25M phosphate solution (Table 8.12). It is clear that the thermal stress provided by domestic hotwater systems cannot be expected to reduce concentrations of sporulated *B. cereus* or other spore-forming bacteria.

8.5.2 Health Significance

The health significance of hotwater samples containing viable spores of *B. cereus* appears minimal even if the hotwater is used for drinking. *B. cereus* is not stressed as a high risk species by WHO drinking water guidelines and is not identified at all in the ADWG (2004), with no current evidence confirming the role of *Bacillus* sp. in waterborne transmission of disease. Even within the food industry, health risks associated with *B. cereus* are considered minor in comparison to risks posed by members of the Enterobacteriaceae family (ICMSF, 1996).

The concentrations of *B. cereus* in rainwater tanks and hotwater systems were far too low to cause illness. The danger concentrations in contaminated foods are typically $>10^6$ cells/gram, while concentrations in harvested rainwater samples were typically <500 cells/mL. Furthermore, the rainwater tank environment is not conducive to the production of toxins, which require solid (non-liquid) growth media. The diarrhoeal toxin is also only produced during the exponential growth phase, which is of limited possibility within rainwater tanks due to nutrient and temperature limitations.

The only feasible health risk posed by the presence of *B. cereus* in harvested rainwater supplies comes from improper storage and refrigeration of foods (particularly rice-based dished) cooked using water sourced from the rainwater tank or hotwater system. *B. cereus* and *P. aeruginosa* have also been implicated in causing infections in patients in burns units (Valentino & Torregrossa, 1995), so neither heated or unheated tank waters would be suitable for treatment of compromised or damaged body parts. Consequently, adherence to basic food preservation hygiene practices as well as not using tank waters to bath skin burns should realistically eliminate health risks posed by *B. cereus* in tank and HWS waters.

8.6 Pathogen Thermal Destruction Analysis

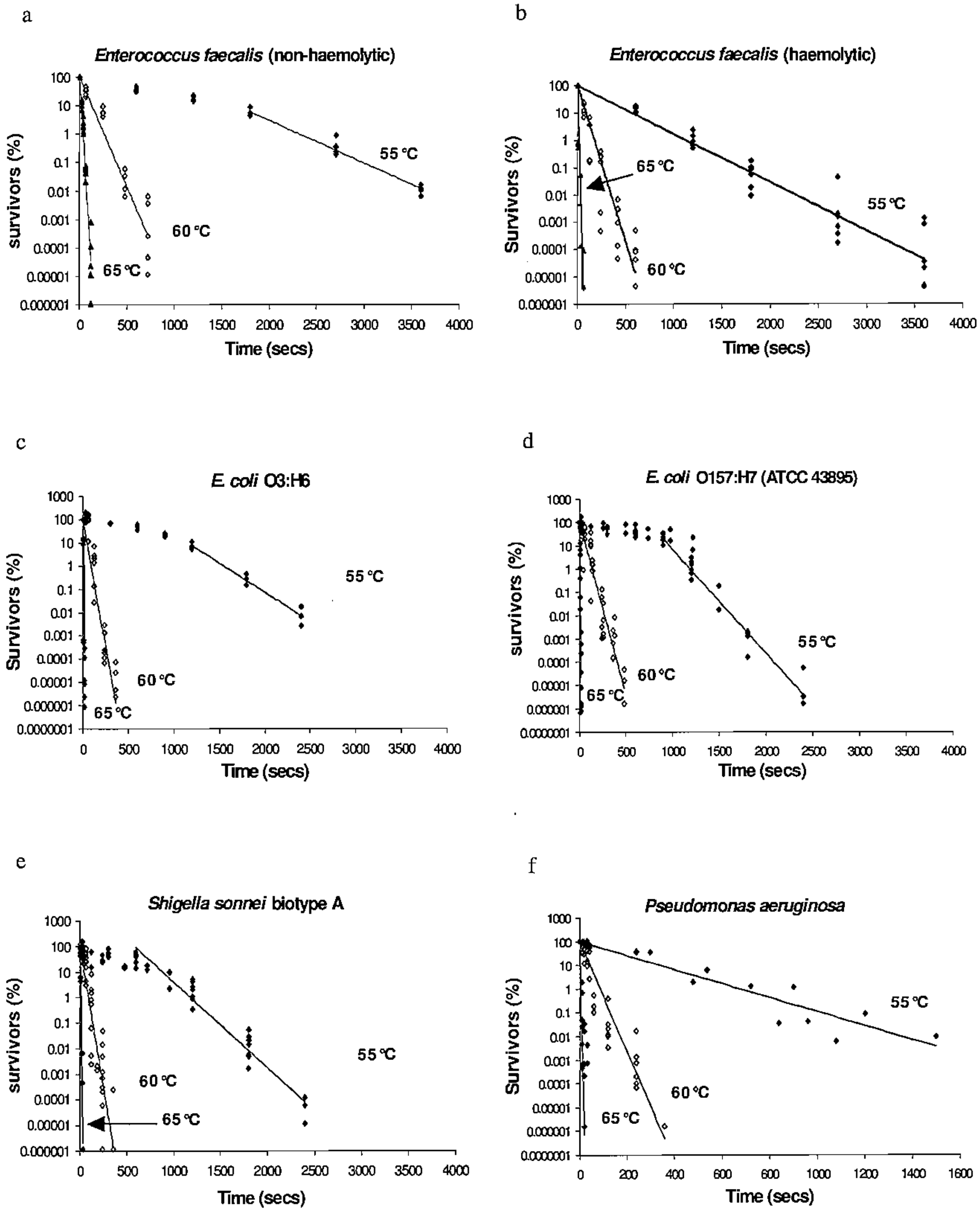
To ensure that domestic HWS would be capable of disinfecting tank waters, thermal destruction experiments were conducted on a range of indicator and pathogenic organisms at temperatures relevant to HWS. The organisms used in the study have been highlighted by the ADWG and WHO–GDWQ as organisms of concern in water supplies. The examination of non-pathogenic indicator bacteria *E. coli* and *E. faecalis*, along with the examination of a number of pathogenic bacteria also enabled the suitability of *E. coli* and *E. faecalis* as indicators of hotwater quality to be assessed.

8.6.1 Heat Inactivation

Significant reductions in viable bacterial counts were observed for all tested bacteria when thermally stressed at 55°C, 60°C and 65°C as shown in Figure 8.6 a-j, which represents a compilation of all data points from at least three replicate experiments for each species. Large variations in thermal inactivation rates were observed between the tested bacterial species as well as between the three test temperatures for each species.

The two strains of *E. faecalis* displayed the greatest heat resistance capacities under these experimental conditions, followed by the two strains of *E. coli* and *S. sonnei.* It was found that at 55°C the non-pathogenic *E. coli* O3:H6 was significantly more heat resistant than the pathogenic *E. coli* O157:H7 ($P<0.01$), while the non-haemolytic *E. faecalis* was more resistant, though not statistically significant, than the haemolytic strain. This is of interest given that it is primarily non-pathogenic strains of *E. coli* that are detected during water quality monitoring. Since indicator organisms are ideally non-pathogenic and possess slightly higher stress resistance capacities than the organisms which they are representing (Feachem *et al.*, 1983), the finding in this study confers a slightly conservative safety measure in cases where hot water samples test positive for *E. faecalis* or *E. coli.*

When challenged at 55°C a number strains exhibited an initial lag period where resistance to heat appeared maximal. In these instances the D-value was taken from the straight line section of the graph where the majority of log reductions occurred, as shown in Figures 8.6 a, c, d, e, and h and summarised in Table 8.13. This demonstrates one of the limitations of


a
Enterococcus faecalis (non-haemolytic)
survivors (%)
Time (secs)
55 °C
60 °C
65 °C
b
Enterococcus faecalis (haemolytic)
Survivors (%)
Time (secs)
65 °C
55 °C
60 °C
c
E. coli O3:H6
Survivors (%)
Time (secs)
55 °C
60 °C
65 °C
d
E. coli O157:H7 (ATCC 43895)
survivors (%)
Time (secs)
55 °C
60 °C
65 °C
e
Shigella sonnei biotype A
survivors (%)
Time (secs)
55 °C
60 °C
65 °C
f
Pseudomonas aeruginosa
Survivors (%)
Time (secs)
55 °C
60 °C
65 °C

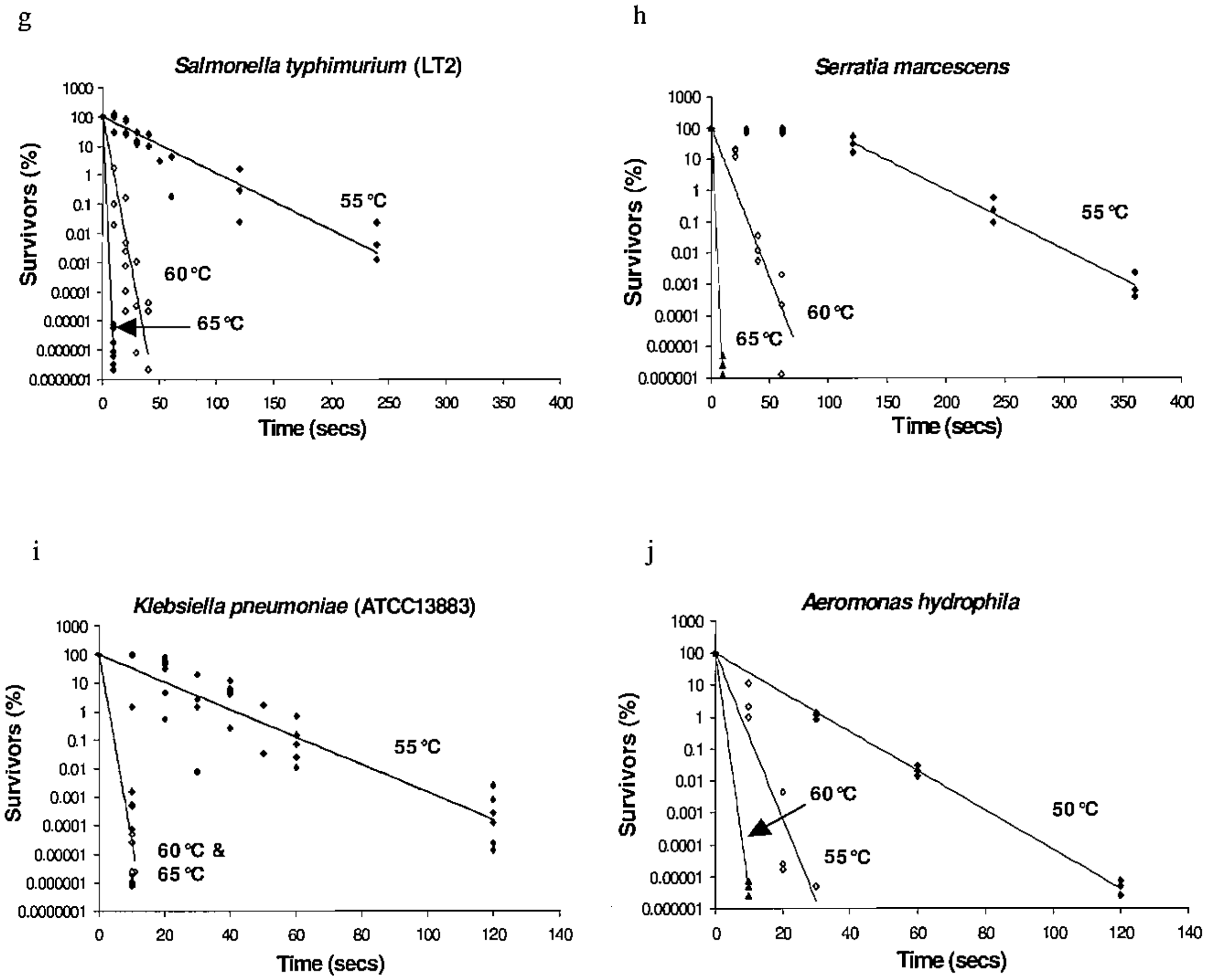


FIGURE 8.6: The reductions in numbers of viable cells were assessed over time following exposure to heat stresses under the defined experimental instantaneous heat system for (a) *E. faecalis* (non-haemolytic) (b) *E. faecalis* (haemolytic) (c) *E. coli* O3:H6 (d) *E. coli* O157:H7 (e) *S. sonnei* (f) *P. aeruginosa* (g) *S. typhimurium* (h) *S. marcescens* (i) *K. pneumoniae* and (j) *A. hydrophila* at 50°C to 65°C. The plots represent cumulative data from three separate replicated experiments, and the corresponding D-values are expressed in Table 8.13.

using the D-value for describing thermal inactivation data, which assumes a consistent reduction rate over time. It is important, therefore, that risk and performance assessments of hotwater systems do not rely solely on D-value data but also acknowledge the inactivation dynamics of the relevant species.

Table 8-13: D-values of non-starved (N) and starved (S) bacteria grown at 37°C and exposed to instantaneous heat treatment. D-values expressed as the time (seconds) required to achieve 1 log reduction in concentration and represents the means (± *Standard Error*) from a minimum of 3 replicate experiments.

	55°C	**60°C**	**65°C**	**55°C**	**60°C**	**65°C**
	N	N	N	S	S	S
Enterococcus faecalis (non-haemolytic)	901 (±26)	131 (±12)	19 (±1.2)	-	-	-
Enterococcus faecalis (haemolytic)	633 (±88)	77 (±8)	7 (±1.2)	509 (±28)	92 (±5)	-
Escherichia coli O3:H6 (wild type)	401 (±30)^{L}	51 (±2)	< 2*	225 (±20) ‡^{L}	41 (±1)	3 (±0.3)
Escherichia coli O157:H7 (ATCC 43895)	223 (±24)^{L}	67 (±7)	3 (±0.3)	232 (±21)^{L}	69 (±4)	3 (±0.2)
Shigella sonnei Biotype A	354 (±32)^{L}	54 (±5)	3 (±0.3)	305 (±18)^{L}	39 (±5)	4 (±0.3)
Pseudomonas aeruginosa (wild type)	304 (±35)	49 (±8)	5 (±0.6)	116 (±9)‡	45 (±3)	< 2*
Salmonella typhimurium (LT2)	77 (±12)	4 (±1)	< 2*	34 (±3)‡	6 (±1)	< 2*
Serratia marcescens (wild type)	71 (±3)	10 (±0.6)	< 2*	-	-	-
Klebsiella pneumoniae (ATCC 13883)	22 (±3)	< 2*	< 2*	19 (±2)	< 2*	< 2*
	50°C	**55°C**	**60°C**			
	N	N	N	S	S	S
Aeromonas hydrophila (wild type)	17 (±0.3)	3 (±0.4)	< 2*	-	-	-

‡Significant difference at P<0.05 compared with the non-starved cells.

* At small D-values, unaccounted-for timing variations may be significant. Because the analysis assumes error arise only in concentrations, only upper bounds on the D-value are reported.

^{L} Lag period exhibited before commencement of inactivation

As temperature increased, variations in thermal resistances between species became less pronounced. The capacity for heat resistance of *S. typhimurium, S. marcescens, K. pneumoniae* and *A. hydrophila* was greatly diminished at 60°C with several log reductions occurring within one minute. The D_{60} values for *E. faecalis*, *E. coli* and *S. sonnei* were two to seven fold faster than those for *L. pneumophila* serogroup 1 (D_{60} 138-288secs) as reported by Stout *et al.* (1986). At 65°C, D-values were less than six seconds for each species with the exception of *E. faecalis* (7-19 secs) which is comparable with the findings of Dennis *et al.* (1984) who reported D_{65} of 9 secs for the relatively heat resistant *L. pneumophila.*

8.6.2 Growth Temperature

Since bacteria present in rainwater systems are likely to be exposed to a temperature range of 12–25°C in most moderate climates of the world, the influence of a lower growth temperature on heat resistance was investigated for *E. coli* O157:H7, *S. typhimurium* and *S. sonnei.* Interestingly, growth temperature does not appear to influence all species but occurs in a species-specific manner. While no statistically significant changes in D-value were associated with growth at 20°C or 37°C for *E. coli* O157:H7 or *S. sonnei*, the results showed that an increase in growth temperature from 20°C to 37°C increased heat resistance of *S. typhimurium* at 55°C by approximately three-fold ($P<0.05$). Manas *et al.* (2003) found that there was a four-fold increase in the thermal resistance of *S. typhimurium* when growth temperature was increased from 10°C to 37°C, and a similar phenomenon was reported for *Yersinia enterocolitica* by Pagan *et al.* (1999). While it has been reported that structures such as the cell wall and the outer membrane can change their functionality in response to changes in growth temperature, the exact mechanisms by which growth temperature influences heat resistance are not fully understood. The degree of saturation of membrane fatty acids increases with increasing growth temperature as a means of maintaining a constant degree of fluidity within cell membranes, and this has been speculated to influence heat resistance (Manas *et al.*, 2003; Pagan *et al.*, 1999; Tsuchiya *et al.*, 1987).

8.7 Legionnaires Disease

8.7.1 Legionella pneumophila

One of the major perceived threats to public health associated with hotwater systems is that of infection caused by *Legionella pneumophila. L. pneumophila* serogroup 1 is the aetiological agent of Legionnaires disease, an acute form of pneumonia, which most commonly infects the respiratory tract of immuno-compromised individuals. *L. pneumophila*-associated infections occur as a result of the inhalation of contaminated bio-aerosols. Ingestion of high concentrations of *L. pneumophila* does not cause harm.

8.7.2 Influence of Hotwater System Temperature

Interest in the colonisation of hotwater systems by *Legionella* arose due to frequent isolations of *Legionella* from hospital and domestic hotwater systems sourced from treated municipal water supplies in areas suffering outbreaks of Legionellosis (e.g. Ezzedine *et al.* 1989; Lee *et al.* 1988; Wadowsky *et al.* 1985; Goetz *et al.* 1998). Martinelli *et al.* (2000) found that *L. pneumophila* were isolated much more frequently from hot water tanks (30%) as opposed to instantaneous hotwater systems (6.4%) in an area of Italy suffering an endemic outbreak of Legionellosis. The study concluded that temperature was critical, as the hotwater tanks in the study had been maintaining water at 50°C °C ±5°C, while the tap water averaged 45°C and dropped to 40°C during flow. Instantaneous systems, however, always maintained temperatures above 60°C (Martinelli *et al.*, 2000).

Ezzeddine *et al.* (1989) found that the main reservoir for *L. pneumophila* were mixing tanks where hotwater at 60-65°C mixed with cold water to achieve 45°C. Maintaining temperatures of 60°C or accelerating flow rate were found to be the most effective methods of controlling *L. pneumophila* (Ezzeddine *et al.*, 1989). Lee *et al.* (1988) discovered that water temperatures in electrically heated tanks were significantly lower than in gas-heated tanks. They concluded that the presence of *L. pneumophila* was associated with systems maintained below 48.8°C. Wadowsky *et al.* (1985) detected *L. pneumophila* in water and sediment samples taken from hotwater systems maintained at 30-54°C, but not from systems maintained at 71 – 77°C.

Hotwater tanks may be a reservoir for environmental pathogens if they are not maintained at adequately high temperatures. Legionella is found in highest concentrations at temperatures around 30-40°C, with an optimal growth rate at around 36°C (Szewzyk *et al.*, 2000). Rogers *et al.*, (1994) found that in hotwater systems maintained at 40°C *L. pneumophila* were at their most abundant. However, at 50°C the diversity of organisms was greatly reduced and numbers of *L. pneumophila* were reduced, and at 60°C *L. pneumophila* were absent from the system (Rogers *et al.*, 1994).

8.7.3 Occurrence in Rainwater–fed Hotwater Systems

Only limited investigation has been conducted on the prevalence of *Legionella* in harvested rainwaters and rainwater-supplied hotwater systems. Lye (1991) found that three hotwater systems supplied by harvested rainwater maintained below 53°C were positive for *Legionella*-like isolates, as shown in Table 8.14. The two systems maintained above 60°C were negative for such isolates. When the operating temperatures of the three hotwater systems operating below 53°C were increased to above 60°C, *Legionella*-like isolates could no longer be isolated.

Table 8-14: Occnrrence of *Legionella*-like Isolates within Cistern Systems

System	1		2		3		4		5	
	Hot	Cold	Hot	Cold	Hot	Cold	Hot	Cold	Hot	Cold
Water Temp. (°C)	52	21	63	22	52	22	52	21	63	21
Total CFU	$2x10^3$	$1x10^6$	$2x10^3$	$8x10^2$	$2x10^3$	$1x10^2$	$1x10^5$	$6x10^3$	$1x10^2$	$5x10^5$
Total Coliforms	2	3	0	0	13	36	0	300	0	0
CFU on BCYE medium	190	34	0	15	225	7	18	25	0	0
Possible Isolates of Legionella	YES	NO	NO	NO	YES	YES	YES	NO	NO	NO

All counts in CFU/mL (Lye, 1991)

8.7.4 Heat Resistance

A limited amount of research has been conducted which investigated the heat tolerance of *L. pneumophila* in a water medium, summarised in Table 8.15. Research findings generally indicated that *L. pneumophila* has little thermal resistance capacity above 60°C, with the widespread recommendation consequently made that hotwater systems should be maintained at a minimum of 60°C to inhibit the growth of *L. pneumophila* (Dennis *et al.* 1984; Sanden *et al.* 1989; Stout *et al.* 1986).

Table 8-15: D-values (mins) for *Legionella pneumophila* serogroup 1

REFS	50°C	54°C	55°C	58°C	60°C	66°C	70°C
[a]Stout					2.3–4.8		1.2–1.4
[b]Dennis	111	27		6			
[c]Sanden	380		13.93		0.74	0.45	

([a]Stout *et al.*, 1986; [b]Dennis *et al.*, 1984; [c]Sanden *et al.*, 1989)

8.7.5 HWS Regulation – AS/NZS 3500.4:2003

As a result of much of the previous research into the prevalence, control and elimination of *Legionella* from hotwater systems, Australian Standards (AS/NZS 3500.4:2003 – Heated Water Services) have been developed to define appropriate installation and operational practices to reduce risks of *Legionella* contamination. The standards apply for domestic hotwater storage services and state that domestic hotwater systems should be maintained at a minimum of 60°C in order to inhibit the growth of *L. pneumophila*. From the findings of this chapter, AS/NZS3500.4 also appears suitable for controlling a wide range of other non-sporing bacteria, providing effective disinfection against a number of microbial pathogens.

8.8 Hotwater System Corrosion

The final concern relating to the performance of hotwater systems supplied with harvested rainwater was that of corrosion. Accelerated corrosion of hotwater systems due to the use of rainwater has been a concern amongst Australian councils and water suppliers. While the performance of HWS to reduce microbial loads relates to human health, the ability of HWS to resist corrosion relates only to the lifespan of the HWS and therefore poses an economic consideration. However, the premature corrosion of domestic HWS is still an important consideration in the assessment of suitable uses of harvested rainwater and was investigated using data from the Brisbane study.

8.8.1 Principles of corrosion

The processes of corrosion often involve complicated chemistry making it difficult to understand and predict which systems will be more vulnerable to corrosion. Corrosion is essentially the oxidation (loss of an electron) of a given metal, resulting in a change of oxidation state. The site of oxidation (damage) is referred to as the anode and the location at which the electron (or rust) is deposited is known as the cathode, with transport of the electron provided by an electrolyte. An important factor in corrosion is the electrical conductivity (related to total dissolved solids) of the medium which surrounds the metal being corroded. Pure water is a very poor electrical conductor, but as substances dissolve in it, particularly those which ionise, conductivity rapidly increases. Probably the most common corrosion-causing electrolyte is dissolved oxygen. Oxygen reacts with and removes reaction products from the cathode during electrochemical corrosion, thereby permitting the attack to continue. In order to restrict the rates of corrosion in HWS, anodes are used. These are usually a magnesium, aluminium or zinc based alloy rod submerged in the tank to absorb the chemical action that causes tank corrosion. The anodes are therefore referred to as sacrificial, as they attract the corrosion that would otherwise attack the walls of the HWS.

8.8.2 Corrosion & Heavy Metal Leaching

Due to concerns over potential rapid corrosion of hotwater systems by a number of local water suppliers and local government authorities, potential corrosion of HWS was investigated in the Brisbane study by measuring heavy metals in hotwater samples to determine levels of metal leaching within the pipework and storage tanks. While the sacrificial anodes within the HWS themselves were not inspected in this project, the levels of iron and copper, which may leach from the HWS tank walls and heating element, and the levels of magnesium, aluminium, and zinc, which may leach from the sacrificial anode, were monitored in hotwater samples. The results from the 2 year study found that these metals were not present in significantly higher concentrations in the hotwater samples and therefore did not indicate significantly elevated rates of HWS corrosion.

Figures 8.7 to 8.12 show the levels of zinc, iron, magnesium, aluminium, copper and nickel in the rainwater tanks, solar hotwater systems, and mains water of the Brisbane City region. Zinc and iron are often bi-products from the corrosion of galvanised iron. Zinc and iron levels in the samples from the solar hotwater systems were lower than in the associated rainwater tanks and were not increasingly over time, shown in Figures 8.8 and

8.9, respectively. The lower concentrations in the hotwater samples indicate that no corrosion and leaching of these metals is occurring in the hotwater system.

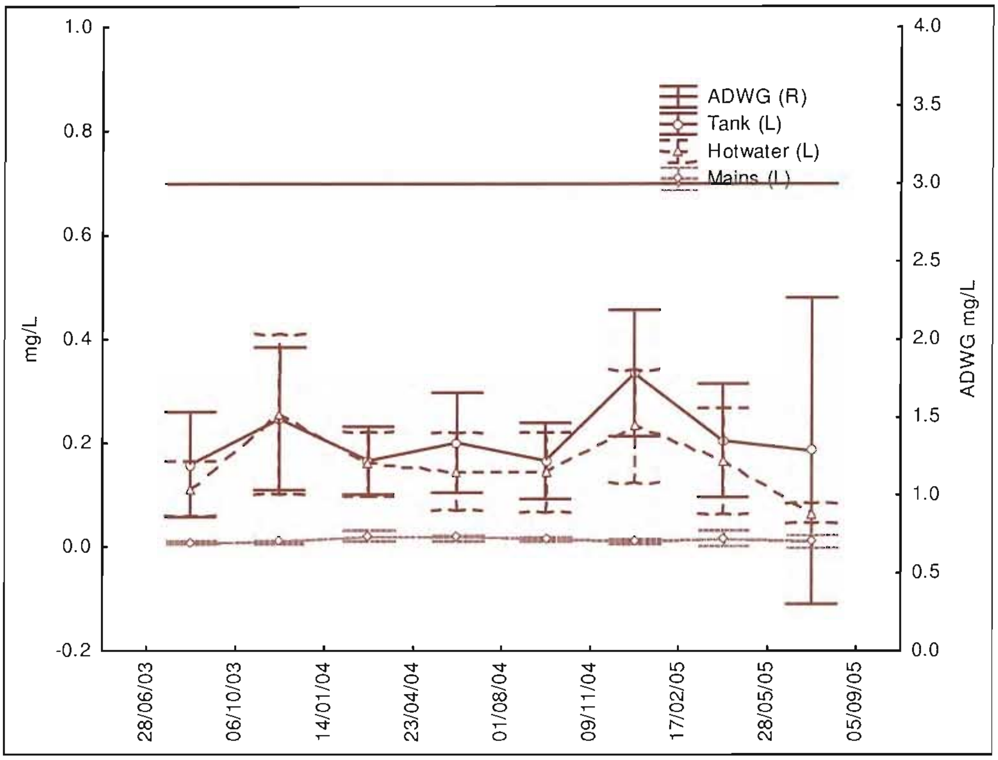


Figure 8-7: Average Zinc concentrations in Rainwater Tank, Mains and Solar HWS (left axis) compared against ADWG (right axis)

Hotwater system anodes are used to absorb the corrosion action that would otherwise attack the HWS walls. Typically HWS anodes are made of magnesium, aluminium or zinc, with the most common being magnesium or a magnesium-based alloy. The levels of magnesium in the hotwater samples were only marginally higher than in the rainwater tank samples, and followed a similar temporal distribution pattern, shown in Table 8.9. The slightly higher concentrations of magnesium in the hotwater were probably a result of magnesium leaching from the anodes, which is to be expected if the anodes are still functional. Levels of aluminium were higher in the hotwater than in the rainwater samples, although these levels were generally lower than in mains water samples, suggesting mains water may be a significantly contributing source. All aluminium levels were well below guideline values (Figure 8.10).

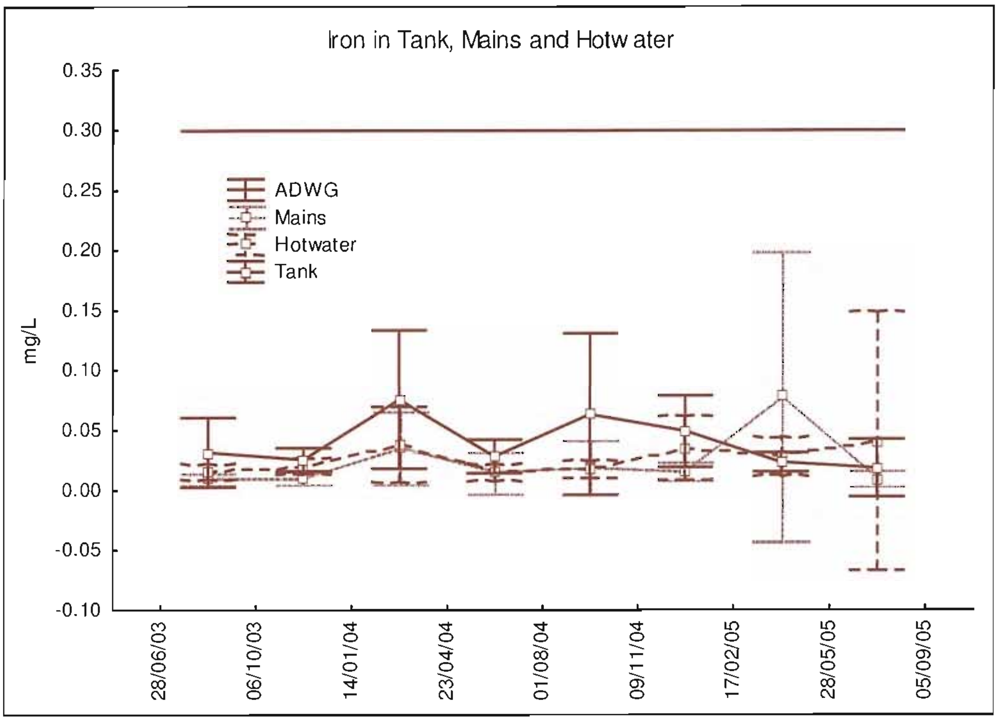


Figure 8-8: Average Iron concentrations in Rainwater tanks, Mains and Solar HWS Systems compared against ADWG

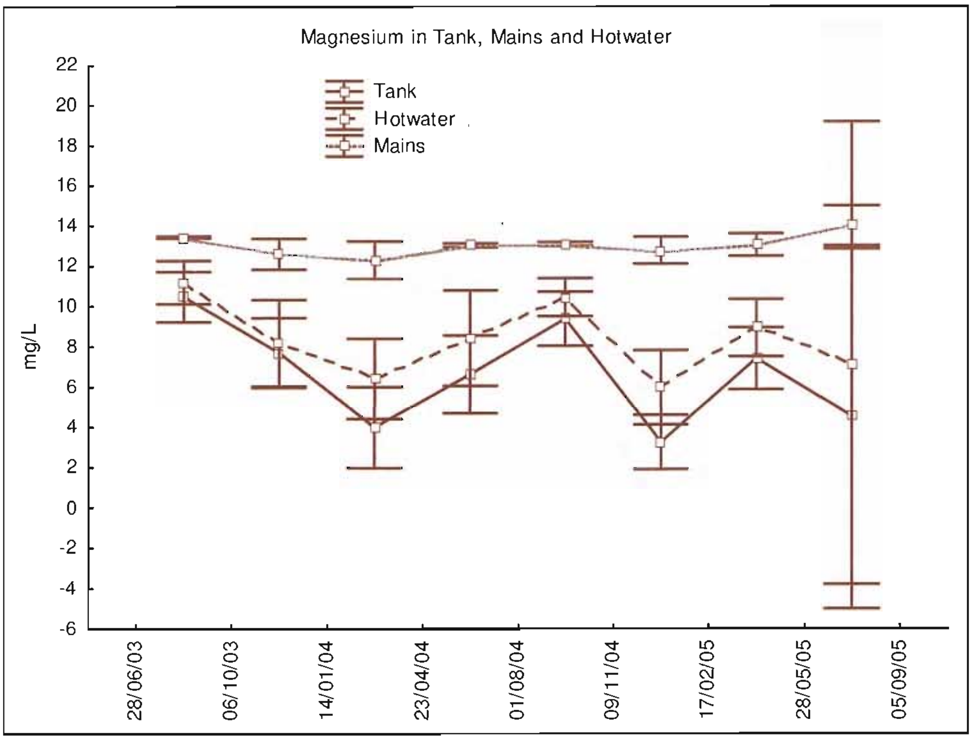


Figure 8-9: Average Magnesium concentrations in Rainwater Tanks, Mains and Solar-Powered Hotwater Systems

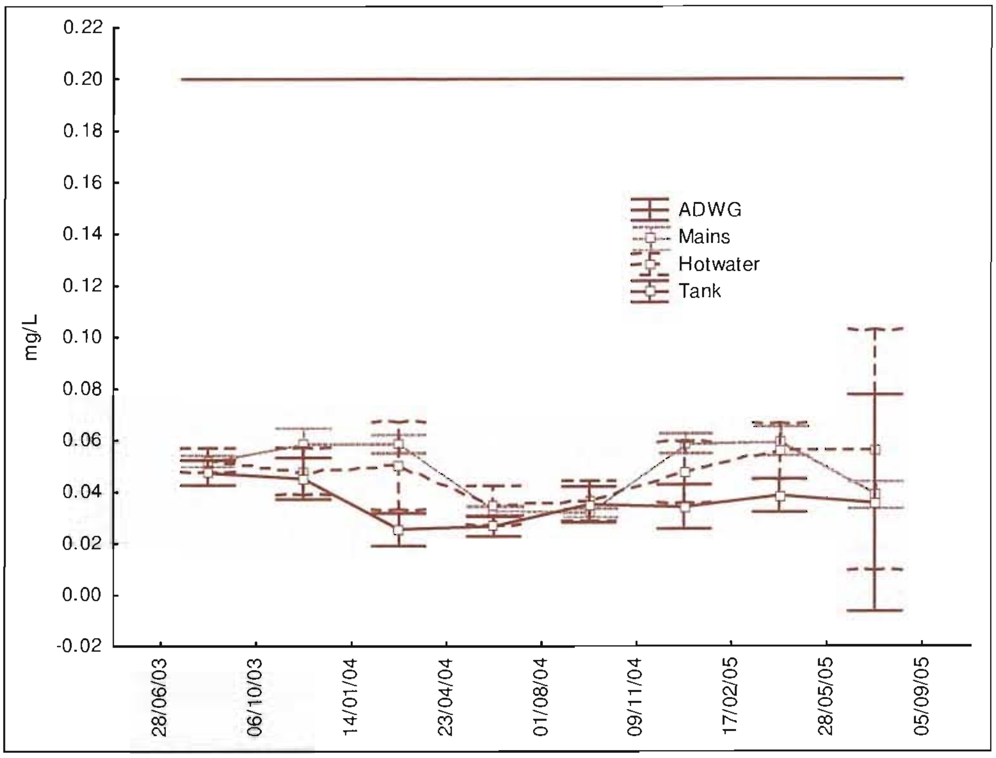


Figure 8-10: Average Aluminium concentrations in Rainwater tanks, Mains and Solar HWS compared against ADWG.

Copper concentrations were consistently higher in hotwater samples than rainwater tank samples, shown in Figure 8.11. This is to be expected as heating elements of hotwater systems are typically composed of copper. The levels of copper leaching from the hotwater system were well within expected background levels (<1mg/L) and as such did not indicate accelerated corrosion (Personal Communication, Dr David Nicholas, Manager – Corrosion Consulting, Hunter Water Australia - nicholasd@labs.hwa.com.au).

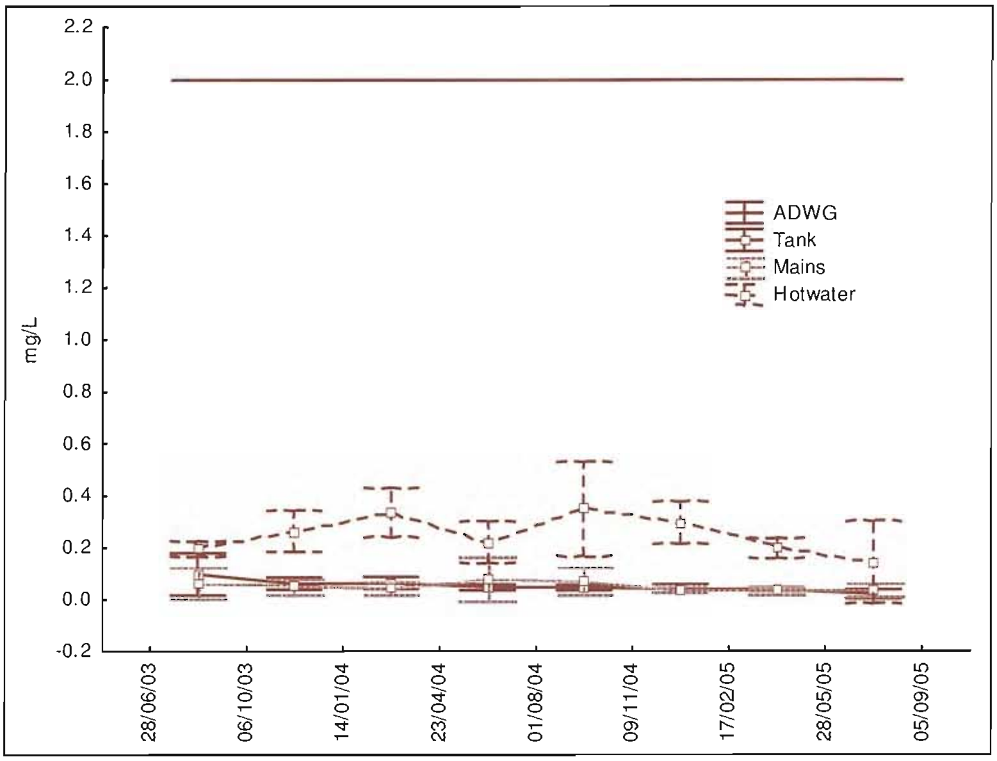


Figure 8-11: Average Copper concentrations in Rainwater tanks, Mains and Solar HWS compared against ADWG

Lead and nickel levels were often higher in hotwater samples than rainwater samples and at times exceeded the ADWG limits of 10 and 20 µg/L, respectively (Figures 8.2 and 8.12). Consistently high levels of lead were only found in a limited number of solar hotwater systems, possibly due to soldering within the pipework. While this is probably not indicative of the degenerating health of the HWS, it does suggest that people should not routinely drink large amounts of water (>1L/d) from the hotwater tap, whether the source of water is rain or municipal supplies, although this seems unlikely given typical water use trends.

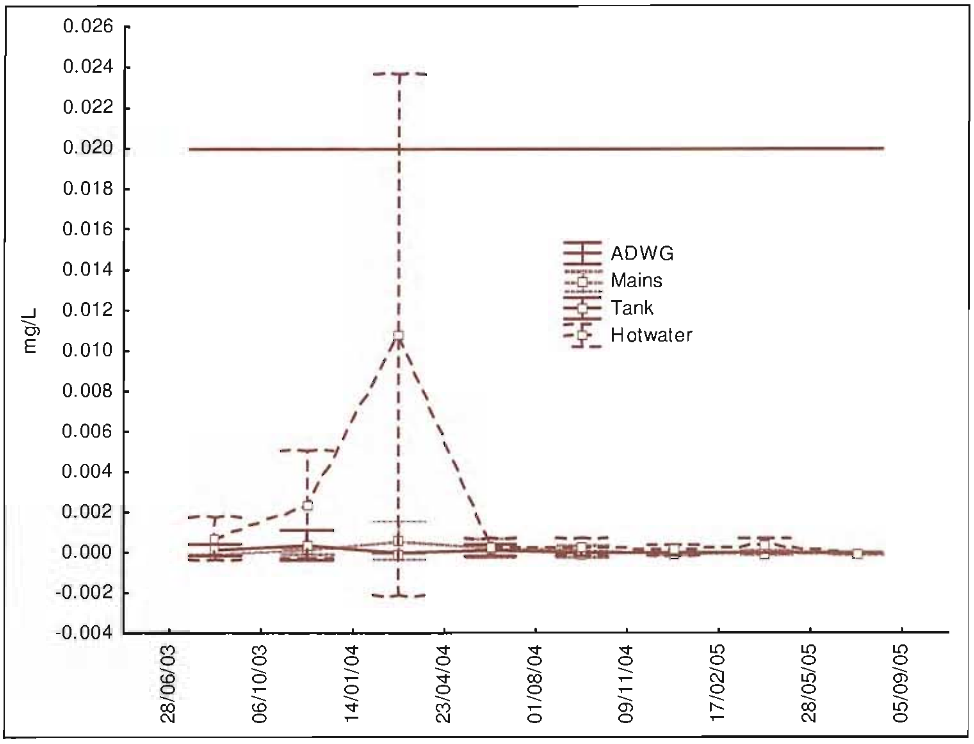


Figure 8-12: Average Nickel concentrations in Rainwater Tanks, Mains and Hotwater compared against the ADWG

One complicating factor is the concentration difference of dissolved solids between rainwater and mains water. Harvested rainwater generally contains low concentrations of dissolved solids, and therefore has a low electrical conductivity. In such environments, anodes with high electrical outputs are required to 'drive' the corrosion circuit toward the sacrificial anode, and hence confer protection. However, the electrical conductivity of mains water is much higher, and therefore requires anodes with a relatively low electrical output. It is suspected that during the switch from rainwater to mains water the high-output anode designed for rainwater will drive the sacrificial process at an accelerated rate, resulting in the rapid degeneration of the anode and subsequently the HWS. As no commercially available anode has, as yet, been designed to handle both water sources, the resolution to this problem has been left balancing on the results of experimental observation.

The investigation of an instantaneous hotwater system supplied by a combination of mains and rainwater by Coombes *et al.* (2003) also did not indicate significant corrosion of the HWS. Instantaneous HWS do not include a storage tank and hence contact time between the water and the hotwater system pipes is minimised. The pipework of the particular instantaneous HWS under investigation did not appear to be leaching into the hotwater. The levels of dissolved solids, lead, iron, zinc and cadmium were higher in rainwater tank samples than the associated hotwater samples (Table 8.16) indicating the absence of corrosion.

Table 8-16: Metal Concentrations in Rainwater supplying Instantaneous HWS

	RWT Average	HWS Average	Guideline
pH	5.7	5.5	6.5-8.5
Dissolved Solids (mg/L)	67.3	15.8	500
Lead (mg/L)	<0.01	<0.01	0.01
Iron (mg/L)	<0.06	<0.06	0.3
Zinc (mg/L)	3.90	3.90	3
Cadmium (mg/L)	<0.002	<0.002	0.002

Coombes *et al.*, 2003

It is not definitively known whether the use of rainwater in hotwater systems increases the rates of corrosion, or whether the standard M2 hotwater system anodes are adequate to protect hotwater systems against both rainwater and mains water containing differing pH and TDS levels. However, the results of this project and the available literature do not indicate that accelerated corrosion of hotwater systems commonly occurs beyond that which occurs through the use of only mains water.

8.9 Conclusions

The results of field sampling of solar and storage hotwater systems showed that the microbial quality of harvested rainwaters is greatly improved when passed through domestic hotwater systems. Substantial reductions in the concentrations of *E. coli*, thermotolerant coliforms, total coliforms, *Pseudomonas* and HPC were achieved through both the solar-powered and storage hotwater systems. Hotwater systems in the Brisbane and Newcastle/Central Coast studies operating at or above 60°C produced waters of comparable quality to chlorine disinfected municipal supplies. All hotwater systems operating below this temperature were still in total compliance with bathing water guidelines for microbial parameters.

Domestic hotwater systems achieved disinfection of harvested rainwaters though did not result in sterilisation. Spore-forming *Bacillus* sp., particularly *B. cereus*, and to a lesser extent *Stenotrophomonas* sp., were commonly isolated from hotwater systems and survived laboratory heating regimes. Thermal stress imparted by domestic hotwater systems cannot be expected to inactivate bacterial spores. However, spore-forming species do not pose significant public health risk and are rarely implicated as the aetiological agents in waterborne illness.

Laboratory thermal destruction experiments conducted on a range of indicator and pathogenic bacteria demonstrated significant reductions in all species at temperatures relevant to domestic HWS. The largest differences in heat resistance between species were observed at 55°C, with *E. faecalis* demonstrating the greatest heat resistance. The increase in temperature by 5°C to D_{60} resulted in a substantial reduction of heat resistance capacity in all species, with eight- to twelve-fold reductions in D-values. Exposure to heat at 65°C resulted in D-values of 7–19 seconds for *E. faecalis* and less than 6 seconds for all other tested organisms. The effects of decreasing growth temperature of *S. typhimurium* and multi-nutrient starvation of *E. coli* O3:H6, *P. aeruginosa* and *S. typhimurium* prior to heat treatment resulted in decreased heat resistance for these bacteria. The results of this study suggested that the temperature range from 55°C to 65°C was effective in the elimination of enteric/pathogenic bacterial components and supported the thesis that hotwater systems should operate at a minimum of 60°C.

Corrosion of the sacrificial anodes and HWS did not appear to be occurring at accelerated rates within the investigated HWS. However, this is a complicated issue, and ongoing observation and experimentation would be beneficial in elucidating some of the corrosion processes occurring within HWS supplied by both harvested rainwater and municipal water. Development of a commercially available anode capable of protecting against multiple water sources would be an ideal solution.

-------- SECTION III --------

Health Risk

CHAPTER 9

Health Risk & Secondary Use Guidelines

9.1 Introduction

In sections I and II, water quality was examined in a range of rainwater harvesting systems and incidental treatment mechanisms within the treatment train were investigated. However, water quality in itself is not the ultimate interest of water quality investigations. The basis of all water quality investigations relates to the health and comfort of the human users or the environment.

In water supply systems, water quality is a surrogate measure for human health with water quality guidelines acting to translate health risks into assessable water quality criteria. In other words, if a water source was found to be uncompliant with a given set of water quality guidelines and subsequently proven through epidemiology to not be causing elevated rates of illness, then the study would not be demonstrating 'poor' water quality but would be highlighting the use of inappropriate guidelines. This is to the detriment of optimal urban water management and leads to a less sustainable set of management solutions.

Therefore, in evaluating the appropriateness of particular water quality guidelines it is essential to incorporate as much relevant epidemiological data as possible within the scope of the intended use of the water resource. The aims of this chapter were therefore three-fold. Firstly, to evaluate health risks from the use of harvested rainwater compared to municipal water supplies based on a review of pathogen transmission pathways and published outbreak data. Secondly, to review the adequacy of currently existing water quality guidelines (including drinking, bathing and greywater reuse guidelines). And finally, to evaluate the health risks posed by the use of roof-harvested rainwater waters for non-drinking purposes and to propose a set of secondary-use water quality guidelines.

9.2 Drinking water epidemiology

9.2.1 Pathogen Transmission Pathways

At present, the contamination of a water supply by pathogenic organisms is typically measured as a potential, rather than an observed, presence. The major surrogate measure for pathogenic organisms has been thermotolerant coliform bacteria, and specifically *E. coli*. However, it has been increasingly found that bacterial index organisms do not accurately indicate the presence of protozoan or viral pathogens and often not even bacterial pathogens (Ashbolt *et al.*, 1997). Consequently new index organisms have been proposed such as the spore-forming *Clostridium perfringens* for protozoan pathogens and bacteriophages for viral contaminants. However, many drinking water guidelines still operate on a pass/fail system primarily according to the absence/presence of *E. coli* or thermotolerant coliform bacteria. This standard is a simple and convenient test, however, there may be significant variations in the degree of correlation between the presence of thermotolerant coliforms and the presence of pathogenic microorganisms for different water supplies.

Hazard Analysis and Critical Control Point (HACCP) is an analytical tool used widely in the food industry as a method for identifying and managing specific points of concern in the processing of consumables. Since the Sydney water crisis of 1997, it has been increasingly adopted by the Australian water industry (Deere and Davison, 2004). In essence, the HACCP approach focuses primarily on the prevention of contamination and understanding its upstream sources. In contrast, the traditional approach has focussed on the treatment of contamination and monitoring downstream water quality. One of the first steps in HACCP management is surveying the water supply catchments for potential sources of pathogenic contamination. Municipal water suppliers typically draw water from surface waters, such as rivers, lakes, or dams, or from groundwater reservoirs. These catchments are vulnerable to human, animal, and environmental contamination, with the major points of contamination including sewage treatment plants (STPs), sewer overflows, agricultural areas, and high water fowl numbers (Medema *et al.*, 2003). Sewage outfalls and agricultural runoff present significant health risks as they are the sources of high numbers of human pathogens, particularly during peak rain events (Atherholt *et al.*, 1998). An example includes the correlation between rainfall and increased levels of *Cryptosporidium* found in water supplies located within cattle grazing lands (CRC, 2004). This is due to cattle being a primary source of *Cryptosporidium* contamination (Walker *et al.*, 1998). Human sewage usually contains viable human pathogens including a number of viruses, bacteria, and protozoa (Medema *et al.*, 2003).

By applying the HACCP approach to domestic rainwater catchment systems, far fewer critical points (entry or multiplication points for pathogens) emerge. This is essentially due to the catchment consisting of only elevated roof area, with the number and type of potential points of contamination reduced to small mammals, reptiles, birds, insects, leaf debris and dust. A variety of bacteria, including *Klebsiella*, *Aeromonas*, *Campylobacter*, *Pseudomonas*, *Legionella*, *Yersinia*, and *Salmonella* are known to have at least one environmental (i.e. soil or water), small animal, or bird reservoir (Szewzyk *et al.*, 2000). However, according to Cunliffe (1998), the transfer of pathogens via small animals, reptiles, amphibians and birds is considered to be less hazardous than contamination from human faeces due to the lower occurrence of human pathogens being carried by these animals.

9.2.2 Disease Outbreaks from Tank Supplies

Despite the fact that untreated harvested rainwater generally does not meet drinking water standards, many people still rely on it for drinking and maintain regular health (Gould, 1999). This is because contamination does not automatically mean sickness. The purpose of water quality monitoring is to assess and minimise potential impact on human health. Water quality data alone should not be substituted for what is of real interest – human health. Millions of people around the world depend on rainwater for all domestic purposes and the number of reported cases of serious illness is very low (Gould, 1999). Furthermore, the concept of acceptable risk must incorporate the cost of increased risk of illness in future generations due to unsustainable water management practices by the current generation.

Many of the studies reporting the isolation of pathogens from rainwater tanks note a lack of illness amongst the water users who drink the tankwater (e.g. Tuffley & Holbeche, 1980). Moreover, potentially carcinogenic disinfection by-products (DBPs) would not occur, or at least in much lower concentrations, in tank water.

However, sometimes illness does occur. An outbreak of Salmonellosis was reported in Trinidad in 1976 amongst 83 members of a camp. The roof of the facility was heavily contaminated with bird faeces and rainwater tanks were subsequently suspected as the cause (Koplan *et al.*, 1978). A more recent outbreak of Salmonellosis was reported amongst 28 construction workers in Australia, with the outbreak attributed to a poorly maintained rainwater tank (Taylor *et al.*, 2000). A small outbreak of infant botulism occurred in 1980-81 in rural Australia, during which *Clostridium botulinum* was isolated from soil, vacuum cleaner dust, and the sludge of rainwater tanks at several sites (Murrell & Stewart, 1983). The use of harvested rainwater was found to be one of several potential minor causes for an increase in notification rates of camplyobacteriosis in New Zealand, although improperly cooked chicken was found to be the major cause of the increase (Eberhart-Phillips *et al.*, 1997). *Campylobacter fetus* isolated from a rainwater tank was attributed as the source of infection in a 64 year old immunocompromised chemotherapy patient (Brodribb *et al.*, 1995). Other cases of illness attributed to the consumption of harvested rainwater are found sporadically throughout the literature generally involving very few people. To the authors' knowledge, all reports of illness have resulted from direct ingestion of contaminated rainwater rather than secondary uses such as hotwater, toilet flushing, laundry or outdoor.

9.2.3 Centralised versus De-centralised Risk

Centralised water supply systems have the disadvantage that contamination of the system means a health risk for all users. A single breakthrough of pathogens, such as the Milwaukee *Cryptosporidium* outbreak, may result in widespread illness and death. In Milwaukee, a single incident resulted in the infection of approximately 400,000 people with approximately 100 deaths (MacKenzie *et al.*, 1994). A framework for risk assessment was presented by Davison *et al:* (2002) where weightings are based on frequency and severity of infection, as seen in Table 9.1.

While outbreaks from centralised water supplies may not occur often, they are restricted to higher weightings for significance due to the potential to infect large numbers of people with a wider range of human pathogens. Conversely, decentralised systems usually supply less than 5 people, resulting in a low weighting for threats to population. This is because while a greater number of rainwater harvesting systems equates to a greater number of potentially failing systems, the likelihood of enough systems failing at a given time to threaten the same population at risk from a single incident in a centralised water system becomes increasingly remote as the population increases. As seen from the literature, reported cases of infection from harvested rainwater are sporadic and not widespread.

Table 9-1: Descriptive weightings for risk assessment

Item	Definition	Weighting
Almost certain	Once a day	5
Likely	Once per week	4
Moderate	Once per month	3
Unlikely	Once per year	2
Rare	Once every five years	1
Catastrophic	Potentially lethal to large population	5
Major	Potentially lethal to small population	4
Moderate	Potentially harmful to large population	3
Minor	Potentially harmful to small population	2
Insignificant	No impact or not detectable	1

(Davison *et al.*, 2002)

The total number of cases of illness associated with drinking harvested rainwater in published literature is less than one thousand. This is certainly not the total number of cases, as many cases go unreported. However, despite these sporadic cases, epidemics caused by contaminated rainwater systems are extremely unlikely given the lack of suitable vectors capable of causing significant widespread contamination. The combined number of reported cases of illness from rainwater tanks worldwide is less than 1000. While municipal water supplies in developed countries typically supply a greater proportion of the total population than rainwater tanks, the high severity of risk from a single mains water outbreak can cause significantly more illness than rainwater tanks, illustrated by the outbreak of *Cryptosporidium* in Milwaukee in 1994 where approximately 400,000 fell ill. This suggests that the higher severity of centralised risk may outweigh the high frequency of decentralised risk associated with harvested rainwater use.

The most comprehensive way to assess the risks associated with rainwater sources is to couple epidemiological studies with ecological data, namely water quality (Lye, 2002). This is rarely done, although in one instance, Kuberski (1980) combined rainwater tank surveillance and epidemiology to demonstrate that rainwater tanks were not the cause of a spread of cholera. Within Australia, a significant epidemiology study was undertaken with 965 participants drinking harvested rainwater, although unfortunately no water quality data was collected (Heyworth *et al.*, 2006). The results found that there was no statistical difference in rates of highly credible gastrointestinal illness amongst the children drinking rainwater compared to the control group drinking mains water.

These health risk evaluations have been conducted on the assumption that harvested rainwaters are intended for drinking purposes. As explained in chapter 1, the negligible proportion of domestic water supply used for drinking (<2%) means that drinking from rainwater tanks is not required for tanks to have a substantial beneficial impact on urban water cycle management. Obviously the health risks posed when tank supplies are used only for secondary purposes will be significantly lower. By utilising tank supplies for non-drinking purposes only, the threat posed by enteric pathogens, which comprise the major group of human pathogen which are required to be ingested for infection to occur, are realistically eliminated. The health risks posed by the remaining range of pathogens, such as respiratory, skin and opportunistic pathogens, are significantly more limited.

9.3 Current Water Quality Guidelines

There are a variety of currently endorsed water quality guidelines covering uses such as drinking, swimming and bathing, recreational water uses and recycling and re-use applications. However, there are currently no officially endorsed guidelines for harvested rainwaters. This is primarily due to the fact that domestic RWH systems are private systems and that the majority of people who use rainwater for drinking purposes do not have a suitable alternative supply to draw from if the guidelines show the tank waters to be of poor quality. The de-centralised distribution of RWH systems also means that the widespread monitoring and regulation of these systems is not practically achievable.

9.3.1 WHO, Australian & NSW Guidelines

However, the use of rainwater tanks is being increasingly encouraged in urban areas where alternative water supplies exist and the assessment of qualities of these waters is therefore becoming increasingly important. Currently in Australia, a variety of authorities have taken responsibility for the development of water quality guidelines and recommendations, including international (WHO – drinking and recreational water guidelines), national (Australian – drinking and recreational water guidelines) and state (NSW – greywater reuse) authorities. Before new guidelines be proposed, it is necessary to review the current guideline framework in order to identify areas of potential extrapolation and areas of where new guidelines are required.

9.3.1.1 Drinking Water Guidelines (ADWG & WHO)

The WHO Guidelines for Drinking Water Quality (WHO-GDWQ, 2004) are the internationally accepted set of guidelines for drinking water supplies. The WHO guidelines are designed to provide guidance for countries developing their own national drinking water quality strategy and act as a surrogate for nations that have not yet developed such guidelines. The Australian Drinking Water Guidelines (ADWG, 2004) are predominantly aligned with the WHO-GDWQ, and where discrepancies arise the ADWG have generally specified a higher water quality standard. The microbial requirements of the Australian Drinking Water Guidelines are the same and are based on the use of coliform indicator organisms, with the guideline values presented in Table 9.2.

Table 9-2: Australian Drinking Water Guidelines

	ADWG
E. coli	0 CFU (>98%)
Thermotolerant coliforms	0 CFU (>98%)

The nature of drinking water guidelines assumes that the presence of coliform bacteria equates to the presence of pathogens. Furthermore, they do not incorporate minimum infective doses which for most enteric bacterial pathogens are greater than 10 000 viable organisms, though for viruses and protozoa are often lower. Given that the excreted concentrations of *E. coli* are usually significantly greater than those of pathogens, the detection of a coliform or *E. coli* organism in a water sample is still far from confirming the water supply as a public health threat. Within the context of centralised water supplies, however, the availability of treatment facilities and the significance of mains water supplies for public health mean that it is necessary to have conservative guidelines capable of protecting the health of the most vulnerable users, including the young, elderly and immunocompromised.

However, the application of drinking water guidelines to urban RWH systems is inappropriate for two main reasons. Firstly, harvested rainwater is not advocated for drinking and therefore should not be scrutinized by the stringency of drinking water guidelines. Secondly, as discussed in section 9.1.1, rainwater harvesting systems are not vulnerable to the high pathogen loading sources of faecal contamination that surface waters are vulnerable to, namely human and cattle faeces, and hence the detection of coliform or *E. coli* organisms in tank waters are less likely to be associated with the presence of human pathogens. However, due to the lack of specific secondary use guidelines, drinking water guidelines are often used to evaluate urban tankwater quality.

9.3.1.2 Bathing Water Guidelines (GRWQA & WHO)

The Guidelines for Recreational Water Quality and Aesthetics (Australia/New Zealand) include two main categories of water quality requirements based on the extent of human contact. The highest quality waters are termed 'primary contact waters' which include those used for swimming, bathing, surfing and direct contact water sports. The guidelines explicitly assume that during interaction with primary contact waters, human users will be submerged in the water and will accidentally ingest small volumes. 'Secondary contact waters' are those termed to be used for activities with less human contact, such as boating and fishing. This category assumes that humans may come in contact with the water though are not intending to be submerged in it. The microbial standards set for these two categories are stated in Table 9.3. WHO have also published Guidelines for Safe Recreational Water Environments (Vol. 1 Coastal and Fresh Waters, 2003). However, the WHO guidelines do not prescribe maximum allowable concentration limits for specific contaminants but describe four categories of water quality based on Enterococci concentrations.

The bathing water guidelines hold relevance for rainwater tank and hotwater supplies intended for showering purposes. While the GRWQA (primary contact waters) appear to be the most relevant guidelines within the current guideline framework for assessing showering waters, they could still be considered as conservative in their application to tank supplies. The microbial standards in the GRWQA were developed primarily from a large amount of epidemiological data from coastal and freshwater areas, including beaches, lakes and rivers (Pruss, 1998). These surface waters are vulnerable to a far more extensive range of contaminant sources than harvested rainwaters, including stormwater runoff, ocean-discharged sewage effluent and faecal contamination from users. As discussed in section 9.1.1, roof-harvested tank waters are not vulnerable to the two most significant sources of human pathogens (i.e. human and cattle faecal contamination) to which many recreational waters are vulnerable. Consequently, the range and concentration of pathogens in tank waters are likely to be lower than in surface waters, which implies the detection of thermotolerant coliforms or enterococci in tank waters are less likely to be associated with the presence of human pathogens.

Table 9-3: The Australian Guideliues for Recreational Water Quality and Aesthetics for the subsection of Primary Contact waters (including bathing and swimming waters)

GRWQA	Thermotolerant coliforms	Enterococci
Primary Contact	Median* <150CFU/100mL & >80% of samples <600CFU/100mL	Median* <35CFU/100mL & Maximum of 100CFU/100mL
Secondary Contact	Median* <1000CFU/100mL & >80% of samples <4000CFU/100mL	Median* <230CFU/100mL & Maximum of 700CFU/100mL

*Median value from a minimum of five samples.

Moreover, the level of exposure to shower waters can be controlled to a greater degree than during swimming and bathing. Within a shower or bath the likelihood of accidental ingestion is significantly lower than during recreational water activities such as beach or pool swimming. Users of public swimming pools and beaches frequently gulp water, expose their eyes to direct contact with the water, and acquire water in their sinus system. The degree of immersion and direct body contact is significantly lower in showers and baths, with the extent of contact between the water and eyes/ears/nose able to be controlled and limited by the user. Therefore, the standards set by the GRWQA (primary contact waters) offer microbial limits that should satisfy a conservative health risk approach to tank waters intended for showering purposes, and are the most appropriate of the currently accepted guidelines to be applied to heated rainwater supplies.

9.3.1.3 Greywater Reuse Recommendations (NSW Health)

Within NSW, Australia, water quality recommendations have been developed for the reuse of domestic greywater (NSW Health, 2000). The recommendations 'Greywater Reuse in Sewered Single Domestic Premises' were intended to provide advice on greywater recycling rather than official guidelines. Three main categories of reuse were defined in the recommendations, each requiring the recycled greywaters to be treated to different standards. The highest category of use includes applications for toilet flushing and laundry use, with the required water quality for such uses specified in Table 9.4.

Table 9-4: Microbial and physicochemical standards for the reuse of domestic greywater for toilet flushing, laundry and irrigation

Parameter	NSW Health (maximum)
Thermotolerant coliforms	$\leq$ 10 CFU/100mL
BOD_5	$\leq$ 20 mg/L
Suspended Solids	$\leq$ 30 mg/L

The application of the greywater quality recommendations could potentially be applied to tank waters intended for corresponding uses (i.e. gardening, toilet flushing, laundry). However, major inconsistencies appear between the three sets of guidelines presented here. It would be logical to express the order of water uses, in terms of human contact and potential disease transmission, as drinking waters as the highest order use, followed by bathing waters and finally water intended for toilet/laundry use. However, the thermotolerant coliform limit recommended by NSW Health of $\leq$ 10 CFU/100mL is significantly lower than that prescribed by the GRWQA (primary contact waters) of a maximum median value of 150 CFU/100mL. Given this discrepancy, it would be unreasonable to apply a higher water quality standard to waters intended for toilet flushing than to waters intended for bathing or showering purposes.

9.3.2 Proposed Rainwater Tank Guidelines

In 1993 formal rainwater tank guidelines were proposed by a leading technical group (J. Hari Krishna, Dennis Lye and Roger Fujioka) through the International Rainwater Catchment Systems Association (IRCSA). These guidelines included water quality standards (Krishna, 1993) as well as eight maintenance standards for rainwater harvesting systems (Fujioka, 1993).

9.3.2.1 Water Quality Guidelines

The microbial water quality standards for harvested rainwaters were set out in *Water Quality Standards for Rainwater Cistern Systems* (Krishna, 1993). These standards categorised tank waters into three classes of quality based on the concentrations of thermotolerant coliforms, summarised in Table 9.5. Class I waters are the highest quality waters, complying with current drinking water standards. It was acknowledged that a range of environmental and non-human sources of bacterial contamination may enter tanks and that tank waters do not often meet the class I standard. Class II waters were therefore proposed as also being suitable for drinking purposes. Class III waters are the lowest quality waters and not considered suitable for drinking.

The principle objective of these guidelines is to distinguish between waters that are acceptable and unacceptable for drinking purposes. Class III waters, implied to be acceptable for non-drinking purposes, do not specify recommended quality limits for secondary uses of such waters. The suitability of harvested rainwaters for uses such as showering are therefore not able to be determined through these guidelines, limiting their applicability to urban harvested rainwater supplies. Furthermore, these guidelines have had limited formal acknowledgement and have not been officially adopted within Australia.

Table 9-5: Proposed drinking water quality standards for harvested rainwater

Class	Thermotolerant coliforms (CFU/100mL)	Recommended Use
Class I	0	Drinking
Class II	1–10	Drinking
Class III	>10	Non-drinking

9.3.2.2 Maintenance Guidelines

Guidelines defining appropriate design and maintenance factors were also proposed at the same time as the water quality standards. Eight guidelines are identified within

Guidelines and Microbial Standards for Cistern Waters (Fujioka, 1993) published by IRCSA, summarised in Table 9.6. Although these guidelines have been formally proposed, they have not been formally adopted by the Australian government.

Within Australia, enHealth have published the most recent set of design and maintenance guidelines for rainwater harvesting systems (enHealth, 2004). These guidelines are similar to those proposed by Krishna (1993) and have been adopted by the Australian water industry. Both of these guidelines provide clear and beneficial advice on how to maximise tank water quality and would make appropriate exclusive guidelines for people who rely on harvested rainwaters as their primary water supply. However, in urban centres where mains water is available, the choice of supply and level of appropriate use must be made on the basis of water quality considerations. In this regard, these design and maintenance guidelines are beneficial though do not enable an assessment to be made of the appropriateness of utilising harvested rainwaters for various secondary uses.

Table 9-6: Guidelines for the design and maintenance of rainwater catchment systems

Cistern Maintenance and Design Guidelines
Guideline 1 – Roofs of structures should be of a smooth material to prevent entrapment of contaminants on the roof. Rainwater flowing off smooth roofs will carry less contaminants into cisterns.
Guideline 2 – Prevent trees from overhanging the house. Establish a programme of cleaning out gutters and washing the roof.
Guideline 3 – Use roof washers or foul-flush devices.
Guideline 4 – Water from the roof should be screened and filtered before it reaches the cistern.
Guideline 5 – Cisterns should be sealed to keep out sunlight and contaminants such as insects (mosquitoes), reptiles (lizards) and animals (birds) from entering them.
Guideline 6 – Cisterns should be periodically cleaned.
Guideline 7 – Water in the cistern should be periodically disinfected.
Guideline 8 – Cistern waters should be monitored for drinking-water quality and evaluated against the drinking-water standards.

9.3.3 Inadequacies of Current Guideline Framework

The inadequacies of the current guideline framework that is applied to harvested urban rainwaters can be summarised in two points. Firstly, many of the current guidelines (including ADWG, WHO–GDWQ and the guidelines published by enHealth and IRCSA) address water quality requirements for drinking waters only. The health risks assumed by non-drinking purposes compared to drinking purposes are relatively minor and have not been seen to warrant specific mention in these guidelines. And secondly, the guidelines referring to non-drinking uses (including GRWQA and the NSW Health greywater reuse recommendations) have been designed to incorporate risks posed by significant human and other faecal contamination sources. Guidelines for secondary use of harvested rainwaters need to acknowledge both the non-drinking intention of such waters along with the lower association of thermotolerant coliforms to human pathogens resulting from the restricted possibility of human or carnivore faecal contamination. The following section identifies components of the existing guideline framework that are suitable for assessment of tank waters intended for particular secondary uses and proposes new water quality standards where there are no appropriate current guidelines.

9.4 Proposed Secondary Use Guidelines for Harvested Rainwater

In response to the deficiency within the current water quality guideline framework, a set of water quality guidelines have been proposed for harvested rainwaters. The proposed guidelines specify different water quality requirements corresponding to the level of intended use identified within the four categories of water use.

9.4.1 Non-drinking Uses

The major limitation within the current guideline framework is the lack of suitable non-drinking water quality guidelines. As such, the three categories of secondary use guidelines proposed here are the major focus of this chapter. A fourth category is proposed for harvested rainwaters intended for drinking purposes, though at this point in time insufficient data is available to propose adequately informed guidelines.

Recommendations are made for the types of further research required to provide the data necessary to develop appropriate guidelines.

9.4.1.1 Category 1 – Amenities Supplies

The first category of non-drinking usage is termed here 'amenities supplies'. This is usage which requires no direct contact with water and includes washing clothes or dishes, toilet flushing and outdoor garden watering. Human exposure is generally therefore limited to indirect contact (touching an object which has had contact with the tank water) and inhalation of water aerosols with direct human contact being infrequent. However, incorporated into the development of this guidelines is the expectation that direct contact and ingestion, although not intended uses of this supply, are likely to occasionally occur as a result of children using the garden tap.

9.4.1.1.1 Human Exposure and Health Risk

Indirect contact facilitates the most limited contact with harvested rainwater and therefore poses the lowest levels of health risk to the user. No reported cases of illness have been identified that have been a result of using contaminated rainwaters for the indirect contact uses defined above. The possibility of disease transmission through contact with objects such as dishes and clothing that have been washed in tank waters is considered here to be negligible. The limited number of viable organisms remaining after desiccation on the dried object and the limited opportunity for ingestion or exposure to wounds negates this pathway as a feasible route of disease transmission.

The inhalation of bioaerosoles produced during outdoor garden watering or toilet flushing may occur to a limited degree. The major pathogen of concern for respiratory infection from water supplies is *Legionella pneumophila* which are found in many man-made water systems. For these bacteria to cause illness they require water supplies to be held at temperatures above those encountered in rainwater tanks (40 – 45°C) in order to facilitate multiplication to concentrations high enough to cause infection. The restricted growth of these organisms by temperature limitations as well as the low probability of significant inhalation of contaminated bioaerosoles produced during gardening or toilet flushing excludes these uses as realistic routes of Legionellosis transmission. A number of other opportunistic pathogens commonly found in tank waters, such as *Klebsiella* and *Pseudomonas*, have also been known to cause clinical respiratory infections. However, due to the ubiquitous distribution of these bacteria in the environment and the regular inhalation of contaminated aerosols and dust particles by all people, the contribution of contaminated bio-aerosols produced from tank waters also appears to be negligible.

It is conceivable that ingestion of water from garden taps (for example by children) may occur which would significantly raise the risk of illness. However, the use of tank waters for drinking purposes is not being recommended and the regular or deliberate use of tank waters for such purposes should require the water supply to be compliant with drinking standards. Concern over the occurrence of this scenario may require the installation of signage to identify the source of the water supply on relevant outdoor taps.

The most limiting factor of the use of indirect-contact waters is probably the aesthetic quality. Waters with objectionable odour or colour will generally not be considered appropriate for cleaning purposes. The presence of high concentrations of iron (Fe^{+3}) may also contribute to colouration and the staining of clothes.
Waters used for garden watering must also not pose eco-toxic or environmental health risks. Levels of some heavy metals may accumulate in plants and some viruses and bacteria are capable of causing infection in plants. Particular care needs to be taken when the tank water is used for irrigating fruits and vegetables, particularly root crops, which are intended for human consumption. Salt levels in irrigation waters have also been known to cause problems concentrations exceed the soil sodium adsorption capacity, although the risk of this from tank waters would be negligible given that tank water usual has a lower TDS than municipal water supplies.

9.4.1.1.2 Proposed Guideline

The proposed microbial and physicochemical guidelines for harvested rainwaters used for 'amenities supplies' are presented in Table 9.7. The microbial limits, based on *E. coli* and enterococci, were adapted from the 'Primary Contact' category of the GRWQA. These guidelines are applied to waters intended for uses, such as swimming and surfing, where full emersion in the water is intended or extremely likely. Although 'amenities supplies' are defined as not being intended for human contact, the application of the 'Primary Contact' microbial standards was considered suitable for tank water used for outdoor purposes including hosing and sprinkler systems given that occasional contact or ingestion of the water is probable albeit generally limited.

Heavy metal (lead, cadmium, arsenic) limits were adapted from the 'Short Term Trigger Values' for irrigation waters from Chapter 4 (Primary Industries) of the Australian and New Zealand Guidelines for Fresh and Marine Water Quality Primary industry (2000). Sodium limits were also adapted from these guidelines from the upper concentration limit stated to cause foliar damage to 'Moderately Sensitive' crops. The heavy metal and sodium limits in these guidelines apply to waters used for commercial irrigation of food crops intended for human consumption. They were therefore determined to be suitable for application to harvested rainwaters used for the watering of garden fruits and vegetables that may be eaten.

Table 9-7: Proposed Harvested Rainwater Quality Guidelines for Waters Intended for Amenities Supplies (Outdoor, toilet flushing, laundry)

Parameter	Guideline Value
E. coli	Median* <150 CFU/100mL & >80% of samples <600CFU/100mL
OR	
Enterococci	Median* <35 CFU/100mL & Maximum 100 CFU/100mL
Lead	5 mg/L
Cadmium	0.05 mg/L
Arsenic	2 mg/L
Iron	1 mg/L
BOD_5	20 mg/L
SS	30 mg/L
Sodium	230 mg/L

* Median value from a minimum of five samples

The aesthetic parameters of five-day biochemical oxygen demand (BOD_5) and suspended solids (SS) were adapted from the greywater reuse recommendations for waters intended for laundry and toilet flushing. While the probability of harvested rainwaters containing high concentrations of BOD5 or SS is low, the use of these guidelines may help identify problematic systems that produce waters not appropriate for toilet flushing and laundry use.

The iron concentration was adapted from the aesthetic guideline value for iron in drinking water supplies in the ADWG. The ADWG guideline for iron is 0.3mg/L, which is defined as the minimum concentration at which iron can be tasted in water and at which precipitation of iron may occur. Iron concentrations of 3mg/L are generally thought to be objectionable. The proposed iron guideline here is based on the visual quality of the water.

9.4.1.2 Category 2 – Showering Supplies

The second category of non-drinking usage is termed here 'showering supplies'. This usage involves a substantial degree of direct contact between the user and the water and includes showering and bathing applications. The level of exposure is much greater than for indirect contact uses of amenities supplies and although these waters are not intended for drinking the chance of ingestion is also higher than for indirect contact waters.

9.4.1.2.1 Human Exposure and Health Risk

The primary mode of exposure to showering supplies is through direct contact during showering and bathing. During these uses, a substantial degree of superficial contact is made with certain exposure of the skin to water contaminants. There is also a high probability of exposure to the eyes, ear cavities, nose, mouth and throat although the degree of exposure will be less than during full immersion activities such as swimming. Direct contact exposure to rainwaters is generally not considered to be a significant threat unless the user is severely immunocompromised or has physical ailments such as serious skin burns or damaged eyes. In these cases, opportunistic pathogens may have the potential to cause secondary infections. The likelihood of skin infections will be greater during bathing than showering due to greater contact time between the user and the bacterial populations if sufficient concentrations are present. As shown in the previous chapter, significant thermal inactivation is achieved in standard domestic hotwater systems and the threat posed by opportunistic pathogens, even to immunocompromised people, will be low.

Inhalation of aerosolised water particles also occurs during showering. A number of species have been associated with respiratory tract infections, such as *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*, though the most common and serious of these is *L. pneumophila*. The required number of *Legionella*-contaminated bioaerosols to be inhaled for infection to occur is largely unknown, though a number of cases have been reported where contaminated shower supplies (non-rainwater) from hotwater systems operating below 55°C have lead to Legionellosis in hospital patients.

Showering supplies are not intended for consumption although there is a strong likelihood that on occasions small quantities of water may be ingested by the user. Due to the cumulative nature of non-microbial toxins and their requirement for long-term ingestion in order to cause illness, only infection from microbial contaminants may be considered a feasible health threat. The likelihood of illness will depend on the dose ingested, though from a typical mouthful containing 20–100mL the risk of infection will be lower than expected if compared to ADWQ standards which assume ingestion of 1L.

9.4.1.2.2 Proposed Guideline

The proposed microbial and physicochemical guidelines for harvested rainwaters used for 'showering supplies' are presented in Table 9.8. The microbial and heavy metal guidelines were adapted from the 'Primary Contact' category of the GRWQA. As described above, these guidelines are applied to waters intended for uses, such as swimming and surfing, where full emersion in the water is intended or extremely likely. The application of these microbial standards was considered suitable for tank water (or domestic hotwater systems) used for showering purposes.

An operational guideline is also included for control of *L. pneumophila* in domestic hotwater systems based on the Australian Plumbing and Drainage Standards (ANZ Standards 3500.4 – Heated Water Services). This standard states that domestic hotwater systems should operate at a minimum of 55°C to inhibit the growth of *L. pneumophila*. Published thermal inactivation data for *L. pneumophila* shows that the organism is inactivated at temperatures above 55°C. Hence, in the proposed guidelines in Table 9.8, an operational temperature of 60°C is recommended with the majority of samples being above 55°C.

Table 9-8: Proposed Harvested Rainwater Quality Guidelines for Waters Intended for Showering Applications

Parameter		Guideline Value
E. coli		Median* <150 CFU/100mL &
		>80% of samples <600/100mL
	OR	
Enterococci		Median* <35 CFU/100mL &
		Maximum 100 CFU/100mL
	AND	
Legionella pneumophila		Operational temperature of HWS[a] 60°C
		75% of samples >55°C
Lead		50 µg/L
Cadmium		5 µg/L
Arsenic		50 µg/L

* Median value from a minimum of five samples [a]Applicable to hotwater storage systems only

9.4.1.3 Category 3 – Cooking Supplies

The third category of water usage is termed here 'cooking supplies' and involves consumption of heated waters. This applies to houses where the rainwater tank is used to supply the hotwater system. As household hotwater taps are all supplied from the hotwater system, the hotwater tap in the kitchen, which may be used for cooking purposes, will contain harvested rainwater. Although the practice of drinking directly from the hotwater tap is rare, it is more common for water from the hotwater tap to be used for cooking purposes. These waters are therefore ingested with the meal after a second exposure to heat stress. Many cooking methods use water during preparation, with the most typical including the boiling of vegetables, pasta, rice etc., and tea and coffee making. The amount of water absorbed into foods during cooking generally only constitutes a minor proportion of total daily water intake, though this will be higher for tea and coffee drinkers who fill their kettles from the hotwater tap.

9.4.1.3.1 Human Exposure and Health Risk

The ingestion of liquids and foods into the body exposes the enteric tract to pathogenic organisms and the body to the absorption of chemical contaminants. However, as shown extensively throughout the published literature, including chapter 8 of this thesis, thermal inactivation of pathogens occurs rapidly at temperatures exceeding 60-65°C. As temperatures used for cooking are typically in excess of 100°C, waters used in cooking will be disinfected (potentially sterilised) by the double exposure to thermal stress (HWS and cooking). The risk of infection caused by the ingestion of enteric pathogens is therefore negligible.

A more feasible health risk comes from the long-term ingestion of chemical contaminants (particularly lead) from waters used for tea and coffee making. As was shown in chapters 3, 4 and 8, lead concentrations in some rainwater tanks were found to consistently exceed ADWG limits and were exacerbated in a small number of systems as a result of leaching from the hotwater systems. The ADWG indicated that the average dietary intake of lead for an Australian adult is about 100µg per day with 80% of this intake coming from food, dirt and dust. The lead limit (10µg/L) in the ADWG is based on concentrations that do not result in increased lead retention in young children assuming the ingestion of 1L per day. Adults absorb significantly less lead than children and the ultimate importance of lead intake from water is dependent upon the age of the user and on the degree of lead intake from alternative sources.

9.4.1.3.2 Proposed Guideline

Due to the significant level of thermal stress applied to cooking waters, the threat of illness from microbial pathogens is considered negligible. Therefore a stricter microbial limit than that prescribed in the GRWQA for primary contact waters was not considered necessary. However, a stricter approach is required for heavy metal contaminants. As heavy metals are cumulative toxins, it is not the ingestion of acute doses of high concentrations of metals that is of primary importance but the total quantity of metal ingested over a long period of time (>10yrs). In other words, the daily consumption of 2L of water containing half the guideline limit will result in the same degree of risk as the daily consumption of half a litre containing twice the guideline limit.

There are number of considerations in determining suitable heavy metal guidelines for cooking applications of tank waters. The importance of lead concentrations in the hotwater is dependent upon the age of the user, with children more susceptible to lead accumulation than adults. The ways in which the hotwater is used are also important considerations, with the regular drinking of tea and coffee sourced from the hotwater tap probably the most significant mode of ingestion. Given that young children (weighing 13kg) with high rates of lead accumulation were used for the calculation of the ADWG lead limit, it is unlikely that such young children would consume significant amounts of tea or coffee on a daily basis. A further consideration is the proportion of time in which the HWS actually contain harvested rainwaters.

In the calculation of the 'cooking supplies' guidelines for heavy metals, presented in Table 9.9, a number of conservative assumptions were made including, firstly, that water from the kitchen hotwater tap was used for cooking and tea and coffee making purposes, secondly, that young children were regular consumers of this water, thirdly, that two-thirds of total daily water intake was from the hotwater tap, and fourthly, that harvested rainwater was present in the hotwater systems 50% of the time. The heavy metal guidelines for tank waters used for secondary ingestion have therefore been proposed as three times the allowable concentration stated in the ADWG for municipal drinking water supplies.

Table 9-9: Proposed Harvested Rainwater Quality Guidelines for Waters Intended for Cooking Supplies

Parameter	Guideline Value
E. coli	Median* <150 CFU/100mL &
	>80% of samples <600CFU/100mL
OR	
Enterococci	Median* <35 CFU/100mL &
	Maximum 100 CFU/100mL
Lead	30 µg/L
Cadmium	6 µg/L
Arsenic	20 µg/L
Iron	300 µg/L

* Median value from a minimum of five samples

It is important that tank owners are aware that if they connect their tank to the hotwater system then the kitchen hotwater tap will also, at times, contain harvested rainwater. It is recommended that for people regularly using tank waters for cooking purposes, a hotwater sample be analysed to determine the lead concentration at the point of use.

9.4.2 Drinking Uses

The WHO is currently developing official drinking water quality guidelines for harvested rainwaters as part of the rolling revisions incorporated within the latest editions of the WHO–GDWQA (2004). However, these guidelines are still currently being formulated and a brief outline of the health issues of drinking harvested rainwaters is therefore presented here. As has been explained throughout the thesis, the primary interest in urban rainwater harvesting systems is as a non-drinking water supply and therefore only limited attention has been paid in this thesis to issues related to drinking water health risk.

9.4.2.1 Category 4 – Drinking Supplies

In many nations the practice of rainwater harvesting provides the sole water supply for a small proportion of the population. Within Australia, approximately 11% (2.2m) of the population rely on harvested rainwater to supply their drinking water needs (ABS, 2004). The use of harvested rainwaters for drinking purposes obviously poses a greater risk of illness than the three non-drinking categories of use. The regular consumption of unheated tank waters exposes the enteric tract to microbial infection and chemical contaminants. Despite the general lack of treatment and non-compliance with drinking water guidelines, only a relatively small proportion of the disease burden from drinking waters comes from harvested rainwater supplies.

9.4.2.1.1 Human Exposure and Health Risk

The regular consumption of tank waters both increases and decreases the risk of illnesses. The utilisation of harvested rainwater as a drinking supply involves the regular ingestion of water that is not typically disinfected (Plazinska, 2001; Chapter 3). Connection of the tank to the kitchen coldwater tap also bypasses the HWS and the disinfection imparted by thermal stress. Therefore, the presence of a limited range of viable enteric pathogens is more likely to be encountered in non-disinfected tank waters than in municipal supplies.

However, the dose of a given pathogen must be above the minimum infective dose for infection to occur. The ingestion of low concentrations of pathogens results in acquired immunity enhancing the body's ability to recognise and destroy such pathogens during future inoculations. Acquired immunity does not provide protection from infection against high doses of pathogens but acts to raise the minimum infective dose required to cause illness. Hence, the regular ingestion of tank waters increases the likelihood of encountering a sufficiently high concentration of pathogens to cause illness while simultaneously providing greater general protection. The frequency of use is therefore an important variable with the risk of illness decreasing over time. This means that frequent users may be protected against concentrations that would result in illness for infrequent users.

This was perhaps a significant factor in the findings of Heyworth *et al.* (2006) where the rates of gastroenteritis were lower (though not statistically) in children drinking tank waters than those drinking mains waters. Many authors who have noted the detection of enteric indicator and pathogenic organisms in tank waters have also noted a lack of illness amongst the tank users. The use of traditional indicator organisms and even the use of pathogen presence/absence tests do not therefore appear to be adequate for use in estimating health risks from consumption of harvested rainwaters.

However, even after an infectious dose of a pathogen has been ingested the resulting infection falls along a spectrum of severities. The severity of the illness is determined by a number of factors including the type of pathogen, the ingested dose, the immunocompetency and nutritional status of the host, and previous exposure to the pathogen. While a limited number of highly virulent pathogens are able to cause relatively high rates of severe illness and mortality in healthy people, the majority of enteric infections, including typical Cryptosporidiosis, result in only a mild form of watery diarrhoea with short duration. The WHO has explicitly acknowledged this in their recent shift to the more health-outcome based risk assessment (Disability-Adjusted-Life-Year system) which accounts for the spectrum of severities and durations of illnesses.

9.4.2.1.2 Proposed Guideline

The rainwater tank drinking water guidelines, currently being prepared by WHO, are being developed essentially for those who rely on harvested rainwater as their principal water supply. Until these guidelines are published, it is recommended that where harvested rainwater is the primary water source, then maintenance guidelines (such as enHealth, 2004) be used to ensure the water is of its highest possible potential quality.

In urban areas of Australia a treated municipal water supply is available with water quality being monitored and regulated by the conditions of operating license agreements between water suppliers and state governments. It is here recommended that the consumption of harvested rainwater not be practised where treated municipal supplies are available.

Many urban residents, however, have expressed the desire to use harvested rainwater for drinking purposes despite the availability of a municipal supply. It is therefore important that in the future appropriate drinking water guidelines be developed for urban rainwater harvesting systems. Such guidelines should be developed using the methodology recommended by the most recent WHO GDWQ (2006).

In short, this process involves conducting a quantitative microbial risk assessment within the context of a risk management plan. For a robust quantitative microbial risk assessment to be conducted, significantly more research will need to be conducted on the diversity, load and prevalence of microbial contamination in rainwater tanks in urban rainwater harvesting systems. The use of Disability Adjusted Life Years (DALYs) is emerging as the metric of choice for quantifying the potential adverse impact of rainwater consumption on the general population. To accurately estimate the DALYs associated with drinking harvested rainwater, further investigations also need to focus on the average extent of exposure (litres of tank water consumed per day) and epidemiological associations (probability of illness resulting from exposure) in conjunction with monitoring microbial loads in tanks.

9.5 Conclusions

The growing interest in roof-harvested rainwater as a complementary supply to municipal supplies means that the need for comprehensive health risk assessments and the development of health based secondary use guidelines is becoming increasingly important. To date, however, a thorough assessment of the health risks associated with various uses of harvested rainwaters has been largely neglected.

The current guideline framework, including international, national and state water quality guidelines covering a range of applications including drinking, recreational and recycling uses, does not sufficiently regulate or provide guidance for acceptable water quality for secondary uses of harvested rainwaters. Conventional drinking and bathing water guidelines, aimed primarily at municipal surface or groundwater supplies, have neglected to consider the significant differences in the range of feasible disease transmission routes in RWH systems. The exclusion of the major faecal-oral disease vectors (being human sewage and cattle faeces) in RWH systems significantly reduces the risk of illness associated with the detection of microbial indicators used in the assessment of surface water supplies, and hence dramatically increases the conservativeness of such guidelines.

Such conservativeness is accentuated by the fact that the majority of urban rainwater users do not intend to utilise such supplies for drinking water purposes. Although non-drinking uses are advocated for urban rainwater supplies, it is typically the drinking water standards that are used to assess the safety of this water source. Secondary-use guidelines have therefore been proposed, addressing water quality requirements for a range of popular uses of harvested rainwater including garden watering, toilet flushing and washing applications (amenities supplies), showering applications and cooking applications. Recommendations were also made for conducting further research into drinking applications focussing on the need for future investigations to couple water quality data with epidemiological data to form the basis of appropriate rainwater tank drinking water guidelines.

CHAPTER 10

Conclusions

10.1 Current Urban Water-Cycle Management

It is becoming increasingly evident that major deficiencies exist within the current urban water management paradigm. Along with the provision of municipal water supplies, the distinguishing factors of current urban water cycle management include low levels of rainwater harvesting and large volumes of contaminated stormwater runoff. Increasing demand on mains water supplies has lead to significant reductions in drought security and has further highlighted the need for change. The practice of rainwater harvesting offers enormous potential for relieving some of these water stresses within the urban environment. Capturing rainfall close to where it lands is an energy efficient practice and more closely mimics natural hydrological processes than current centralised designs. The level of beneficial impact that rainwater harvesting can have on the urban water cycle is largely dependent upon the level of utilisation of the harvested rainwaters. Higher utilisation rates of harvested rainwater supplies result in more rapid draw-down of tank supplies which increases the harvestable yield during subsequent rain events. This increases both the level of savings on mains water supplies as well as maximises reductions in stormwater runoff.

The extent that harvested rainwater is utilised, however, depends upon the designated end uses of the tank supply, which in turn is governed by water quality. Due to the supply/demand mismatch between tank supplies and outdoor garden demands, the popular use of tankwaters for irrigation alone does not result in optimal utilisation of tank supplies. Optimal utilisation only occurs when tank supplies are drawn down during rain events in order to maximise harvested yield. This requires tank waters to be used for supplying substantial indoor uses, including hotwater applications. Despite the general comfort with using harvested rainwater supplies by many urban residents, extensive water quality monitoring of urban rainwater systems had not been conducted nor an understanding of the incidental treatment train been developed. The increasing use of rainwater tanks in many urban locations throughout the world has raised the need for an increased level of understanding of water quality issues within these systems.

10.2 Field Assessment of Harvested Rainwater Quality

The central objectives of this study were to examine the dynamics of water quality in urban rainwater tanks and to elucidate the mechanisms responsible for causing changes to water quality along the collection and storage train. Two major Australian rainwater tank retrofit projects were monitored over a two-year period to assess a variety of water quality issues. Thirty rainwater tanks in the Brisbane City Council district were monitored monthly for a variety of microbial and physicochemical parameters over a two-year period. A complementary study in the Newcastle region was conducted with a more investigative approach to examine intra-system water quality changes and to identify incidental treatment mechanisms operating within these systems acting to improve water quality.

Water quality in the rainwater harvesting (RWH) systems was evaluated against both the Australian Drinking Water Guidelines (ADWG) and the Guidelines for Recreational Water Quality and Aesthetics (GRWQA) for 'Primary Contact' waters. The harvested rainwater quality in both the Brisbane and the Newcastle/Central Coast studies often did not meet the microbial standards of the ADWG. In the Brisbane study, slightly more than 3 out of every 4 samples contained no *E. coli* organisms and were hence compliant with ADWG, while in the Newcastle study this was only slightly more than 1 in 4 samples. However, microbial water quality in both studies did meet the less stringent requirements of the GRWQA. Lead was found to be the heavy metal of most concern in tank waters with some tanks suffering regular lead contamination. The majority of other heavy metals being present in trace concentrations usually below detectable limits.

A number of design variables were investigated for their influence of water quality, although surprisingly few were found to have any explainable significance. The influence of roof material was seen to influence pH, iron and zinc concentrations. Tank materials were found to influence coliform levels, although this was most likely related to the capacities of these tanks. Aquaplate® tanks, on average, contained the lowest coliform levels but also averaged the lowest storage capacity. Stainless steel tanks averaged the second highest coliform concentrations and averaged the second largest capacities, while polyethylene tanks had the largest capacity on average and contained the highest levels of coliform contamination. The influence of tank storage capacity on microbial quality probably related to the proportion of mains water being stored in the tanks. Smaller tanks emptied more quickly and therefore held chlorinated municipal waters for greater periods of time reducing coliform concentrations more rapidly through the actions of free chlorine residual and dilution. Non-microbial parameters did not follow the same pattern of reduction in smaller tanks, suggesting that the action of chlorine entering the tanks during top-up plays a significantly role in reducing bacterial levels.

Clear seasonal trends were observed in the Brisbane study for rainfall, ions and heavy metals. The high summer rainfall and low winter rainfall distribution pattern correlated with the distinct seasonal distribution pattern of coliform, thermotolerant coliform, *E. coli*, HPC and lead concentrations in tanks, suggesting that these contaminants are transported into the tank through rainfall. The converse seasonal distribution patterns displayed for a range of ions, including calcium, magnesium, potassium, sodium and barium, strongly indicating that mains water is the major source of these. The influence of rainfall on water quality was observed in a number of the Newcastle RWH systems where the sampling regime was determined by rain events. The concentrations of microbial contaminants and lead were generally higher immediately after rain events with concentrations decreasing over subsequent dry days. The same finding could not be statistically concluded from the Brisbane study, where only very weak correlations were found between contaminant concentrations and the number of days since the previous rain event. These correlations were likely hidden by the statistical noise resulting from the time-based monitoring program, where sampling was conducted monthly irrespective of rainfall.

10.3 The Incidental Treatment Train

The ability of natural water bodies to undergo self-purification is a phenomenon that has been extensively relied upon though in the past has not been equally understood. The concept of bioremediation is inherent in all natural systems including water courses, where chemical and biological processes lead toward an equilibrium unique to the specific circumstances of a given water body. Natural systems have been exploited for millennia for their ability to remove or decrease pollutant levels, such as the disposal of sewage into rivers and ocean outfalls. Within rainwater harvesting systems, a limited number of previous studies had observed improvements in stored rainwater quality over time and at different points in the collection and distribution system suggesting that a degree of self purification also occurs. This thesis identified and elucidated many of the major treatment mechanisms involved in this incidental rainwater treatment phenomenon. A range of beneficial mechanisms were identified at various locations throughout the RWH systems as well as points of possible contamination.

10.3.1 Roof Catchment

The first component of RWH systems, and possibly most influential for water quality, is the roof catchment. The roof catchments of two RWH systems suffering from significant water quality problems were investigated to identify the processes contributing to the deterioration of the waters. The four most significant factors in these two cases studies were identified as overhanging vegetation, lead flashing, gutter design and maintenance practices. Overhanging vegetation at both sites resulted in aesthetic water quality problems and high bacterial concentrations. Overhanging vegetation provides a source of bacteria and organic matter while also providing roof access to birds, bats, small mammals and reptiles which may further contaminate the roof catchment. The use of lead flashing on one of the roofs was also found to be highly inappropriate and resulted in chronic lead contamination. Laboratory experiments revealed that older oxidised flashing contributed more to lead contamination than newer unoxidised flashing and that low flow events mobilised the greatest concentrations of lead. This case study reaffirms the recommendation that lead flashing not be used on roofs intended for rainwater harvesting.

A minimal roof slope was also identified as a factor which may exacerbate water quality problems. The type of gutter screening is important on these roofs to minimise the accumulation of organic debris which may be trapped by the gutter screen. The use of alternative gutter protection, such as those based on surface tension designs, may be more effective on such roofs. As regular inspection and maintenance of roof catchments is generally not conducted by householders, the use of self-cleaning devices is encouraged, although it is important that these components are installed and operating properly. When cleaning and maintenance are conducted by householders it is essential that these are conducted appropriately so as to avoid the mobilisation of contaminants in the system which may lead to increased contamination.

10.3.2 Rainwater Tank

Within the rainwater tanks, water quality was not found to be static but to be continually changing as it interacted with the physical processes and ecology of the tank. Both temporal and spatial variations in water quality were observed in many of the investigated tanks. Microbial stratification was observed in some tanks, with higher concentrations of microbes observed in the surface layers of the water columns. Temporal variations were also apparent, with concentrations of bacteria and lead typical decreasing in the days following rain events. These improvements in water quality indicated that the existence of a functional rainwater tank microbial ecology may be an important part of producing optimal water quality. Many of the environmental organisms identified within the water columns have the potential for beneficially contributing to tank ecology and improving water quality. These bacteria can increase competitive pressures on pathogens by utilising nutrients, can form biofilms which potentially have the ability to remove toxic metals and compounds from the water column, and some species have insecticidal properties, such as *B. thuringiensis* and *B. sphaericus*, which are capable of destroying mosquito larvae.

10.3.3 Biofilms

An important and novel discovery in this thesis was the potential for biofilm growth in rainwater tanks. The development of biofilms was found to occur on glass, metal and polyethylene slides suspended in a rainwater tank, indicating that the inner surfaces of rainwater tanks may provide a suitable structure for biofilm development. A number of insights were gained from this research relating to the composition and functioning of rainwater tank biofilms. The concentrations of bacteria and heavy metals were much higher in the biofilms than in the water column, with concentrations greatest in the biofilms grown in the lower levels of the tank. Approximately two thirds of biofilm-associated cells were concentrated in the basal layer of the biofilm and were firmly attached to the substrate, with the remaining one third loosely attached to the surface of the biofilm. The finding that heavy metals, and in particular lead, may be removed from the water column by biofilm uptake demonstrates that biofilms are an important part of a beneficial microbial ecology capable of improving tank water quality.

10.3.4 Sludge

Passive settlement of particulate matter resulting in the build up of sludge at the base of the tank was observed in every tank examined. Sludge accumulation rates varied widely between tanks, ranging from an estimated 0.2kg to 2.6kg dry weight accumulation per year. The settling rates of different sludges also showed large variation, with a humus-based sludge taking 30mins to completely settle out of a 200mm water column compared to 7 days for a clay based sludge. The process of sedimentation was found to be the single most beneficial incidental treatment mechanism for the removal of heavy metal and organic contaminants from the tank water columns. While concentrations of bacteria were also found to accumulate in the sludge, ranging from one to three orders of magnitude greater than the water column, the magnification rates of heavy metals were the most significant. This was particularly so for lead, where concentrations in the sludge were $6.7 \times 10^4 - 3.4 \times 10^5$ times greater than in the respective water columns. Fractioning of the sludge revealed that lead was more bound up with the finer particulate matter than the course sediment. Under some experimental conditions resuspended sludge demonstrated the ability to enhance flocculation and settlement of suspended bacteria. However, due to a number of factors the re-suspension of sludge is not generally considered beneficial to water quality.

10.3.5 Hotwater Systems

The use of tank waters to supply domestic hotwater systems had been previously shown to produce significant improvements to the security of municipal water supplies. However, hotwater applications, such as showering, also pose a significant degree of human exposure to the waters. Very little relevant data had been published prior to this thesis expounding on the influence of hotwater systems (HWS) on microbial and chemical water quality. Massive reductions in bacterial concentrations were observed as harvested rainwaters passed through a range of domestic hotwater systems. *E. coli* populations underwent several orders of magnitude, and often total, inactivation in all HWS. HWS operating above 50°C produced significantly higher quality waters than those below 50°C. The water quality produced by domestic HWS in both the Brisbane and Newcastle studies was regularly compliant with ADWG microbial standards and was in total compliance with bathing water guidelines (GRWQA – Primary Contact Waters). Water samples from HWS systems operating above 60°C were found to be of comparable or better quality than the local municipal water supplies. Low concentrations of HPC were found to survive HWS and these were often identified as *Bacillus sp.* or *Stenotrophomonas sp.*

Laboratory thermal inactivation experiments demonstrated that enriched rainwater tank bacterial communities can be rapidly reduced by several log reductions at temperatures relevant to domestic HWS. Non-sporing environmental bacteria were found to be the most rapidly inactivated, followed by enteric bacteria and finally sporing-forming *Bacillus*. This was a logical progression of inactivation given that most environmental species have optimal growth temperatures around 24°C, while enteric bacteria prefer 37°C. Spores of *Bacillus sp.* are capable of prolonged survival at temperatures above 90°C and are not expected to be inactivated by typical HWS temperatures.

A number of non-sporing pathogens were also examined for their thermal resistance capacities and were found to be susceptible to inactivation at temperatures above 55°C. Indicator organisms were generally found to contain greater heat resistance than non-sporing pathogens. Strains of *E. faecalis* were found to be the most heat resistant followed by *E. coli*. A variety of other pathogens, or opportunistic pathogens, contained lower heat resistance capacities including, in order of heat resistance, *Shigella sonnei*, *Pseudomonas aeruginosa*, *Salmonella typhimurium*, *Serratia marcescens*, *Klebsiella pneumophila* and *Aeromonas hydrophila*. The heated rainwaters were therefore found to be of very high microbial quality, with systems maintained at 60°C in accordance with Australian Standards (ANZ3500.4) posing negligible health risks to users.

The potential for accelerated corrosion of HWS was also assessed in the Brisbane study by examining differences in heavy metal concentrations in hotwater system and rainwater tank samples. The results of this study did not provide evidence that harvested rainwater increases the risk of accelerated corrosion of hotwater systems, although a small minority of HWS appeared to be a source of lead and cadmium contamination. Concentrations of zinc, iron and magnesium in the HWS were generally marginally higher, though not significantly, than concentrations in the rainwater tanks. The concentration trends of these metals in the HWS were also not found to be increasing over the course of the 2-year experimental period.

10.4 Health Risk and Secondary-Use Guidelines

Ultimately, the interest in water quality relates to the protection of human health. Concern for public health from the use of harvested rainwaters for drinking and direct contact applications has been expressed by a number of health authorities and researchers based on water quality findings. Despite this, there remains little evidence to suggest that harvested rainwaters are a significant means of disease transmission. RWH systems hold a topographical advantage over surface catchments in that the roof catchment of RWH systems is vulnerable to far fewer routes of faecal and pathogenic contamination. While it is accepted that only a minority of illnesses resulting from the ingestion of tank waters are reported, the combined global incidence of illness from the ingestion of tank waters is less than 0.25% of that recorded from a single mains water outbreak event (Milwaukee – 1994). This is a significantly lower proportion than the typical proportion of populations in many countries relying on tank waters for their drinking supply, such as in Australia where 11% of the population use tank waters for drinking. The only significant epidemiology study conducted on illness associated with tank water use, involving an experimental group of nearly one thousand children, found that the rates of gastroenteritis were lower, though not statistically significantly, amongst those drinking harvested rainwater compared to those drinking municipal supplies (Heyworth *et al.*, 2006).

Although there is currently a deficit of evidence confirming that harvested rainwaters pose a high level of health risk, it is not currently recommended that harvested rainwaters be used for drinking purposes whenever a treated municipal supply is available. However, a variety of potential secondary uses exist which may provide substantial benefits to urban water management. Clear recommendations as to which secondary uses are suitable for tank waters have been missing however, largely due to the lack of appropriate secondary use guidelines and compounded by the inherent nature of published literature to focus on studies that have detected pathogens rather than those that have demonstrated their absence.

The secondary use with the greatest human exposure is that of showering and bathing. While water quality will vary between tanks, the use of harvested rainwaters to supply domestic HWS operating above 60°C[a] is an acceptable and beneficial practice. This conclusion is based on the following summarised research findings.

1. Although harvested rainwaters are not intended for drinking purposes, they have been shown to:
   - be less vulnerable to pathogenic contamination pathways than municipal supplies,
   - cause lower rates of gastrointestinal illness amongst those drinking the tank water compared to those drinking municipal supplies, and
   - achieve complete compliance with bathing water guidelines (GRWQA).
2. After passing through domestic HWS, harvested rainwaters have been shown to be of significantly improved microbial quality, with massive reductions in concentrations of indictor bacteria resulting in regular compliance with drinking water guidelines (ADWG) and total compliance with bathing water guidelines (GRWQA).
3. Despite the rare detection of single indicator bacteria in hotwater samples, the major categories of indictor bacteria (*E. coli* and *E. faecalis*) were found to be significantly more heat resistant than a variety of other non-sporing bacterial pathogens. The absence of these organisms in hotwater samples therefore emphasises the unlikelihood of hotwater samples containing an infective dose of non-sporing pathogens.
4. In the remote chance that an infective dose of a pathogen was to survive the thermal stress of a hotwater system, heated rainwaters are not intended for human ingestion and rarely do people drink directly from a hotwater tap without further boiling or cooking, making the risk of illness negligible.
5. HWS have not, to date, shown signs of increased rates of corrosion when supplied with combinations of mains and harvested rainwater.

There are currently no water quality guidelines designed specifically for secondary uses of harvested rainwater. Secondary-use guidelines were therefore proposed for three categories of use. The first category of secondary-use was indirect contact, such as garden watering, toilet flushing and washing applications, which require waters of the lowest quality. The second category was direct-contact, including showering and

[a]Based on current data, 55°C is an acceptable minimum temperature for maintaining waters of high microbial quality. The recommendation of 60°C is a conservative recommendation in accordance with current Australian Standards (AS3500.4) for HWS operation.

bathing uses, requiring a higher microbial and physicochemical quality. The third category was secondary-consumption, relevant in cases where tank-supplied HWS are used for cooking applications. Drinking applications were the fourth category. While insufficient health and water quality data have prevented the proposal of scientifically informed guidelines for the fourth category, recommendations were made as to the need for further epidemiological studies linked with water quality monitoring to establish valid rainwater drinking guidelines.

In summary, in order to more sustainably manage our urban water cycle, freely available resources, such as rainwater, should not be discounted on the basis of oversimplified interpretations of water quality and health risk. Harvested rainwater is a high quality natural resource that many people currently use without treatment and without consequential illness. A shift in the judgement of harvested rainwaters from that of a finished drinking water to that of a source water would result in an increased willingness to accept this resource as a high quality secondary-use water supply. In essence, this thesis has argued that a series of incidentally occurring treatment mechanisms contribute significantly to producing a high quality freshwater resource and that with an appropriate guideline framework, the fuller and more appropriate utilisation of this sustainable water resource for secondary uses including showering and bathing, toilet flushing, laundry and washing, and outdoor irrigation applications, should be achieved.

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Appendix A: Design Variables of Brisbane RWH Systems

SYSTEM NUMBER	SUBURB	ROOF AREA (m^2)	ROOF MATERIAL	TANK CONNECT TO HWS	HWS TYPE	TANK MATERIAL	TANK SIZE (L)	TREES* OH/<5/NO	No. of TOILET/ BATHRM
1	Tarragindi	169	Tile	+	Edwards-solar	Aquaplate	3380	OH	2 / 1
2	Kedron	192	Metal	+	Beasley-solar	Aquaplate	3240	OH	2 / 1
3	Paddington	137	Metal	–	Elgas-gas (inst)	SS	3000	OH	1 / 1
4	Mt Gravatt	172	Tile	+	Rheem-solar	Aquaplate	4850	NO	1 / 1
5	Red Hill	82	Metal	+	Beasley-solar	Aquaplate	3240	OH	2 / 1
6	Tarragindi	151	Metal	–	Rinnai-gas (inst)	SS	4500	OH	-
7	Enoggera	257	Zinc	+	Saxon-solar	Aquaplate	4850	<5	2 / 2
8	Everton Park	166	Tile	+	Beasley-solar	Polyethylene	5000	OH	2 / 2
9	Carindale	245	Tile	+	Quantum-heatpump	SS	4500	OH	2 / 2
10	Morningside	206	Metal	+	Solarhart-solar	Aquaplate	3820	<5	2 / 2
11	The Gap	138	Tile	+	Edwards-solar	Polyethylene	4540	<5	1 / 1
12	Hendra	140	TBA	+	Saxon-solar	SS	3000	OH	3 / 2
13	Kedron	157	Tile	–	Bosch-gas (inst)	Polyethylene	4540	<5	1 / 1
14	Toowong	141	Metal	–	Bosch-gas (inst)	SS	4500	OH	2 / 1
15	Upper Mt Gravatt	167	Metal	+	Solco-solar	Aquaplate	3820	OH	1 / 1
16	St Johns Wood	217	Tile	–	Bosch-gas (inst)	Aquaplate	3380	<5	2 / 2
17	Toowong	226	Tile	+	Edwards-solar	Aquaplate	3240	<5	2 / 1

18	Annerley	189	Metal	–	Rinnia-gas (inst)	Polyethylene	5000	<5	1 / 1
19	Tarragindi	158	Tile	–	Bosch-gas (inst)	Aquaplate	3930	OH	1 / 1
20	Wavell Heights	191	Metal	+	Rheem-solar	Aquaplate	3240	<5	2 / 2
21	Alderley	189	Tile	–	Rheem-gas	Polyethylene	5000	<5	2 / 2
22	Bardon	132	Metal	+	Solarhart-solar	Aquaplate	3930	<5	1 / 1
23	Yeronga	217	Metal	+	Solarhart-solar	Aquaplate	3380	OH	2 / 2
24	Northgate	128	Metal	+	Rheem-heatpump	Aquaplate	3380	<5	2 / 2
25	Camp Hill	189	Tile	+	Rheem solar (Elgas-LPG Booster)	Aquaplate	3240	<5	2 / 2
26	Stafford Heights	267	Tile	+	Edwards-solar	Aquaplate	3380	OH	1 / 1
27	Mitchelton	143	Metal	+	Beasley-solar	SS	4500	OH	1 / 1
28	Wavell Heights	136	Metal	+	Dux-solar	Polyethylene	4540	OH	2 / 2
29	Red Hill	255	Metal	–	Dux-gas (inst)	SS	4500	<5	3 / 2
30	Norman Park	118	Metal	+	Edwards-solar	Aquaplate	4850	OH	1 / 1

* TREES
OV = At least one tree directly overhanging the roof catchment
<5 = At least one tree within 5m of roof catchment
NO = No trees within 5m of roof catchment

Appendix B: Design Variables of Newcastle RWH Systems

SYSTEM NUMBER	SUBURB	TANK-WATER USES	ROOF MATERIAL	TANK MATERIAL	TANK SIZE (L)	TREES OH/<5/NO
1	Kotara	ALL	Metal	Aquaplate	9000	OH
2	Kotara	G, L, T, H	Metal	Aquaplate	5500	OH
3	Kotara	G, L, T	Tile	Polyethylene	2500	<5
4	Lambton	G, L, T, H	Tile	Polyethylene	5000	OH
5	Carrington	ALL	Metal	Aquaplate	2000	OH
6	Ourimbah	ALL	Tile	Conerete	17000	OH

G = garden; L = laundry; T = toilet; H = hotwater; ALL = includes drinking

APPENDIX C

Newcastle tanks have been shown for comparison, although these tanks were not sampled using the systematic approach employed in the Brisbane study. In the Newcastle study, specific tanks with water quality problems were targeted which effectively skewed the data towards the higher concentration ranges. Furthermore, only the larger tanks were sampled more than a week after rain events to reduce the incidence of sampling added mains water in the rainwater tanks.

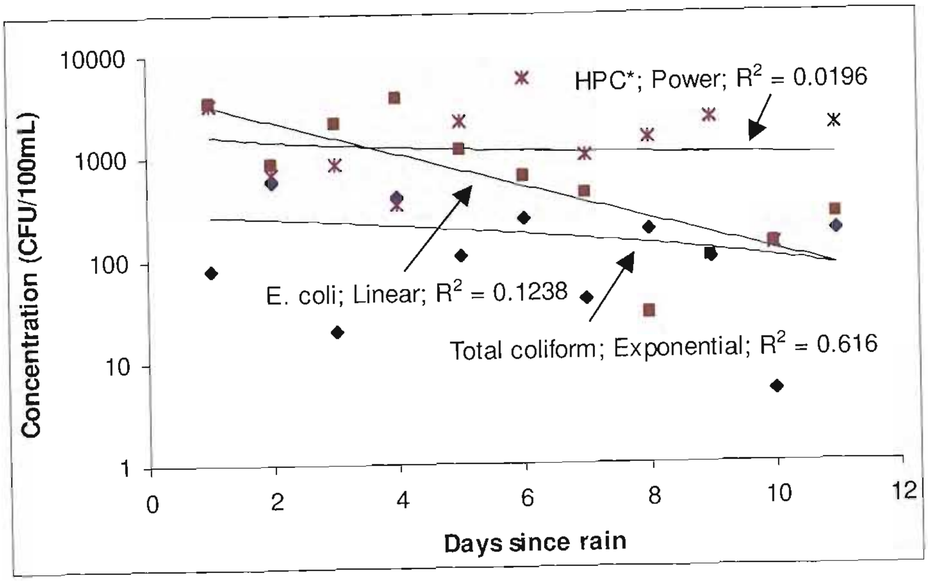

Figure C1: Concentrations of bacterial parameters in Newcastle RWH systems as a function of the length of time between the previous rain event and sampling date (*HPC concentrations in CFU/mL)

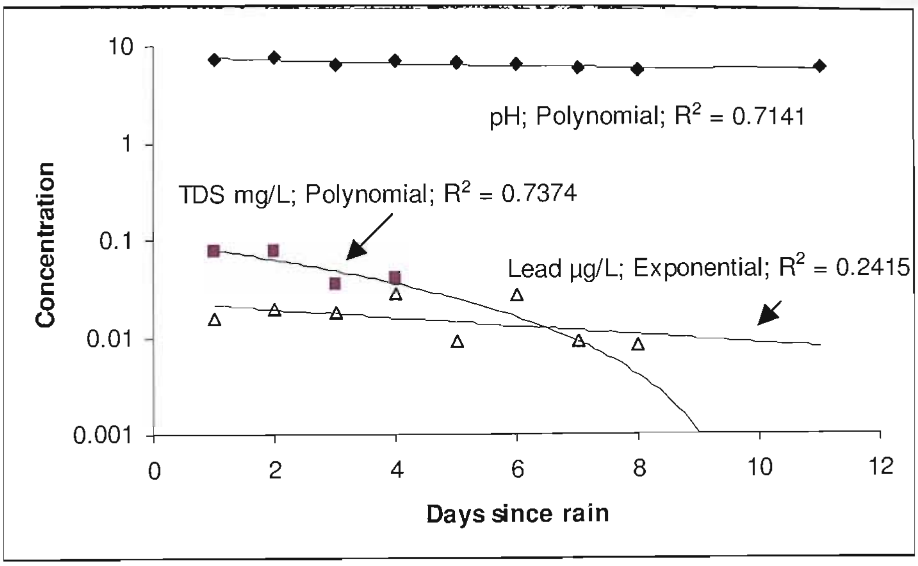

Figure C2: Concentrations of physicochemical parameters in Newcastle RWH systems as a function of the length of time between the previous rain event and sampling date

Appendix D

Reductions in total coliform and lead concentrations in Brisbane harvested rainwaters were non-linear, with the most rapid reductions occurring in the first few days immediately following rain events.

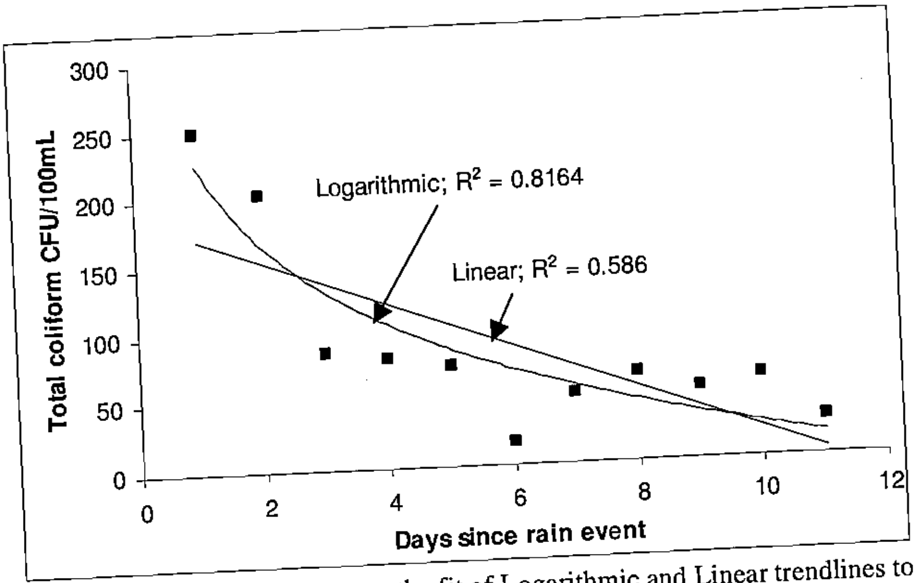

Figure D1: Comparison between the fit of Logarithmic and Linear trendlines to total coliform concentration.

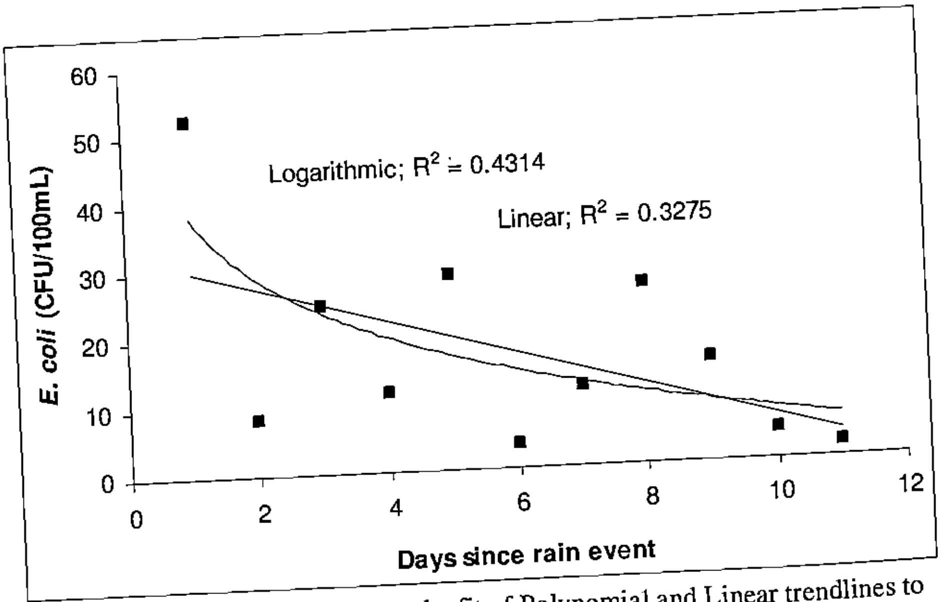

Figure D2: Comparison between the fit of Polynomial and Linear trendlines to *E. coli* concentration.

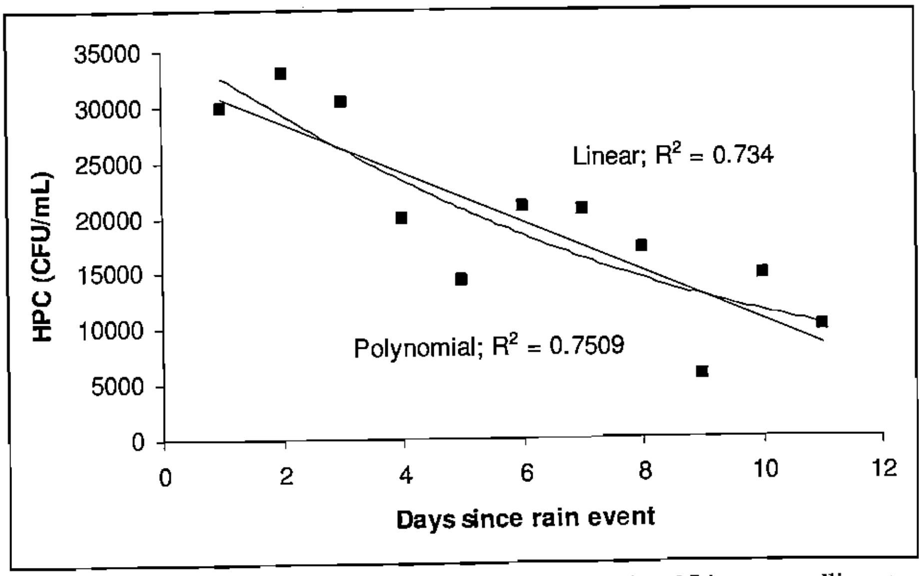

Figure D 3: Comparison between the fit of Polynomial and Linear trendlines to HPC concentration.

In contrast to bacteria and lead concentrations, the pH and electrical conductivity of Brisbane harvested rainwaters increased over time with storage, shown in Figure D4 and D5, respectively. Both of these parameters were best described by power curves

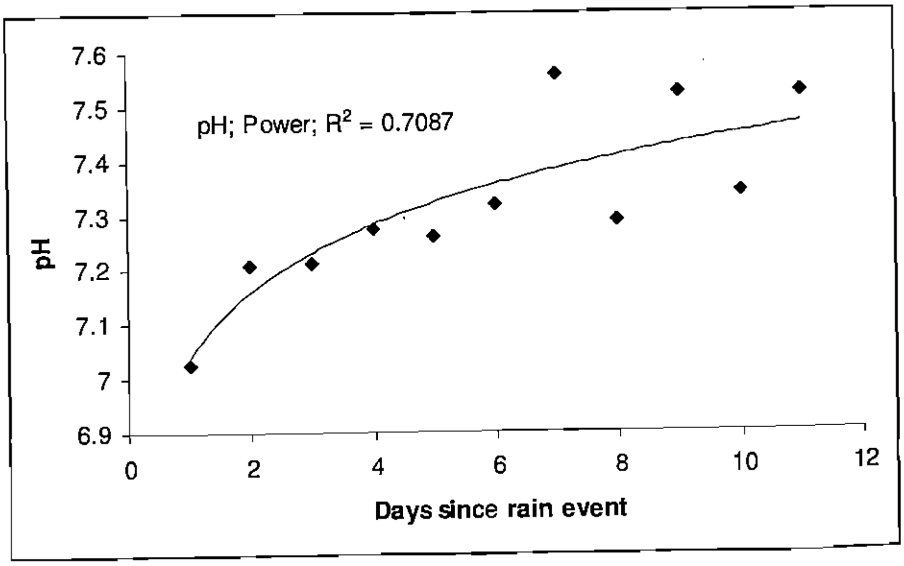

Figure D4: pH in Brisbane RWH systems as a function of the length of time between the previous rain event and sampling date

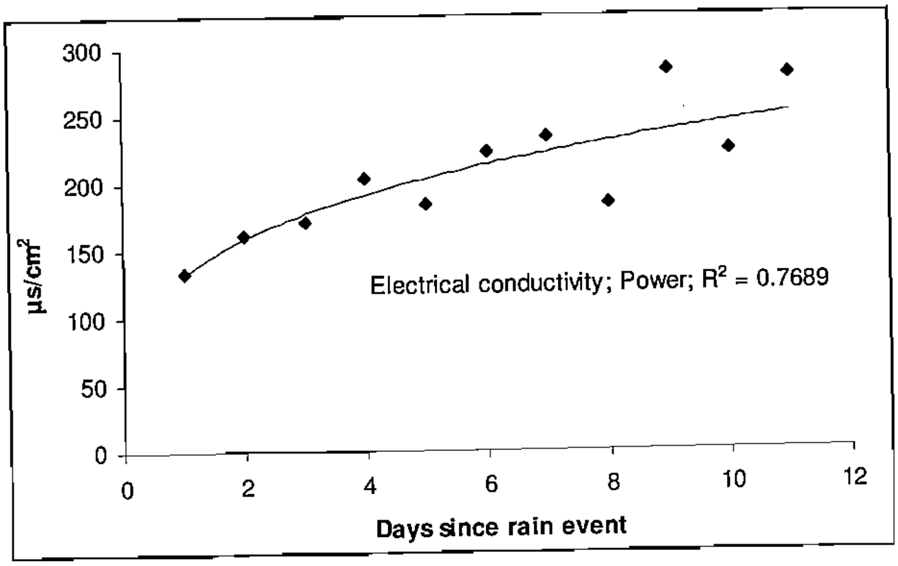

Figure D5: Electrical conductivity in Brisbane RWH systems as a function of the length of time between the previous rain event and sampling date

Appendix E

Sludge depths in six rainwater tanks in relation to inlet and outlet positions.