Quantifying the Significance of Faecal Contamination in Yarra River due to Dry Weather Sewer Spills

A thesis submitted in fulfilment of the requirements for the degree of Masters of Engineering

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DECLARATION

I certify that except where due acknowledgement has been made, the work is that of the author alone; the work has not been submitted previously, in whole or in part, to qualify for any academic award; the content of the thesis is the result of work which has been carried out since the official commencement date of the approved research program; and, any editorial work, paid or unpaid, carried out by a third party is acknowledged.

Sainath Tavate
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ABBREVIATION

a  Calibration coefficient for the catchment
S08  summer 2008
W08  winter 2008
S09  summer 2009
EPA  Environmental Protection Agency
E. coli  Escherichia Coli
WWTP  Waste Water Treatment Plant
FRNA coliphage  F- specific RNA coliphage
ERS  Emergency relief structures
NHMRC  National Health and Medical Research Council
SS  Suspended solids
DO  Dissolved oxygen
MST technique  Microbial source tracking technique
OEPA  Ohio Environmental Protection Agency
IMS  Immunomagnetic separation
ATP  Adenosine triphosphate
CRC  Cooperative Research Centre
SEPP  State Environmental Protection Policy
WHO  World Health Organisation
EMC  Event Mean Concentration
CSIRO  Commonwealth Scientific and Industrial Research Organisation
P_w(t)  Pollutant present at time t
k_w  Empirical pollutant removal coefficient
R  Runoff flow rate
CO_2  Carbon dioxide
TDS  Total dissolved solids
BOD  Biological oxygen demand
MUSIC  Model for urban stormwater improvement conceptualisation
CRCCH  Cooperative Research Centre for Catchment Hydrology
CTRs  Continuously stirred tank reactors
MOPUS  Micro Organisms Prediction in Urban Storm Water
UV  Ultraviolet
BOM  Bureau of Meteorology
R²

Coefficient of Determination

SEW

South East Water

APHA

American Public Health Association

mm

millimetres

hr

Hour

T

Temperature

PET

Potential evapotranspiration

PET(t)

Potential evapotranspiration on a specific day

RH

Relative Humidity

VP

Vapour Pressure

Pₛˌloss(t)

Loss of pathogens during a time period 't'

Pₛ(t-1)

Pathogen concentration at the end of the previous day

Pₛ(0)

Concentration of pathogens at '0' time interval

mL

Millilitres

Cₛ(t)

Concentration of microbes at stormwater drain

Pₛ(t)

Number of microbes present on surface before storm event

SEW

South East Water

t

Time period (h)

TN

Total Nitrogen

TP

Total Phosphorus

TSS

Total Suspended Solids

WSUD

Water Sensitive Urban Design
Abstract

The Yarra River is considered to be an important environmental and recreational asset by the Melbourne community. The upper reaches of the Yarra River provides water for drinking and agricultural purposes, the lower Yarra is mainly utilized for recreational purposes and is a focal point for tourism in Melbourne. According to State Environmental Protection Policies (SEPP) When compared with the upper reaches of the river water quality of the Yarra River in its lower reaches is relatively poor due to urbanisation. Faecal coliform levels especially have been observed to be high in the lower sections of the Yarra River, even during dry weather periods. One of the contributors to faecal contamination during dry weather has been identified as dry weather sewer spills due to structural collapses or blockages from tree roots.

The main aim of this study is to investigate the effect of dry weather sewer spills on river water quality and to estimate the survival rate of microbes on pervious and impervious surfaces after a spill event. In addition, the number of faecal microbes carried to the waterways will depend on the volume of spill, magnitude of the storm generating runoff, elapsed time after the spill as well as the antecedent climate conditions before the storm.

To develop a predictive model for the mobility of microbes after the spill, it was important to understand their survival with time. Field experiments were carried out at the Mt. Martha Sewage Treatment Plant, Victoria to investigate the survival rate of the microbes (E.coli, enterococci and FRNA coliphages) on pervious and impervious surfaces after a dry weather spill. In addition, the availability of nutrients (Total nitrogen and Total phosphorus) with elapsed time on the surface after a spill was also examined. The experiments were carried out in summer 2008, winter 2008 and summer 2009. From the experiments carried out during summer, the presence of E.coli and enterococci on pervious surfaces was evident even 14 days after a simulated spill. The FRNA coliphages did not survive 24 hours after the simulated spill. The E.coli and Enterococci had survived on the pervious surfaces in spite of the hot weather and low moisture conditions. However, the nutrient concentration levels dropped significantly with time. The microbe levels on the impervious surface were very low. Furthermore, microbes did not exist on the impervious surface 4 days after the simulated spill.

This retained concentration of microbes on the surface after a dry weather spill provides a source of microbes ready to be washed off into waterways following a rainfall event. Surface runoff experiments carried out at Mt. Martha wastewater treatment plant also demonstrated
the variation of \textit{E. coli} and enterococci concentrations within the collected samples on the same day during the experimental period and within a collected sample from the same experimental plot. The coefficient of variation of the samples collected and tested for microbial parameters from the same pool of surface water is as high as 170\%. The concentrations of nutrients present on the experimental surface showed no relationship to the availability of microbes.

A multiple regression analysis was carried out between survived microbe concentrations, climate variables and elapsed time after a spill to develop a predictive model for simulating the survival rate of \textit{E. coli} and enterococci on a pervious surface after the occurrence of a dry weather spill. The information from field experiments during winter 2008 and summer 2009 experimental periods was used to develop the model. The climatic data was collected from the Bureau of Meteorology web site for the nearest meteorological site. A successful model was developed between microbes, elapsed time after a spill and average relative humidity data between spill and the storm event. This model was further verified with an independent set of data and the performance of the model was deemed acceptable. The sensitivity of the model to the variation in relative humidity was also examined. However, the developed model should be used with caution to predict the enterococci organisms with time due to the variation of these microbes on the pervious surface.

This predictive model was coupled with the simple microbial transport model to estimate the microbial concentration at the stormwater drain inlet. A relationship was successfully developed between different storm events and the percentage of microbes at the stormwater drain inlet.

The actual spill data during the year 2007 were collected from archived information for two catchments in Melbourne. This research concludes with a discussion on the potential effects on river water quality due to dry weather spills, the impact of rainfall intensity and their potential to mobilize the microbial contamination and move towards a stormwater drain and be transported to a nearby water course. Overall, the objectives of this study were achieved and the transport of microbes can be estimated at different elapsed times depending on the relative humidity after a dry weather spill event, providing its impact on waterways.
1.1 Introduction

The Yarra River flows across the heart of the Melbourne city and provides water for a number of purposes, including drinking water for Melbourne and its suburbs. The total length of the river is 242 Kilometres from the source which is about 40 kilometres east of Warburton on the flanks of Mt. Baw Baw. The river ends in the Port Philip Bay at Newport. Although the water quality in the upper reaches is excellent it deteriorates as the river approaches the heavily urbanised lower section. The name “Yarra Yarra” means ‘ever flowing’ in the Wurundjeri Aboriginal language. In addition to water supply, the Yarra catchment supports agriculture, forestry, recreation and tourism. The catchment covers 4078 kilometres, includes 24 tributaries and a population of about 2 million people live within its catchment. As a result of commercialisation and industrialisation in the middle and the lower Yarra catchment, the colour of the water turns brown because of the pollutants entering from settlements, especially sediments (Melbourne Water, 2007).

The Yarra River with its origin in the southern slopes of the Great Dividing Range in the forested Yarra Ranges National Park is an important environmental and recreational asset for the Melbourne community. This river is used for various purposes in different reaches (Figure 1.1) i.e. upper reaches of the Yarra predominantly provides water for drinking, some agriculture/horticulture use by industry and for domestic purposes. Human settlements tend to gravitate to water courses since early years. The Yarra River was no exception. The Yarra River was used as an open ‘drain’ transporting sewage and dumping ground for industrial waste since early European settlement, and has always been under pressure for human development. 70 % of the drinking water supply for the Melbourne city is provided by the non polluted upper reaches of the Yarra River. Water quality in the Yarra River is monitored by the EPA Victoria (State Government Authority) and Melbourne Water. Melbourne Water is the water authority suppling drinking water to the three of the water retail companies (Yarra Valley Water, South East Water, and City West Water) across the Melbourne region. The Yarra River itself is divided into three main sections called Upper Yarra, Middle Yarra and Lower Yarra sections (Figure 1.1).
The Upper Yarra catchment is mostly a protected area (Millgrove to Warrandyte near Lilydale) to fulfil the provision of drinking water for the Melbourne metropolitan area. The activities from settlements nearby the river region below the water supply catchments such as agriculture, vineyards and stormwater from townships influence the water quality downstream of this section of the Yarra River. The Middle Yarra section is defined as the section in between Warrandyte and Dight falls in Kew. This section of the river is however dominated by human activities due to urbanisation. Significant impervious surface area contributes to increased runoff and stormwater inputs to waterways. The lower Yarra section continues from downstream of Dight falls to Docklands. Increased loads of pollutants enter the main stream in this section as a result of the increased number of stormwater drains entering the river in this area.

River health is getting further affected with increasing population or settlements in catchment areas. Increasing urbanisation, commercialisation and industrialisation plays a major part by increasing pollutant loads significantly to waterways (Melbourne Water, 2007). Water quality monitoring is an essential element of keeping waterways clean and thus attracting more people for recreational activities.
The lower Yarra region is mainly utilised for recreational purpose and it is a focal point for tourism in Melbourne (Victorian Government, 2005). Over the years, sewerage infrastructure throughout Melbourne has served the community well by improvising river health. The health of the Yarra River has improved since 1970’s and 1980’s, after the investment in sewerage infrastructure and industrial waste diverted away from stormwater drains to the sewerage system. More recently, gains have been achieved through further system upgrades. Councils and the development industry are working with water authorities to reduce stormwater pollution. Local community groups and water authorities are also becoming partners in a range of Yarra River improvement programs (Victorian Government, 2005) and through a structured approach for investing in a ‘sewer backlog’ program to progressively connect areas with septic tanks to centralised treatment. Melbourne’s drainage system was historically constructed in a way to remove stormwater quickly and efficiently to reduce the risks to public health and safety from local flooding. However, this allowed the entry of a range of pollutants to Melbourne’s streams and rivers unhindered.

The stormwater and drainage system consists of a complex network of underground pipes and a series of retarding basins and a number of wetlands constructed recently. The city’s extensive sewerage system carries sewage through series of pipes to two large sewage treatment plants. Faecal contamination from human sources either from sewer spills or leaking septic tanks can find its way into the stormwater system and subsequently to waterways. According to Melbourne Water’s Yarra River Action Plan, the major focus of the water authorities’ energy over the next ten years is to manage sewerage and stormwater and carry out research and investigations to trace key areas contributing pollution to the waterways. Melbourne Water is planning to invest around $600 million to secure a healthier Yarra River for the community (Victorian Government, 2005).

According to the Yarra River Action Plan (Victorian Government, 2005) and (Melbourne Water, 2004) the water quality in the lower reaches of the Yarra River is poor. The quality of water within the Yarra basin is depicted in Figure 1.2 (Victorian Government, 2005).

Based on “Our Water Our Future” (Melbourne Water, 2004) a number of long term projects have been identified to improve water quality in the Yarra River. One of these projects is to identify and eliminate key sources of faecal pollution. EPA Victoria is undertaking the Yarra Watch program which involved daily monitoring of water quality in the river. The objective of this program is to provide the information to the community, identify short term recreational water quality problems, target actions to
improve water quality and monitor the effects of catchment management by tracking changes in bacterial levels.

Figure 1.2: Water Quality in the Yarra Catchment (Victorian Government 2005)

1.1.1 Yarra River water quality

Unsuitable recreational water quality restricts recreational activities such as swimming, diving and water skiing, boating, fishing and wading. Yarra Watch program is especially developed to monitor river water quality, which monitors the river water quality each week providing details about the microbial activities within the water body. According to the state recreational water quality standards E.coli load limits should be less than 200 E.coli organisms/100ml for primary contact and 1000 E.coli organisms/100ml for secondary contact (Yarra Watch, 2007).

Yarra Watch (2007) categorises the water quality as high, medium, low and not suitable for recreational activities based on the E.coli levels. Water quality is defined as:

- High water quality: 200 organisms per 100 mL or lower. These sites are generally considered suitable for all forms of recreational activities.
- Medium water quality: 201 to 1000 organisms per 100 mL. Sites with water quality in this range are considered suitable for boating, but not generally suitable for swimming.
- Low water quality: 1001 to 5000 organisms per 100 mL. Sites with water quality in this range may be used for boating, but they are not suitable for swimming.
- Unsuitable for recreation: Greater than 5000 organisms per 100 mL. Sites with water quality in this range are considered unsuitable for any kind of recreational activities, with greatly increased potential risk to human health.

Haydon (2006) reported that the World Health Organisation (WHO) has identified 3.4 million deaths per year due to waterborne illnesses and diarrhoea was considered to be a prime reason (WHO, 2001) with 2.2 million deaths across the world, mostly in the developing world. Recreational water consisting high pathogen concentrations is a threat to human life, as it spreads gastrointestinal diseases or skin infections.

**1.1.2 Current focus of Yarra River water quality**

Melbourne Water and EPA Victoria (Victorian Government, 2005) stated that there is significant contribution of pathogens from urban stormwater systems to the waterways. The current research concentrates on the contribution of faecal contamination from dry weather sewer spills on the Yarra River water quality. Dry weather sewer spills in catchments occur due to:
- Sewer blockages due to solidified fat blocks;
- Roots finding their ways into sewer pipes; and
- Sewer collapses and cracks

There are complimentary studies carried out by other researchers to investigate the faecal contamination due to animal droppings wash off, illegal connections of sewer pipes into stormwater drainage, poorly maintained septic tanks and wet weather overflows (Victorian Government, 2005 and Wong, 2006).

The sewage that overflows during a dry weather spill is retained on the land surface and will get washed off to the stream during the storm event that followed the blockage. The growth of indicator organisms in water in the waterways or sediments is harmful to human health. The lifespan of faecal bacteria is less outside the body of an animal or a human being, and will decay faster and after some time, pathogens will become unnoticeable and ineffective (Wong, 2006). Many factors influence the depletion of
these bacteria’s lifespan outside the animal or human intestine: for example

temperature, UV exposure and salinity. Human pathogens contact to waterways has

significant influence as it can spread several diseases i.e. non gastrointestinal

illnesses, mild gastroenteritis, to severe and sometimes fatal diseases. The levels of

bacterial indicators after a storm may return to background levels within a day or two,

but in the absence of washout or proper amount of dilution, bacteria can stay active for

longer periods. The faecal coliforms can survive up to 2 to 3 weeks on grass after

sludge deposition depending on several environmental conditions and rainfall (Brown

et al., 1980). The report mentioned that municipal sludge deposition to soils is the most

hazardous as it transmits pathogenic bacteria and viruses.

The amount of faecal coliform that will be carried to the stream after a dry weather

sewer spill will depend on the magnitude of the storm as well as on antecedent rainfall

conditions that determine surface run-off and the weather during the period between

the dry weather spill and the wash off event. Minimising dry weather sewer spills is an

important aspect of protecting water quality for recreation and in general, a key

contribution to maintaining river health.

1.2 Objectives of Current Research Study

The objectives of this study are to:

• Determine the die-off or survival rate of microbes post – dry weather spill under
different climatic conditions;

• Model the movement of microbes from the location of the dry weather spill into
the stormwater system; and

• Estimate the significance of the dry weather sewer spills on stormwater quality
and loads transported to the waterways.

1.3 Research Plan

Based on the above objectives there are two phases of data collection for the research.
Both phases were carried out concurrently.
Phase I:
Controlled conditioned experiments at the Wastewater Treatment Plant (WWTP) to understand the survival rate of microbes on different types of surfaces (permeable and impermeable).

To determine the transport and die-off rate of microbes present in surface waters due to dry weather sewer spills, controlled conditioned experiments were conducted. These controlled conditioned experiments were carried out at Mt. Martha WWTP located in the South East Water’s region in metropolitan Melbourne. Two different kinds of surfaces (Figure 1.3) were used for the study. They are:
1) Grass cover on clay soil – Pervious Surface
2) Cement Sheets – Impervious surface

Under control conditions raw sewage was sprayed on field plots to simulate a sewage spill. Rainfall was simulated on these plots after predetermined elapse time periods (1 day, 2 days, 4 days post – spill etc.) to examine the die-off rate of microbes between the dry weather spill and the wash off event. The surface runoff from the plots was collected and sent to Ecowise Environmental commercial laboratories to determine the microbe and nutrient concentrations. The detailed experimental procedure is outlined in Section 3.4. The experiments were carried out in the summer of 2008 (S08), winter 2008 (W08) and summer 2009 (S09). The learning and results from the first set of experiments were used to plan the experimental setup of the second set and the results from the second set were used to plan the third set of experiments (i.e. designed to cover gaps). Experimental set up will be explained later in Section 3.4.1.
Phase II:
Actual dry weather sewage spill data collected from archived information to determine the significance of dry weather sewer spills on stormwater quality.

The dry weather sewage spill data collection was carried out at South East Water (one of the three water authorities in the Melbourne metropolitan area) with the assistance of an affiliated company, Utility Services. Usually, if a dry weather blockage (or sewer collapse) occurs the public will inform the water retail company. However, if the spills occur within a household property the blockages go unrecorded as it is the responsibility of the owner to get the problem fixed. Dry weather spill data from the catchments’ reticulation system will be extracted from the South East Water data base.

1.4 Outline of the Thesis
This thesis consists of seven (7) chapters and additional information in the support of this material is included as appendices and references.

Chapter 1 presents the background to Yarra River water quality issues, the research problem, sets objectives of the current research and provides the outline of the thesis. The research that has been carried out in microbial survival rates, impacts of sewer spill on waterways, various methods of tracing faecal contamination in waterways and pathogen modelling is discussed in Chapter 2.

Chapter 3 presents the climatic data at the site during the experimental period and field experimental procedure conducted to understand the survival rate of microbes after a spill event. The analyses of field data are presented in Chapter 4. Chapter 5 presents the relationships between the survival rate of microbes with climatic variables and time.

The collection of actual sewage data are presented in Chapter 6. The relationships developed earlier in Chapter 5 to estimate microbes’ survival rates are used to determine the impact of household sewer spills towards stormwater drains. Chapter 7 presents the summary, conclusions and recommendations for future investigations based on the current study results.
2.1 Introduction

As reported in Chapter 1, this chapter demonstrates a general overview with regards to recreational water quality, relevant Australian water quality standards (NHMRC, 2000). On top of this, the information of waterborne pathogens and related diseases, various methods to trace faecal coliform in waterways have been illustrated. Furthermore, the survival pattern of the microbes with time and other climatic variables along with the available stormwater quality models that can predict the contribution of microbes towards quality of waterways in catchments have been discussed.

This chapter concludes by selecting critical indicator organisms for testing, planned field experiments, and selecting the appropriate stormwater model with a view to predicting the microbes decay rate.

2.2 Recreational Water Quality

Environmental protection agency (EPA) is the governing body for the protection of waterways and other environment related issues in Victoria, Australia. EPA Victoria monitors the water quality in all the rivers, creeks, coastal and marine waters to maintain the safety of the public (EPA Victoria, 2007). Recreational water quality in Victoria is assessed by measuring physical, chemical and biological indicators. Yarra Watch program was developed to monitor the water quality of the Yarra River and its reaches (Yarra Watch, 2007). Above report lists the importance of water quality parameters and their role in determining recreational water quality.
Table 2.1: Water quality indicators and its impact on water quality (Yarra Watch, 2007)

<table>
<thead>
<tr>
<th>Indicators</th>
<th>Role in water quality</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>A measure of the water acidity or alkalinity which defines the toxicity levels of water for organisms in water body.</td>
</tr>
<tr>
<td>Salinity</td>
<td>Amount of dissolved salts in water bodies. Low salinity indicates fresh water source and indicates the water is suitable for agricultural purpose.</td>
</tr>
<tr>
<td>Toxicants in sediments</td>
<td>Many of the trace elements are required for microbes or aquatic animals. However higher concentration indicates contamination of water and is hazardous.</td>
</tr>
<tr>
<td>Suspended solids</td>
<td>It is the particulate material suspended in water such as soil, plankton or organic debris. Benthic environment gets lack of solar rays and light as a result of high particulate matter in water.</td>
</tr>
<tr>
<td>Turbidity</td>
<td>Turbidity is measure of light scattering due to suspended particles. Light penetration is measured by turbidity of water</td>
</tr>
<tr>
<td>Oxygen</td>
<td>Dissolved oxygen is the most important factor for the life of aquatic animals and is important in eutrophication process.</td>
</tr>
<tr>
<td>Faecal coliforms</td>
<td>Faecal coliforms indicate threat to human health. Various sources are animals, human excreta, wildlife, emergency relief structures (ERS) and sewer spills. E.coli, enterococci organisms are used as indicator organisms to trace faecal pollution in waterways.</td>
</tr>
</tbody>
</table>

Water quality issues in the Lower Yarra catchment are due to high concentrations of heavy metals, nitrogen, sediments and microorganisms (Yarra Watch, 2007). Studies conducted by Melbourne Water during 2003 and 2004 indicate that there is a significant variation in E.coli levels for different stream flows and storm events. The study further noted that the faecal indicator levels vary significantly due to variation of flow.

Stormwater contains contaminants or pollutant loads, source of which is urban runoff. Pollution loads consist of Suspended Solids (SS), nutrients, oils and Surfactants, litter and micro-organisms. These pollutants have significant ecological and aesthetic impact.
on water bodies. Wong (2006) reported that the common bacteria found in stormwater are faecal coliform and specific pathogens such as salmonella. The most common sources of micro-organisms in urban catchments are from sewer spills and animal faeces, such as dog faeces. Roof of a household can be considered as the prime contributing factor of microbe’s deposition because it is directly connected to the drainage system. Faecal coliforms are invariably used as an indicator of faecal contamination of water. Faecal coliforms are a subset of total coliforms, and are more closely associated with faecal contamination than the total coliforms. Escherichia coli (E. coli) and enterococci are members of this group, and are widely used as an indicator of faecal contamination. The variation of bacterial contamination within the catchment could be due to:

1. Animal (eg. bird and dog) droppings washed off
2. Illegal connections of sewer pipes into the stormwater drainage system.
3. Poorly maintained septic tanks
4. Dry weather sewer overflows due to structural collapses or blockages from tree roots; or
5. Wet weather overflows via the emergency relief structures (ERS).

### 2.2.1 Recreational water quality standards set by NHMRC (2000)

Water bodies attract the tourists and also are used mainly for recreational purposes such as swimming, bathing, boating, fishing, and other water sports. To protect the health and safety of the user water bodies should be free from faecal contamination, pathogens and other hazards. National Health and Medical Research Council (NHMRC, 2000) stated that the water quality guidelines would facilitate the protection of water bodies to maintain their standards. This will ensure safe ambience for recreational activities. Recreational water quality standards set by (NHMRC, 2000) are defined as:

- **Primary contact** – The median bacterial content in fresh and marine waters during bathing season should not exceed 150 faecal coliform organisms/100mL or 35 enterococci organisms/100mL. Pathogenic free-living organisms should be absent from bodies of fresh water.

- **Secondary contact** – The median value in fresh and marine waters should not exceed 1000 faecal coliform organisms/100mL or 230 enterococci organisms/100mL.
• **Nuisance organisms** – Algal species above 15,000 – 20,000 cells/mL suggest that there should be restriction on direct contact activities such as swimming. Macrophytes, phytoplankton scums, filamentous algal mats, sewage fungus, leeches etc. should not be present in excessive amounts.

### 2.2.2 Water quality indicators

Pathogens are considered to be a major threat in recreational waters because they can propagate number of harmful diseases. Berg (1978) reported that presence of microorganisms in waters indicate the existence of pathogens. Pathogens may enter waterways through different sources such as animal droppings, poorly maintained sewer pipes, stormwater system, dry and wet weather overflows. Urban catchment contributes significant amount of pathogens in waterways. There are several microbial indicators of pathogens in waterways. In particular, *Escherichia coli* (*E.coli*) is one of the members of the faecal coliform group and recommended as an indicator organism all over the world. U.S. Environmental Protection Agency and E.P.A. Victoria recommends to used *E.coli* as an indicator organism to trace the faecal contamination in recreational waters or fresh water bodies (Kimberly et al., 2005) and enterococci for fresh as well as saltwater. *E.coli*, enterococci or streptococci bacteria are related to faecal material (Human, animal or birds). *E.coli* or enterococci could be used in water quality assessment as these pathogens have the tendency to multiply mainly in human or animal intestines (US EPA, 2007).

### 2.2.3 Waterborne diseases

The presence of indicator organisms indicates the threat of possible occurrence of diseases like throat, eye, ear infections or weakening immune system. Nevertheless, *E.coli* or enterococci monitoring may be inappropriate to trace the human faecal source. It is essential to monitor faecal microbes, virus or chemicals that do not strengthen in the environment i.e. spores of *Clostridium perfringens* or male specific F-RNA coliphages to trace this kind of source. Human traces of faeces can occur due to recreational activities, cross connections of sewer pipes into stormwater drains and faecal loads caused by animal excreta. Studies on stream water and microbial organisms to maintain water quality of several water bodies conducted by USEPA establish *E.coli*, enterococci, and *Clostridium perfringens* as indicator organisms and developed water quality standards and tests to maintain reservoir water quality. Carillo
et al. (1985), Wright R. (1989), Desmaris et al. (2002) and Shibata et al. (2004) reported that faecal coliform, E.coli and enterococci can be found in an environment without any known source of contamination of raw sewage and multiple within warm tropical environments. E.coli itself can be pathogenic or non-pathogenic organism and in some circumstances can be harmful for human health. To trace the faecal contamination from human source microbes or virus like Clostridium perfringens and FRNA coliphages have been used in recent years as a result of densely populated areas in urban catchments and increasing commercialisation and industrialisation. The description of some of the indicator organisms currently in practice to trace faecal contamination is given below:

1. **Faecal coliform**

Faecal coliform is a subgroup of coliform bacteria. Total coliform contains faecal coliform and several other microorganisms. These microbes have an ability to grow at high temperatures (44.5°C). The presence of faecal coliform suggests the source of the contamination is from warm-blooded animals (WHO, 2003). The recent studies of these organisms haven't found any relationship between pathogenic diseases and presence of specific faecal coliforms in waterways.

2. **Escherichia Coli (E.coli)**

This type of micro-organism is present in gastrointestinal tract and faeces of warm-blooded animals. The determination of E.coli is carried out by counting the number of yellow and yellow brown colonies on 0.45 micron filter placed on m-TEC media and incubated at 35°C for 24 hours (WHO, 2003). The presence of these bacteria's in freshwater indicates faecal contamination.

3. **Enterococci**

This is a subgroup of faecal streptococci bacteria. It is commonly found in human intestinal tract. The indication of these bacteria in waterways suggests a human source of pollution. This is a preferred indicator organism for freshwater and marine recreational waters. The determination is done by counting pink to red colonies containing black or reddish brown precipitate at the bottom of a 0.45 micron filter placed on m-E media for 41°C for 48-50 hours, then for 20 minutes on EIA media (WHO, 2003).
4. **Clostridium perfringens**
This bacteria is found in high concentration in human and animal faeces as well as sewage, its presence in waterways indicates faecal pollution from human sources. *Clostridium perfringens* does indicate remote, intermittent and point source pollution as it can form the spores which have more resistance to disinfection and environmental stress than other indicator bacteria (WHO, 2003). This indicator organism must be analysed under anaerobic conditions and by highly trained technicians.

5. **FRNA coliphages**
Havelaar et al. (1993) suggested that it was not sufficient to measure only *E.coli* and enterococci as an ecological stressors and indicator organisms in waterways. There are number of viruses present in waterways which can harm human beings by faecally polluting water bodies. Host specific nature of enteric viruses derived by human waste leads to enteric viral diseases (Calci et al., 1998). Somatic coliphages have been reported as being a heterogeneous group of organisms (Havelaar et al., 1990; Calci et al., 1998). The source of these coliphages can be a faecal stressor. The presence of these viruses in faecal matter may indicate the simultaneous presence of pathogenic viruses.

FRNA coliphages have been recommended as a useful substitute to the traditional bacterial indicators as their morphology and survival characteristics closely resemble those of some of the important human enteric viruses (Havelaar et al., 1993). FRNA coliphages can serve as model organisms and suitable indicators to indicate the possible presence of human pathogenic enteric viruses as they behave like water-borne viruses for monitoring purposes (Havelaar et al., 1993). These coliphages are resistant to UV than other micro-organisms (Wiedenmann et al., 2002) and can survive longer in hot climatic conditions.

2.2.4 **Water quality levels in the Lower Yarra River**

Figure 2.1 shows the variation in *E.coli* levels in the Lower Yarra between 1977 and 2005 (Wong, 2006). Figure 2.2 shows the annual geometric means at sites along the Yarra River from 2005 to 2007. The graphical representation of data shows that the *E.coli* levels are above 200 organisms/100ml, in the Lower Yarra reaches suggesting that the levels are higher than the recommended standards for primary recreational contact.
Figure 2.1: Annual geometric mean of *E.coli* levels in the lower Yarra River (Wong, 2006)

Figure 2.2: Annual geometric means at sites along Yarra River over the first two years (2005-2007) of Yarra Watch (Yarra Watch, 2007)

Figure 2.3 graphically represents the faecal coliform in storm runoff from a range of land uses (Wong, 2006). The faecal pollution from urban areas varies from 600 to 10,000 *E.coli* organisms/100ml. Faecal coliform levels in residential areas vary between 10,000 to 110,000 *E.coli* organisms/100ml. McCarthy et al. (2006) also suggested that faecal contamination was higher in runoff in urban areas than industrial or commercial areas. The microbe contribution from residential areas (Figure 2.3) identifies that it is important to monitor and minimise faecal contamination from residential (or heavily urbanised catchments) to streams.
Robinson et al. (2007) carried out a screening study on Yarra River and its catchments. The study noted that in the Lower Yarra section, the *E. coli* contamination is high during dry weather as well as during wet weather spill event. During wet weather period, sewerage systems can exceed the original design capacity. This can sometimes lead to spills of diluted sewage into rivers, drains and creeks. Spills can occur in dry weather when sewers become blocked or a sewer collapse occurs. Melbourne Water and the retail water companies have invested heavily in infrastructure in the past 10 to 15 years to reduce emergency relief structures (ERS) overflows during wet weather surcharges.

Based on the above water quality levels of the Yarra River, *E.coli*, enterococci and, FRNA coliphages are identified as critical indicator organisms to investigate the survival rate after a dry weather spill.

### 2.3 Behaviour of Indicator Organisms with Climate Variables and Soil Parameters

As mentioned in Chapter 1, sewage effluent leaking from septic tanks and sewer spills, livestock, industrial process, farming activities, domestic animals, excreta of birds, wildlife and recreational water can itself contain free living micro-organisms contributing towards the faecal contamination of waterways. Micro-organism or microbes index in recreational waters is divided into bacteria, viruses, protozoa and Helminths. Within all
these groups, presence of bacteria and viruses in the environment is dangerous as they are a risk to human health. For information on bacterial life cycle outside of human or animal body (i.e. survival rate of different pathogens) factors that enhance or affect the growth of bacteria are important. The survival of these microbes can vary from half an hour to several years depending on factors such as the soil type, soil moisture, pH, antibiotics, toxic substances, nutrients, organic matter and climate variables like temperature, sunlight, relative humidity, vapour pressure etc. Reddy et al. (1981) reported that the survival rate or die-off rate of microorganisms is dependent on several climate variables such as sunlight, UV, and rainfall. Chamberlin and Mitchell (1978) reported that the intensity of sunlight is a significant factor as it affects the survival of pathogens and indicator organisms under natural conditions. The author further stated that microorganisms in turbid waters and at the bottom of sediments survive longer due to low sunlight intensity and UV rays. However, viruses and protozoa can tolerate sunlight and UV rays better than bacteria (Johnson et al., 1997). Gantzer et al. (2001) stated that salinity of water does not affect the survival of total coliform, *E.coli*, enterococci, faecal coliforms, faecal streptococci and viruses. Predation and competition of microorganisms is a complex and nutrient supply in nature influences the die-off of the indicator organisms in the natural environment (Gauthier and Archibald 2001, Russel and Walling 2007). Transport of microorganisms from soil surface to waterways under different climate conditions determine the effect on water quality and risk to human health (Muirhead et al., 2006). It was decided to incorporate climate variables such as relative humidity, temperature and vapour pressure in the analysis with time to identify a possible relationship with the die-off rate of microbes. Additionally, potential evapotranspiration was also considered to develop relationships as it depends on the several climate variables in nature. Time is the most important factor for the estimation as the die-off rate of microbes under natural condition changes with time as they are exposed to the above mentioned parameters within that frame.

Soil characteristics must be understood and studied to understand the survival of microorganisms in different catchment areas. Soil pH and soil moisture play an important part in survival or die-off of indicator organisms and pathogens transportation on soil surface. Essington (2004) reported that the pH of soil below 4.0 to 4.5 and more than 8.5 usually indicates human activities. On the other hand, pH values less than 5.0 to 5.5 are a concern for the environment. Moreover, the study stated that the soil particles hold many living organisms. Energy, soil structure and organic compounds present in the soil helps these microorganisms to hold on to soil particles and survive longer in nature. Ellis and McCalla (1976) and Reddy et al. (1981) stated that *E.coli*, S.
faecalis, salmonella etc are known to survive better in pH range 6 – 7. Nutrient concentration of soil also acts as a beneficial component for microbe’s survival. The addition of nutrients such as nitrogen and phosphorus will help understand the effect of land use pattern and its impact on waterways.

Based on the above information, it can be asserted that it is important to observe the total nitrogen and phosphorus concentration in addition to indicator organisms. It was also decided to incorporate soil parameters such as pH and soil moisture in the analysis with a view to identify a possible relationship with the die-off rate of microbes.

2.4 Impact of Sewer Spills on Waterways

‘Our Water Our Future’, (Victorian Government, 2005) reported that the basic aim of the Environment Protection Agency (EPA) and Melbourne Water is to secure the sustainable water future for the community. To achieve the goals or targets maintaining public health or ecosystem, Melbourne Water and EPA Victoria monitors its waterways on weekly basis (Yarra Watch, 2007). Sewer overflows cause significant impact on water bodies and potential risk to human health as this untreated sewage contains harmful pathogens (Pollard et al., 2004). Wet weather sewer spills and dry weather sewer spills contribute towards the pollution of waterways.

A study conducted by the Brisbane City Council (Pollard et al., 2004) revealed that the impact of dry weather sewer spill is considerable and is a higher public health hazard as compared to the impact from wet weather sewer overflows and it is known as dominant stressors of ecological health. The above statement highlights the importance in tracing faecal loads in stormwater systems after a spill event.

2.5 Various Methods of Tracing Faecal Coliform

Seurinck et al. (2005) configured faecal pollution containing both human as well as animal faeces as a serious environmental problem that affects many coastal and inland waters all over the world. Seurinck et al. (2005) reveals that every city council or Environmental Protection Agency (EPA) of a city has used several methodologies to determine the presence of faecal contents in catchment areas. Anderson et al. (2005) and Seurinck et al. (2005) reported about the microbial source tracking (MST) technique studied in Europe to identify faecal pollution in the aquatic environment.
Chemical microbial source tracking methods can be used to trace mainly the sewage pollution. Source specific bacteria or viruses are cultured using the MST method and with the help of it the source of faecal pollution can be traced. The report “Microbial source tracking for identification of faecal pollution”, Seurinck et al. (2005) observed that no single technique had been able to consistently identify all possible sources of faecal pollution in the water environment.

The Ohio Environmental Protection Agency (OEPA) is responsible for looking after the water quality monitoring of Cuyahoga River in Ohio (Plona, 2002). Parameters considered in the monitoring program of river sites are dissolved oxygen (DO), conductivity, turbidity, pH and temperature, alkalinity, chloride and faecal coliform. According to OEPA standards, faecal coliform for recreational activities should not exceed 1000 \(E. coli\) organisms/100ml. faecal coliforms for primary contact recreation, should not exceed 126 \(E. coli\) organisms/100ml. since 1984, the faecal coliform level exceeded the standards for primary contact recreation in the Cuyahoga River in Ohio, especially after a rainfall of 5 mm or more (Plona, 2002) during wet and dry weather events. The microbial data’s in between 1998-2002 were analysed for trends in \(E. coli\) levels. All streams were showing bacterial contamination higher than 1000 \(E. coli\) organisms/100ml. High \(E. coli\) levels suggest sewage pollution due to point or non point sources. Tinkers Creek in Ohio which is a highly urbanised watershed showed high levels of bacteria which is considered as the indication of anthropogenic sources. From investigations they found that wastewater from treatment plants was discharging into the stream. As a result, they stopped discharging wastewater into streams except from a small treatment plant in Dover Lake Water Park.

Determining levels of faecal indicator bacteria by conventional methods requires at least 18 hours to process and culture samples before results are available. Bushon et al. (2002) reported 18 hours to be too long with a view to assessing water quality and implementing adequate control measures to warn recreational users of a health hazard. Decay, dilution, dispersion, and transport of faecal indicators bacteria in water cause concentrations to change greatly over a short period of time. Lee and Deininger (2001) introduced a rapid assessment method ‘Immunomagnetic separation (IMS)/Adenosine triphosphate (ATP)’ to determine the water quality parameters. US Geological Survey scientists, in partnership with the National Park Service in Ohio tested the above rapid method that provides estimates of \(E. coli\) concentrations in approximately one hour. Bushon et al. (2002) compared the data collected from conventional method (US Environmental Protection Agency 2002) with the above rapid
Immunomagnetic separation (IMS)/Adenosine triphosphate (ATP) method. It consisted of 206 samples from 3 sampling locations. Fifty-nine percent of the samples collected exceeded the recommended standard for \textit{E.coli}. Strong statistical correlation relationships were developed between data obtained from the conventional method and IMS/ATP method. As a result of this study Bushon et al. (2002) concludes that the IMS/ATP method could be used as an alternative method to using the conventional method in determining \textit{E.coli} concentrations at a river, especially if results are required in a very short period of time.

Investigations of sewage overflows in the Lota creek in Brisbane were carried out by the Coastal CRC in collaboration with the Brisbane City Council to determine risks of sewer overflows to public and ecosystem health (Pollard et al., 2004). The sewer overflow due to dry and wet weather was monitored from 7 overflow structures in the lower Lota catchment in Brisbane. Above authors reported that during both the dry and wet weather overflow event the faecal indicators increased by several orders of magnitude above public health guidelines for primary contact (swimming). Based on measurements of the human sterol biomarker all of the faecal contamination was of human origin during the dry weather overflow event. During the wet weather event, stormwater contributed 80\% of the indicators; only 20\% being human origin. However, above authors reported that the unacceptably high public health hazard remained during the wet weather overflow despite the dilution due to the rain.

2.5.1 Methods used for recreational water quality analysis

EPA Victoria is implementing the Yarra Watch program which consists of monitoring water quality on a daily basis. The aim of this project is to provide the information to the community, identify short term recreational water quality problems and to target improvement actions and monitor the effects in catchments management by tracking changes in bacterial levels. Primary and secondary contact objectives for recreational water quality are set by State Environmental Protection Policy (SEPP), both SEPP and Yarra Watch used the weekly geometric means of \textit{E. coli} measurements to assess the recreational water quality (Yarra Watch, 2007). Weekly conditional ratings are given to each Yarra Watch site based on the geometric mean of water quality of the latest five weekly samples. The information of recreational water quality data collected by Melbourne Water between 1999 and 2004 indicates high levels of \textit{E.coli} at Heidelberg, Kew and in the lower Yarra region. Follow up \textit{E. coli} samples taken by Yarra Watch on above locations showed highly variable amounts of contamination. A relative program called, “Screening investigation of faecal pollution sources of the Yarra River” was
carried out at the same time by EPA Victoria and Melbourne Water to trace loads of pollution through input sources. This program has identified intermittent faecal contamination at few locations along the Yarra River. The study also has identified that most variable loads (i.e. 31 to 52000 E.coli organisms/100ml) of pollution enters the river system at the Prahran Main Drain and the Gardiner’s Creek and has high E.coli loads throughout the catchment (annual geometric means from 2000 to 2005 is in the range between 913 to 1984 E.coli organisms/100ml).

Robinson et al. (2007) screening study consisted of monitoring pollutant concentrations on a set number of occasions which covered both dry and wet weather conditions. Flow rates were measured to provide an indication of the relative contributions to the faecal load in the river from various sources of faecal coliform inputs. Robinson et al. (2007) discovered bacterial contaminated sites or locations and ranked them on the basis of E. coli concentrations. To identify the sampling locations, a team of investigators visited each reach. Melbourne Water conducts weekly water quality sampling for estimation of amount of E.coli contamination along 12 sites on the Yarra River from docklands to Warburton. Yarra Watch monitoring program sites were also included to this screening study and a total of fifty two sites were selected (13 on Yarra River, 29 on stormwater drains and 10 on significant tributary systems). Parameters analysed at each sampling site were turbidity, dissolved oxygen, pH, conductivity and temperature. Recreational risk assessment was based on E. coli concentrations.

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For water quality analysis, the bacterial types measured were E.coli, enterococci and Clostridium perfringens. The World Heath Organisation guidelines for coastal and freshwaters were used to provide a weighting for human and non-human faecal inputs. Drain and Tributary ranking method (Robinson et al., 2007) was used to determine the relative significance of inputs on recreational values of the Yarra River. During this investigation two kinds of ranking procedures were used, initial ranking and overall input ranking to derive the value of E.coli concentrations in input loads. The potential impact of each input was estimated by combining load and flow estimates for a drain or tributary and with load and flow estimates for the Yarra River site immediately upstream. Overall ranking was set on the basis of initial rankings given to the sampling sites. Sampling was intentionally biased towards dry weather as the river was in a dry weather flow state for most of the time. McCarthy et al. (2006) reported on uncertainties in the field measurements on flow, pollutant loads and concentrations. The above authors reported that the errors due to uncertainties of event mean concentrations (EMC) measurement of pollutants were negligible. However, these
uncertainties could affect correlations between successive flow measurements considerably.

Robinson et al. (2007) showed that, in dry weather, the majority of input drains have got much higher loads and concentrations of *E.coli* bacteria than either tributaries or the Yarra River. In wet weather, both the drains and tributaries had much higher concentrations and loads of *E.coli* than in dry weather. Moreover, it was observed that tributaries got more concentration of bacterial load than drains in wet weather. This investigation found a significant human faecal coliform component in the Yarra River contributed from local catchments. Harper Street and Elizabeth Street main drains were clearly contaminated with a high proportion of human faecal matter. The levels of *E.coli* in the Yarra River are constantly changing all the time and may exhibit high spatial variability. The river carries greater *E.coli* loads than its tributaries and drains. A working group comprising personnel from EPA Victoria and Melbourne Water assigned priorities for action requiring detailed follow-up investigations on each of these high priority drains and tributaries. From these studies, 12 stormwater drains and three tributaries were considered as high priority sites as they have the greatest impact on the recreational value of the Yarra River. Secondary investigations had been carried out on the Prahran Main Drain as a result of EPA investigation findings. In this study, according to Coleman (2001) and Rooney (2007) there were significant variations in catchments and further research is necessary to track down the source and load of the spills in the catchments.

Yarra River Action plan (Victorian Government, 2005) recommends providing better management of urban drainages and sewerage infrastructure in the Yarra Catchment. The middle and lower Yarra River is an important reach of the river which is used for recreation more intensely under dry weather conditions. EPA Victoria (2007) recommends focusing on tracing and remediating human faecal sources discharged during dry weather conditions to the Yarra River. It has also stated that human faecal contaminations are more harmful to the environment than others. Urban catchments consist of water supply, stormwater and sewerage infrastructure. Stormwater and sewerage systems get finally discharge to the ocean; stormwater untreated and wastewater after treatment at a wastewater treatment plant (Lloyd et al., 2002). The contamination of pathogens in recreational waters is dangerous to human health. Lloyd (2002) observed that some measures should be taken to improve stormwater run-off quality before discharging it into waterways. Inland water bodies are often monitored for microbial pollution as they are an important natural and recreational resource.
Russell and Walling (2007) reported that pathogens are of major concern for water resource management as it causes diseases. Recreational river water has a mixture of pathogenic and non-pathogenic micro-organisms. Pathogens can cause gastrointestinal disease through infection or ingestion of the body parts or skin. These organisms have an ability to multiply outside the host body if they get favourable environmental conditions (Fujioka et al., 1981). Pathogens can remain in water after its treatment and remain infectious for a considerable length of time (i.e. from a few hours to a week). The numbers of pathogens vary according to the time span and depend on climatic conditions and the die-off rates under different circumstances.

Russell and Walling (2007) noted that the human and the animal population contributed a lot towards the pathogen contents in sewage or stormwater. According to Seurinck et al. (2005), recommended combination of multiple methods to estimate the targeted faecal pollutant. A technique developed by CSIRO named Sterol analysis (Leeming et al., 1998) has been used in the screening study undertaken by Robinson et al. (2007) to identify human sewage contamination. Sterol analysis indicates the presence of the human faecal contamination source. Standards set for sterol analysis were 550 $E.\text{coli}$ organisms/100mL for the Yarra River and 1000 $E.\text{coli}$ organisms/100mL for all drains and tributaries, as it indicates presence of human faeces.

Drain tracking techniques were used to further trace $E.\text{coli}$ contamination at drains where continually high concentrations of bacterial loads are found. This is a novel approach. The water samples are collected from small drains after walking inside the main drain and locating the once that are smaller draining to the main drain. These samples are in turn analysed to identify the small drains which contribute high loads of pollutants to the main drain (Rooney, 2007).

Large scale rainfall simulator experiments were carried out by Government of New Zealand (2002) in Pukemanga catchment, within the Whatawhata Research Station, west of Hamilton. The prime aim of these experiments was to quantify the delivery of microbes under heavy rainfall to waterways and examine the variation with grazing livestock. The experiments were conducted on hill area during summer and winter before and after grazing of sheep on rye grass vegetation to understand the loads of microbes in mainstream water. Outcome from this experiments indicated that the surface runoff is an important mechanism for the delivery of microbes towards
waterways. The results from experiments confirmed that the surface runoff process could play a dominant part in transport of faecal microbes during large storm events.

Collins et al. (2002) carried out a field study to understand the effect of riparian buffer strips on entrapment of faecal microbes during surface runoff. The study involved spraying dairy farm effluent and cow pats onto 4 marked grass plots, following rainfall event to generate surface and sub-surface runoff. It was then collected at the lower end of the plot and analysed further for microbial contents remained in a collected sample. Experimental soil plots were established on Ruakura campus farm, Hamilton. The experimental set up used for this study was on riparian buffer and cow pats with the marked experimental plots and rainfall runoff as explained above. Current study is concentrated on the surface runoff after spill events in urban areas of Melbourne, Victoria. Modification of this experimental set-up will help to achieve the objectives of this study. Therefore, it was decided to conduct controlled conditioned surface runoff experiments during summer and winter periods to understand the die-off rate of selected indicator organisms (E.coli, enterococci and FRNA coliphages) with time under antecedent climate conditions. The field set up for experimental purpose was based on the study called," Riparian attenuation of faecal microbes" (Collins et al., 2002) and is described in the following chapter.

2.6 Urban Stormwater Quality Models

Zoppou (1999) and Wong et al. (2001) reported a simple first order decay function to estimate the removal of pollutants. In addition to this, Driscoll et al. (1979), and Huber (1992) mentioned that the concentration of pollutants predicted with regression models and statistical models can be used in combination to predict the quality of water. Zoppou (1999) mentioned that the first flush of storm event contributes the most towards the transportation of accumulated pollutants. The simple wash-off equation is expressed below:

\[
\frac{dP_w}{dt} = -k_w r P_w 
\]  \hspace{1cm} \text{…………………………………………………………………… (2.1)}

where,

- \( P_w(t) \) is the pollutant present at time \( t \)
- \( k_w \) is empirical pollutant removal coefficient and
- \( r \) is runoff flow rate
(Zoppou 1999) reported a number of stormwater models which are available to simulate water quality and quantity in urban catchment. Zoppou (1999) mentioned that all the models need flow rate to estimate the water quality. As a result all water quality models incorporate a hydrologic component. The above author reported that subsurface flow in urban catchment is very low as a result of large impervious area. The author recommends 1 in 10 year storm event as an ideal storm to use for modelling purposes in urban catchments. Zoppou (1999) referred to eight models specifically designed to simulate urban storm water quality:

- DR₃M-QUAL (Alley and Smith 1982)
- HSPF (Bicknell et al. 1993, Johanson et al. 1980 and Johanson et al. 1984)
- MIKE – SWMM
- QQS (Geiger and Dorsch 1980)
- STORM (Hydrologic Engineering Center 1977)
- SWMM (Huber and Dickinson 1988, Huber et al. 1984, Roesner et al. 1988)
- SWMM Level 1 (Heaney et al. 1976)
- Wallingford Model (Bettess et al. 1978, Price and Kidd 1978)

Nevertheless, most of the above stated models are developed by the United States government funded agencies and are commercially available but are expensive. The models are available with very little support. However, many of the above stated models are based on build-up and washoff process where washoff rate is directly proportional to the generated runoff. Besides this, Zoppou (1999) further informed that these stormwater models need the separate washoff functions. Table 2.2 presents the comparison between the stormwater models, predictive methods and time scale. This tabular format will facilitates to understand the circumstances predictive models can work in, and the functions they are dependent on for predicting stormwater quality. The above stated stormwater models can simulate total dissolved solids (TDS), chlorides, pesticides, temperature, pH, CO₂, algae, nitrate, total inorganic carbon, DO, BOD, ammonia, phytoplankton, zooplankton in streams and storages. However, not all the above stated stormwater models can be used to model faecal coliforms or total coliforms as indicated in Table 2.2. On the other hand, the above stated models were not considered for the current study as these statistical models are expensive packages with limited technical support.
Table 2.2: Characteristics of the mentioned stormwater models (Zoppou 1999)

<table>
<thead>
<tr>
<th>Model</th>
<th>Total coliform</th>
<th>Pollutant Predictive Method</th>
<th>Time Modelling Scale</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Empirical</td>
<td>Build-up and washoff</td>
</tr>
<tr>
<td>DR$_3$M-QUAL</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>HSPF</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>MIKE – SWMM</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>QSS</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>STORM</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>SWMM</td>
<td></td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>SWMM Level 1</td>
<td>✓</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wallingford Model</td>
<td>✓</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Water Sensitive Urban Design (WSUD) is not only an innovative concept but also an effective one to carry out at site water cycle management when planning or designing urban catchments. Wong and Eadie (2000) identified that WSUD was accepted and used across Australia to plan or design urban catchments. Model for Urban Stormwater Improvement Conceptualisation (MUSIC) which was developed by the Cooperative Research Centre for Catchment Hydrology (CRCCH) is widely used in Australia as a conceptual design tool to assess the stormwater quality or quantity transported to waterways and maintains WSUD standards (Melbourne Water, 2004). A simplified mass balance equation by Chiew and McMahon (1997) was used to estimate urban runoff in the MUSIC model. The MUSIC model helps determining water quality from specific catchments, design the stormwater management plan and evaluates the benefits of specific treatments within the catchments. One of the significant characteristics of this model is that, it can be applied from small ($0.01\text{km}^2$) to large catchments (over $100\text{km}^2$) and is based on a time continuous simulation process with time steps from 6 mins to 24 hours to match catchment scale. The removal of
pollutants within the catchment is simulated by a combination of first order kinetic
model and a continuously stirred tank reactors (CTRs) model. MUSIC conceptual tool
predicts water quality based on the removal of suspended solids through swales,
wetlands, ponds and other filter barriers. It incorporated algorithms to determine the
removal of pollutants from above mentioned filter barriers. However, this model does
not incorporate algorithms to estimate the removal of faecal coliforms or indicator
organisms that were collected on catchment surface. Furthermore the objective of the
study is to determine the survival rate of microbes on the catchment surface after a dry
weather spill and to estimate the loads transported to the waterways from the location
of spill to the urban drainage system.

Haydon (2006) developed a pathogen model (EG model) to determine the
concentrations of pathogens through runoff from catchment. The EG model is based on
two major processes in a catchment i.e. build up of microbes and wash-off of microbes
(Figure 2.4). When developing the model Haydon (2006) considered the build-up of
microbes in a catchment is dependent on the deposited faecal material due to sewage
effluent leaking from septic tanks, dry and wet sewer spills, livestock, industrial process
waste, farming activities, domestic animals, excreta of birds and wildlife. Furthermore, it
was considered that the die-off of pathogens is highly dependent on number of factors
such as temperature, relative humidity, rainfall intensity and predation. The
accumulated pathogen load on surface then gets washed off with rainfall event.
Haydon (2006) also considered pathogen wash-off to be a function of the kinetic
energy of rainfall which breaks the bonding between pathogens and surface store
eventually resulting into the flow transporting pollutants off the catchment (Novotny and
Olem (1994); McCarthy (2008)). Haydon (2006) considered pathogen deposition,
storage, movement and decay from surface and sub-surface as these are main
components in large catchments for prediction of microbial contamination. The above
author collected the historical pathogen and hydrological data from three different
catchments for the study (O'Shannassy (Victoria), Myponga (South Australia), and
Aldgate (South Australia)). Haydon (2006) further coupled this data with EG model to
examine the pathogen survival and transport within the catchment. Large number of
pathogens can be transported with surface runoff due to storm event, the study
observed. The potential evapotranspiration and soil moisture were considered as two
important factors in determining survival rate of pathogens on the surface storage.
However sensitivity analysis of the above stated EG pathogen prediction model
showed that the pathogen transport process is more important than the pathogen
decay process.
The simplified EG pathogen model was coupled with SymHyd rainfall runoff model (Chiew et al., 2002) to simulate the pathogen concentrations transported to waterways from contaminated water catchments. The developed EG model was tested on rural catchments to estimate the pathogen concentration from surface and sub-surface storages (Figure 2.4). Haydon (2006) reported that the developed model was applicable to rural and forested catchments than for urban catchments. The author also mentioned the catchment size, event based pathogen data and modelling time step are vital factors for this model. The current research is concentrating on dry weather sewage spills in urban catchments. This model cannot be applied to estimate the loads from dry weather sewer spills as it assumes continuous deposition of pollutants with time.
McCarthy (2008) developed a microorganism model, “MOPUS” (Micro-Organism Prediction in Urban Storm Water). This model predicts the microbial concentration in an urban catchment based on the catchment details such as animal and human density in catchment, vapour pressure and relative humidity. The experiments were conducted to gather the data from selected catchments for *E. coli* and other hydrological data. The origins of the microbes were detected using simple microbial source tracking method in selected urban catchments. This data was mainly concentrated from wet weather events; however the data from dry weather events were also collected for developing the microbial prediction model. McCarthy (2008) simplified SymHyd model (Chiew et al., 2002) and developed a spatially lumped model in one minute time step to estimate microbes contributed from pervious and impervious surfaces to waterways (Figure 2.5). The variables in Figure 2.5 are as follows:

- Simp(t) = Impervious surface store (mm)
- I(t) = Rainfall depth (mm) at time t
- ImpEvap(t) = Amount of water removed from the impervious store by evaporation based on time (mm)
- Simpmax = Capacity of the impervious store (=1mm)
- Sperv(t) = Pervious surface store (mm)
- PervEvap(t) = Amount of water removed from the store due to actual evapotranspiration (mm)
- Qimp(t) = Outflow from the impervious store (mm)
- Qperv(t) = Outflow from pervious store
- Qseep(t) = Amount of water lost from the pervious store to deep seepage (mm)
- Spervmax = Capacity of the pervious store (mm).

Figure 2.5: Rainfall - Runoff model (McCarthy, 2008)
The above stated rainfall runoff model is then coupled with the microorganism model (MOPUS) to predict the concentration of microbes. The simple concept of build up and washoff was simulated with surface and sub-surface components to estimate the number of microbes in the stormwater system. As mentioned earlier, the pathogen load in the catchment is calculated based on domestic animals, wildlife, collapsed septic/sewer systems, illegal sewer/septic connections etc. In addition to McCarthy (2008), Crane and Moore (1986) also reported that the temperature, pH, moisture content, nutrient levels, salinity, and toxicant could play a significant role in microbial die-off on the surface component. The MOPUS model calculates the micro-organism levels on surface store from Equation 2.2.

\[
P_s(t) = 10^{PsCoeff} \times \left[ \frac{VP(t-1)}{14} \right]^{VPCoeff} \times \left[ \frac{RH(t-1)}{97} \right]^{RHCoeff} \]

where,

- \(Ps(t)\) = Microorganism levels in surface store (organisms/L)
- \(VP(t-1)\) = Previous days vapour pressure (hPa)
- \(RH(t-1)\) = Previous days maximum relative humidity (%)
- \(PsCoeff\), \(VPCoeff\), and \(RHCoeff\) are all calibration parameters based on catchment area and 14 and 97 are scaling factors based on catchment type.

A simple surface wash off equation was used to generate the concentration of microbes after rainfall events from surface store towards the water bodies.

\[
C_s(t) = \frac{P_s(t) \times RI(t)^{1.293}}{RI(t)} \text{ (orgs/L)} \]

where,

- \(Cs(t)\) = Level of microbes at stormwater outlet from surface store (orgs/L)
- \(RI(t)\) = Routed rainfall intensity (mm/min)

In addition, Haydon (2006), McCarthy (2008) also considered that the removal of microbes from a surface store is proportional to rainfall intensity. MOPUS model cannot be used in the current study due to insufficient amount of data to calibrate the model parameters, in particular to obtain the \(PsCoeff\). \(PsCoeff\) accounts for deposition rate of microbes on the catchment. However, the simple surface wash off component
from this model can be utilised in combination with the prediction model to estimate the amount of microbes after a spill event in a catchment. It was decided to use surface wasoff component of this model with minor changes to estimate the amount of microbes enter the drains at each location after certain time intervals.

2.7 Summary

In the Lower Yarra section the faecal coliform contamination is high. There is a significant contribution of pathogens affecting the quality of urban stormwater. Some of the examples of contamination could be due to wet weather or dry weather sewer overflow, animal droppings, illegal connections of sewer pipes into the stormwater drainage or poorly maintained septic tanks. The scope of the current study is to investigate the effects on river water health due to dry weather sewer overflows. The dry weather spills occur due to blockages or system breakdown in the sewer network. This could be either inside or outside a household property. High amount of nutrients, pathogens, organic toxicants and heavy metals enter the waterways during a spill event. The \textit{E.coli} loads vary from 2000 to 160000 organisms/100ml. These values exceed the primary and secondary contact levels recommended by the National Health and Medical Research Council (NHMRC, 2000).

The sewage that overflows during a dry weather spill is retained on the surface and will get washed off to the stream during the following storm event. The amount of faecal coliform that will be carried to the stream will depend on the magnitude of the storm as well as on antecedent rainfall conditions that determine surface runoff. Based on the literature it was planned to carry out some field work to investigate the effect of above mentioned rainfall effects on the mobility of faecal coliform including its migration through smaller creeks to the river.

The above literature review has identified \textit{E.coli}, enterococci, FRNA coliphages as critical indicator organisms for the current study. It was also identified that nutrient concentration of soil is also important as it is related to microbial survival.

The microbes and nutrient data collected in the field will be used to obtain simple decay relationships between microbe concentrations, time and climate variables. The EG model developed by Haydon (2006) to estimate pathogen transport from catchments to waterways was developed and tested on rural catchments. This model was not further considered for the current study. It was decided to develop a microbial model with time
and climate variables (i.e. relative humidity, vapour pressure and temperature). Surface wash off can then be calculated with a simple washoff equation used by McCarthy (2008) to predict the concentration of microbes at stormwater drains.
Chapter 3
Site Description and Experimental Procedure

3.1 Background

The current research concentrates on the contribution of faecal contamination due to dry weather sewer spills. The sewage that overflow due to sewer blockages during non-rainy periods is considered a dry weather spill and is retained on the surface, and will get washed off to the stream during the next storm event. Reddy et al. (1981) reported that the survival rate of bacteria was the most important factor while determining the quantity of organisms transported to the stream after a rainfall event. Important climatic factors that need to be considered for survival of microbes are sunlight, ultraviolet rays and rainfall (Jamieson et al., 2002). The intense ultraviolet light on the surface will kill and prevent bacterial growth. The presence of sunlight will help increase the temperature of the water bodies controlling bacteria growth. Rainfall will wash-off all the bacterial concentration to the waterways. Other than these factors, turbidity also plays an important role in the bacterial life cycle. When turbidity of the receiving water is high, the sunlight can not penetrate through it, and hence, the bacterial population can survive longer as UV rays can not reach down to lower depths. Based on the information gathered during the literature review, Escherichia coli (E.coli), Enterococci and FRNA coliphages were considered as indicator organisms because the presence of them would indicate the presence of pathogens and faecal coliforms in waterways. On top of this, the presence of faecal coliform will verify the possible contamination from human and animal sources. This will help identify the survival rate and the movement of pathogens in the environment. Furthermore, the relationship between the decay rate and field data will be simulated with an appropriate stormwater model to predict the concentration of microbes from dry weather sewer spills moving towards the stormwater drain.

Reddy et al. (1981) stated that die-off rate of microbes on soil surface after a spill event was one of the controlling factors determining the presence of indicator organisms in stormwater after a rainfall event. Based on the literature review, it was
decided to focus on the survival rate of microbes with time after a dry weather sewer spill event under different climatic conditions.

This chapter incorporates the experimental procedure used to test the die-off rate of pathogens after a dry weather sewer spill and examines the movement of microbes into the stormwater drain after the spill event. The procedure outlines selection of the sampling period, experimental plot setup, sample collection method, analysis of microbes in the laboratory and the sampling program for different seasons.

3.2 Details of the Experimental Site

3.2.1 Experimental site

The initial step of this research was to select a site for field experiments which provides an area with identical slope and no tree cover as it could prevent direct contact of sunlight. In addition, it was important for the grass cover to be consistent on all the experimental plots. The site selected for experimentation is located on the property of, “Mt. Martha treatment plant” which is located at Craigie Rd, Mt Martha, Vic. Melway reference 146 B 11 (Figure 3.1). This site was chosen as this was the only South East Water owned site which had soil characteristics similar to Gardiner’s Creek and the Prahran Main Drain catchments (Pers. Comm. Kristy Bebend, South East Water).
3.2.2 Selection of sampling period

The selection of sampling period for controlled conditioned experiments was determined on the basis of historical climate data of Victoria. The climate data was one of the important factors in determining the survival rate or die-off period of indicator bacteria. As mentioned earlier in the literature review, the most important factors which affect the die-off rate of the organisms in waterways are sunlight (temperature), UV index and rainfall. Temperature and ultraviolet radiation play the most important roles respectively in the die-off rate of bacteria as these rays penetrate through water and are very effective in controlling the survival of the microbes. Die-off rate of microbes is high if the UV index is high or extreme. UV index is generally measured in scale of 1 to 11+. The UV index of summer and winter season differs as the presence of sun or duration of daylight is less during winter than summer. Figure 3.2 shows that the intensity of these rays is at peak during the early afternoon and is moderate to high between 9am-11am and 3pm to 5pm respectively. The graphical representations published in the report “Forecasts for Sun Safety” by the Bureau of Meteorology (BOM) of Victoria (2007), described that the intensity of UV is extreme during summer and this reduces to moderate intensity throughout winter. Figure 3.3 depicts the mean maximum temperature for each month of the year between 1855 and 2006; in addition
it presents the available data of the highest temperatures for the year 2007 (BOM, 2007).

![UV Index graph during daylight saving](http://www.bom.gov.au)

Figure 3.2: UV Index graph during daylight saving. ([http://www.bom.gov.au](http://www.bom.gov.au), accessed on 15th of November 2007)

![Highest temperature data for year 2007](http://www.bom.gov.au)

Figure 3.3: Highest temperature data for year 2007 (Bureau of Meteorology, [http://www.bom.gov.au](http://www.bom.gov.au), accessed on 15th of November 2007)
Based on the historical climatic data (Figures 3.3 to 3.4), summer sampling was scheduled in March 2008 and 2009 as the temperature is comparatively high during this month and rainfall is low for the year. Sampling for winter analysis was carried out during the month of September 2008 considering low temperature values. Sampling days were selected based on the rainfall forecast to ensure that there is no rain during the experimental period.

Figure 3.4: Annual Mean rainfall and Temperature data for period 1971-2000 (Bureau of Meteorology, [http://www.bom.gov.au](http://www.bom.gov.au))

### 3.3 Climate and Soil Data

Daily rainfall, temperature and soil moisture was measured on site during the sampling days. Relative humidity and vapour pressure during the experimental period were obtained from the Frankston weather observation site {Station 086371} (BOM, 2007) located approximately 17 kilometers from Mt. Martha and is the nearest weather observation station. The entire climate data during the experimental period is enclosed in Appendix - A. Weather details during experiments will help to understand the relationship between the natural environment and the die-off rate of microbes.
3.3.1 Rainfall

The amount of rainfall was measured at site using the rain gauge which was fixed on site (Figure 3.5). However, the sampling period was carefully selected to ensure that there was no rainfall during the whole sample collection period. Also rainfall data was gathered from BOM website for Frankston weather observation station (Table A1). Tipping bucket was installed in the field to collect the rainfall data (Figure 3.5). Table A1 depict that the rainfall was recorded only twice on site during W08 and S09 experiments.

![Tipping drop bucket to measure the rainfall installed on Mt. Martha experimental site](image)

Figure 3.5: Tipping drop bucket to measure the rainfall installed on Mt. Martha experimental site

3.3.2 Temperature profiles

Temperature was measured on field during sample collection days for all three experimental periods. However, on the days that the sampling was not carried out, temperature values at the filed could not be measured. As such, temperature values for the whole period of the experiments were obtained from the BOM web page
BOM temperature profiles were examined to understand the difference between temperature variations during the three experimental periods. Figures 3.6, 3.7 and 3.8 show that the variation between the maximum and minimum temperature values within 24 hours are high. Over the summer periods (Figures 3.6 and 3.8), the range of ambient temperatures varied between 15°C and 40°C throughout 24 hours period. Figure 3.6 depict the difference between temperature values at the actual experimental site and Frankston weather site during S08. It can be seen that the temperature values differ by 5 to 10°C during most of the experimental period. This differentiates the difference in temperature values during the day and night which varied significantly over the period and showed the marked differences between summer (Figure 3.6 and 3.8) and winter (Figure 3.7) data. The data also show that there was a high variation in temperature profiles between measured temperatures and BOM values for the same period.

Figure 3.6: Summer 2008 temperature profiles
Figure 3.7: Winter 2008 temperature profiles

Figure 3.8: Summer 2009 temperature profiles
Table 3.1 provides with the measured and BOM values for S08 experimental period. As could be seen, temperature measured on site is higher than the maximum temperature values obtained from the BOM site. As a result a regression analysis was carried out between measured data and BOM data to obtain the missing temperature values in the field for experimental periods. Equation 3.1 below gives the regression line between the measured and BOM values.

\[ T_{\text{field}} = 1.104 \ T_{\text{BoM}} + 0.47, \quad R^2 = 0.87 \] \hspace{1cm} (3.1)

where

- \( T_{\text{field}} \) = temperature measured on field during experiments
- \( T_{\text{BoM}} \) = temperature values collected from Bureau of Meteorology

Temperature values on field during the three experimental periods were calculated based on the data from BOM values for Frankston {station 086371} (Table 3.1 and A2).

<table>
<thead>
<tr>
<th>Date</th>
<th>Max</th>
<th>Min</th>
<th>Measured</th>
<th>Adjusted</th>
</tr>
</thead>
<tbody>
<tr>
<td>13/03/2008</td>
<td>37.1</td>
<td>15.2</td>
<td>38</td>
<td>41.4</td>
</tr>
<tr>
<td>14/03/2008</td>
<td>30.9</td>
<td>16.1</td>
<td>42</td>
<td>34.6</td>
</tr>
<tr>
<td>15/03/2008</td>
<td>29.9</td>
<td>17.2</td>
<td>40</td>
<td>33.5</td>
</tr>
<tr>
<td>16/03/2008</td>
<td>36.4</td>
<td>18.6</td>
<td>-</td>
<td>40.7</td>
</tr>
<tr>
<td>17/03/2008</td>
<td>33.8</td>
<td>20.7</td>
<td>41</td>
<td>37.8</td>
</tr>
</tbody>
</table>

### 3.3.3 Relative humidity

As mentioned in Chapter 2 literature review, relative humidity plays a substantial role in the die-off rate of microbes. As a result, BOM values of relative humidity during the three experimental periods were obtained from Frankston {Station 086079} and are presented in (Table A3).
3.3.4 pH

The pH values of the soil at Mt. Martha experimental site were obtained from South East Water personnel (Pers.Comm. Kristy Bebend). The pH of the soil at Mt. Martha site ranges from 5.5 to 6.5 and is acidic. Leiendecker (2007) stated that a pH of 9 or above is favourable for the survival of faecal coliform, whereas pH varying from 7.5 to 9 shows little die-off. Also Essington (2004) reported that the pH 4.5 to 5.5 is acidic and depict hazard for environment.

3.3.5 Soil Moisture

Soil moisture was measured on the soil near established plots on experimental site (Figure 3.9) with a soil moisture meter. Soil moisture plays an important role and has an ability to contain or boost the survival of E.coli within soil surface (Solo-Gabriele et al., 2000). In addition, above author reported that E.coli and enterococci organisms can survive longer with moist conditions as this helps the microbial predation and multiplication outside host’s body.

Figure 3.9: Measurement of Soil moisture beside experimental surface
The soil moisture data obtained during three experimental periods is provided in Table 3.2. As seen in Table 3.2, soil moisture content on all days was low except on the 15th day during summer 2009 sampling period (13%). This may be due to the rainfall in between the sampling days, values are provided in Table A1. Soil moisture helps the survival of the microbes on the soil surface.

Table 3.2: Soil moisture data for the three sampling events

<table>
<thead>
<tr>
<th>Sampling Day</th>
<th>Soil Moisture (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Summer 2008</td>
</tr>
<tr>
<td>Day 0</td>
<td>5</td>
</tr>
<tr>
<td>Day 1</td>
<td>5</td>
</tr>
<tr>
<td>Day 2</td>
<td>5</td>
</tr>
<tr>
<td>Day 4</td>
<td>5</td>
</tr>
<tr>
<td>Day 7</td>
<td>-</td>
</tr>
<tr>
<td>Day 14</td>
<td>-</td>
</tr>
<tr>
<td>Day 15</td>
<td>-</td>
</tr>
</tbody>
</table>

3.4 Experimental Design

The experimental design was developed from following methodologies of National Research Project Protocol, U.S.A. and findings of Collins et al. (2002). As mentioned earlier the experiments were carried out on pervious and impervious surfaces during the three experimental periods.

3.4.1 Preparation of experimental plots

The experimental site was fenced (Figure B1) to get protection from animal intrusions that can contaminate the experimental plots. The following steps were carried out to prepare experimental plots required for field experiments:

1. The selected area has a sufficient slope (>2%) to generate surface runoff.
2. Each plot was 100cm in length and 100cm in width.
3. This area was bordered with the help of nails and ropes (Figure 3.10 & Figure B2) to prevent any propagation outside the marked boundaries at the time of distribution of sewage and the simulation of rainfall.
4. At the lower end of the plot, trenches were dug along the width of the plot to a depth 5 cm below the soil surface to collect the surface runoff samples,

5. Cement sheetings were used to simulate the impervious surface (Figure 3.11). These plots were made up of cement sheetings, nails, treated pine and multipurpose glue prepared at RMIT University. The cement sheets will not have any impact on the survival rate of organisms as the material is not alkaline in nature.

All the experimental plots were established on the selected land as shown in Figures 3.12 and 3.13.

Figure 3.10: Experimental Pervious plot marked and the trench at the lower end to collect surface runoff
Figure 3.11: Impervious experimental plot established on site

Figure 3.12: Pervious plots along with trenches at the lower end of the plot
Figure 3.13: Impervious (cement) plots on experimental site

(i) Experimental plan

Three sets of field experiments were carried out to examine the die-off pattern of selected indicator organisms (*E.coli*, enterococci and FRNA coliphages) on pervious and impervious surfaces (S08, W08 and S09). Under control conditions, raw sewage was applied on field plots to simulate sewage spill. Rainfall was simulated on these plots after predetermined lapse time periods to examine the die-off (or survival) rate of microbes between the dry weather spill and the wash off event. The flow chart (Figure 3.14) illustrates the sampling days for each season. Results from S08 were used to design the W08 experiments and the S09 experiments were designed on the basis of W08 obtained data. The experiments were not carried out on impervious surface during S09 as depicted in Figure 3.14.
(ii) Sample collection plan – Summer and winter 2008

On each selected day, rainfall was simulated on 4 randomly selected plots on pervious as well as impervious surfaces (1 control and 3 treated plots). Figure 3.15 below illustrates the selected number of plots on pervious and impervious plots on each sampling day during S08 and W08 experimental periods respectively.

The information on experimental plots used during each sampling period is given below:

1. 32 plots (pervious and impervious) were prepared during S08 (Figure 3.16) with 4 control and 12 treated plots for each surface. A summary of the number of samples collected on each day is presented in Table 3.3.
2. 32 experimental plots were established on site during W08 as well (Figure 3.17). However, on this occasion there were 20 pervious plots and only 12 impervious plots. Based on the results from S08 experiments it was decided not to collect samples from both surfaces, 2 hours after application of raw sewage on the plots. This was due to the presence of considerable number of microbes in collected samples during S08 experiments, as it may not provide with the required output. Furthermore, it was decided to extend the sampling period to 14 days only on the pervious surface. The number of samples collected on each day during W08 is listed in Table 3.4.

Figure 3.15: Number of sampling plots on each day during the S08 and W08 experimental period.
Figure 3.16: Layout of the pervious and impervious plots for summer 2008
Table 3.3: A summary of the type of surfaces and number of samples taken at each time step for S08

<table>
<thead>
<tr>
<th>Surface Type</th>
<th>Number of Samples</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2hrs</td>
</tr>
<tr>
<td>Pervious (clay and grass cover) - control</td>
<td>1</td>
</tr>
<tr>
<td>Pervious (clay and grass cover)</td>
<td>3</td>
</tr>
<tr>
<td>Impervious (cement sheeting) - control</td>
<td>1</td>
</tr>
<tr>
<td>Impervious (cement sheeting)</td>
<td>3</td>
</tr>
</tbody>
</table>
(a) Pervious Surface

Day 1 Contr
Day 7 Treate
Day 4 Treate
Day 2 Treate
Day 7 Contr
Day 1 Treate
Day 14
Day 7 Treate
Day 14

(b) Impervious Surface

Day 2 Control
Day 4 Treate
Day 2 Treate
Day 4 Control
Day 2 Treate
Day 1 Treate
Day 4 Treate
Day 1 Treate
Day 1 Control
Day 1 Treate
Day 2 Treate
Day 4 Treate

Figure 3.17: Layout of the pervious and impervious plots for winter 2008
Table 3.4: A summary of the type of surfaces and number of samples taken at each time step for W08

<table>
<thead>
<tr>
<th>Surface Type</th>
<th>Number of samples on each day</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1&lt;sup&gt;st&lt;/sup&gt; day</td>
</tr>
<tr>
<td>Pervious (clay and grass)</td>
<td>1</td>
</tr>
<tr>
<td>Pervious (clay and grass)</td>
<td>3</td>
</tr>
<tr>
<td>Impervious (cement sheeting) - control</td>
<td>1</td>
</tr>
<tr>
<td>Impervious (cement sheeting)</td>
<td>3</td>
</tr>
</tbody>
</table>

(iii) Sample collection plan – Summer 2009

Field experiments during the S09 period consists of only on pervious surfaces as shown in Figure 3.20. It was decided to collect triplicates of samples for *E.coli* and enterococci organisms to examine the variation of microbe concentration within one pool of water (Figures 3.18 and 3.19). One sample on each experimental plot was collected and tested for nutrients (TN and TP). It was decided not to collect samples for FRNA coliphages during S09 experiments based on S08 and W08 experiments. The results from these experiments showed the negligible amounts present on pervious and impervious surfaces on day 1, indicating higher die-off rate of FRNA coliphages. Figure 3.18 depicts the sampling plan on Day 1 (after 24 hours) during S09 experiments. As shown in Figure 3.18, Sample 1 was further divided into 3 sub-samples. As mentioned before, this was done mainly to examine the variation of microbes within one sample.

Figure 3.19 explains the sampling plan for the rest of the experimental period. Additional set of experiments were also carried out to understand the relationship between washoff of microbes with rainfall intensity.

As shown in the layout of the plots (Figure 3.20), a total numbers of 20 pervious plots were prepared. Table 3.5 provides the information on selected pervious surfaces and collected number of samples on each sampling day.
Figure 3.18: Number of sampling plots on 1st day during S09 experimental period
Figure 3.19: Number of sampling plots on day 2, 4, 7 and 15 during S09 experimental period
Figure 3.20: Layout of the pervious plots for summer 2009
Table 3.5: A summary of the type of surfaces and number of samples taken at each time step for S09

<table>
<thead>
<tr>
<th>Surface Type</th>
<th>Parameters</th>
<th>1st day</th>
<th>2nd day</th>
<th>4th day</th>
<th>7th day</th>
<th>15th day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>No. of plots</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>E.coli + enterococci</td>
<td>5</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>Nutrients</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Treated</td>
<td>No. of plots</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>E.coli + enterococci</td>
<td>11</td>
<td>9</td>
<td>9</td>
<td>9</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>Nutrients</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
</tr>
</tbody>
</table>

Experiments were designed to investigate the effect of microbial movement after a storm event. Multiple storm event experiments were designed for this period during the S09 experimental period. The main aim of these experiments was to examine the movement of microbes after a simulated storm event on pervious surface (multiple storm events on a single plot). The same control plot and treated plot 1 used on day one of the experiments (Figure 3.18) were used for the multiple storm event experiments on 1st day of the S09 experiments. The process was repeated on the same plot for another 3 hours on hourly basis. As shown in Figure 3.12, triplicates of runoff samples were taken from the central surface and from the treated Plot 1 on multiple storm event experiments as well. Furthermore, three sub-samples were taken from Sample 1. Figure 3.21 explains the sampling plan for multiple storm event analysis.
3.4.2 Raw sewage collection and application to treated plots

Raw sewage was applied to all the treated plots on the initial day (0 time interval) and simulated storm events after selected time intervals to observe the die-off rate of microbes with time. This will simulate the dry weather spill in an urban area. Raw sewage was collected from the Mt. Martha treatment plant for the study. Raw sewage used to simulate spill event was pumped out after the primary sedimentation process from the WWTP. The procedure for applying raw sewage to treated (pervious and impervious) surfaces is given below:

1. Figure 3.22 shows the raw sewage stored in a plastic container (125litres).

2. Three samples of raw sewage were collected from different time intervals and different depths (i.e. top, centre and bottom of the container) and tested for indicator organisms and nutrients (Figure 3.23).

3. Raw sewage was sprayed uniformly over the experimental surface other than a strip of 10 cm width which was located close to the sample collection trench to prevent run-off of the raw sewage into the collection trench. (5L of raw sewage was applied to all previous treated surfaces and 1.5L was applied to impervious surfaces)
Figure 3.22: The raw sewage container (125 L) from Mt. Martha wastewater treatment plant

Figure 3.23: Raw sewage samples collected from top, middle and bottom layer of the container
3.4.3 Rainfall simulation

1. Plastic sheets were fixed at the bottom of the plot within the trenches to collect the surface run-off from simulated rainfall events. Figures 3.24 and B3.

2. Potable tap water was used to simulate the rainfall. Plastic water collection cans of 20 litres each were filled with the potable water from the tap located near the site and then transported to the site.

3. Rainfall was simulated on four randomly selected plots each from pervious and impervious plots per day, (i.e. 1 control and 3 treated plots from each surface) at different time intervals (Figure 3.25). Section 3.4.1 explained the selection of plots for rainfall simulation each day (Figures 3.16, 3.17 and 3.20).

4. 15 litres and 4.5 litres of water was applied on pervious and impervious surfaces respectively for approximately 10 – 15 minutes, in an on/off method of application to allow infiltration into the soil and to replicate duration of the simulated storm event. On the pervious surface, a 1:10 year storm event for Melbourne (60mm/hr for 15 minutes) was simulated and on impervious surface a 1:1 year storm event (18mm/hr for 10-15 minutes) was simulated.

Figure 3.24: Plastic sheet fixed in a trench before simulating rainfall event
3.4.4 Collection of samples

The surface runoff was collected on fixed plastic sheets in trenches (Figure 3.26). Those collected samples were transferred to marked plastic buckets (Figures 3.27, B5 and B6) in order to get the volume of surface runoff generated. The volume of the spills in trenches were estimated and added to the total amount of runoff to estimate the runoff volume on the soil plots. However, on the impervious surfaces amount of water used to simulate rainfall was all collected in a plastic tray as a result of this there was no loss.
Figure 3.26: Collection of surface runoff on a fixed plastic sheet in sample collection trench

Figure 3.27: Collected surface runoff sample transferred to marked plastic bucket
3.4.5 Sample collection and analysis

Samples were collected from four each of pervious (soil) and impervious (cement) surfaces into the marked plastic buckets on each selected day. These samples then were transferred to the sample collection bottles (500ml each for *E.coli*, enterococci and FRNA coliphages, 250ml for Total N and Total P) as seen in Figures B8 and B9. Figure 3.28 shows the samples collected from pervious treated plots during the S09 experimental period.

These sample bottles were stored in an ice box (Figure B10) and transported to Ecowise laboratory within 4 hours (<4hrs) for further analysis. Microbial and nutrient analysis for this study has been conducted by Ecowise laboratories, Scoresby, Victoria, Australia. All Ecowise laboratories are NATA accredited to ISO/IEC 17025 and also they are equipped with modern analytical laboratory instrumentation. Location of Ecowise Environmental laboratories was also one of the important factors from transportation view as this laboratory is situated near the experimental site. The samples were analysed for *E.Coli*, enterococci, FRNA coliphages, Total N and Total P. All physio-chemical analyses were carried out using standard American Public Health Association methods (APHA, 1998).

![Figure 3.28: Collected samples from treated soil plot on day 2 of S09 sampling period](image-url)
3.5 Contamination of Experimental Plots

The selected experimental site had been used for cattle grazing before the S08 experimental period. Experimental site had dried faecal matter on surface (Figure 3.29). Guber et al. (2005) mentioned that manure is a source of several pathogens and can potentially contaminate surface and groundwater. These dried faecal pats were removed before the commencement of experiments. The experimental site was also fenced (Figure B1) to prohibit intrusions by livestock. However, Serrano-Garcia et al. (2007) reported that an indicator organism in dry matter (cow dung) takes approximately 7 days to reduce by 6% of its initial concentration. Based on the above statement it could have been possible for the bacterial stains to survive on the soil surface even after removal of the dried faecal pats. This may have affected the results on some of the experimental surfaces.

The experimental site was further restricted for grazing by cows to prevent any kind of contamination for the following sets of experiments. Figure 3.30 and 3.31 shows the presence of kangaroo droppings on 25th of April 2009 on some of the experimental surfaces. Although all necessary steps were taken to avoid contamination, some surfaces could have been contaminated with kangaroo droppings after experimental plots were established. However, the dried faecal matter on the surface was removed before the commencement of the experiments. Although small this contamination may have had some impact on the obtained results during S08, W08 and S09 experimental periods.

Figure 3.29: Faecal pats (dried cow dung) on experimental site before summer 2008
Figure 3.30: Evident kangaroo droppings on marked pervious control surface

Figure 3.31: kangaroo droppings on experimental surface
3.6 Summary

Surface runoff experiments were designed to understand the die-off rate of selected indicator organisms. The experimental procedure for field experiments, sample collection plan and transportation of samples to Ecowise Environmental Laboratories has been explained in this chapter. The following chapter will present and discuss on the results obtained from the S08, W08 and S09 field experiments.
4.1 Background

Field experiments were carried out on the experimental site established on Mt. Martha wastewater treatment plant under controlled conditions. Three sets of experiments were carried out during summer 2008 (S08), winter 2008 (W08) and summer 2009 (S09) to understand the effects of antecedent conditions and the prevailing climate on the survival of microbes. It should be noted that each experimental plot was used with simulated rainfall only once and was discarded after a single use. Rainfall events were simulated on pervious and impervious experimental plots and the run-off was collected as described in Section 3.4.3. Transporting the samples within 4 hrs to the laboratories gave with superior results and confidence in obtained results. The results obtained have high confidence as the quality control maintained throughout the experimental period was high.

This chapter presents the results from each sampling period. Data obtained from the experimental site (microbes and nutrients) over different seasons in 2008 and 2009 provided a better understanding of the relationships between microbes, time and climate conditions on both pervious and impervious surfaces.

4.2 Mapping the Survival of Pathogens with Time

As mentioned in Chapter 3, field experiments were carried out to understand the survival rate of \textit{E.coli}, enterococci and FRNA coliphages with time, after a dry weather spill. The collected water samples were analysed by Ecowise commercial laboratories. Nutrient levels in the water were also determined as the survival rates of pathogens are dependent on the presence of nutrients in the soil (Tong and Chen, 2002). The experimental set up was designed based on literature (Collins, 2002). W08 and S09 experimental plans were modified based on the outcomes from S08 and W08 experiments respectively as explained in Chapter 3.
4.2.1 Raw sewage

Raw sewage used to simulate spill event was collected after a primary sedimentation process from the Mt. Martha wastewater treatment plant. Samples were collected from top, middle and bottom of the raw sewage container to obtain the values of *E.coli*, enterococci, FRNA coliphages and nutrients. The values of *E.coli* and enterococci organisms in raw sewage samples for S08 were reported as greater than (> 2,400,000 orgs/100mL for all three obtained samples instead of a specific value (Table C1). This occurred because the approximate range of pathogen numbers (i.e. concentrations) provided to the testing lab (Ecowise Environmental Laboratories) were not large enough. As a result, sufficient dilution levels for the samples were not carried out while analyzing the above mentioned indicator organisms. Actual values for *E.coli* organisms were obtained from the Mt. Martha wastewater treatment plant after primary sedimentation process. Raw sewage collected for experimental period S08 had *E.coli* levels varying from 5,150,000 orgs/100mL to 9,150,000 orgs/100mL (Personal Communication, Kristy Bebend, South East Water). These actual values for *E.coli* organisms were much higher than reported value of >2,400,000 orgs/100mL. On the other hand, FRNA coliphages values obtained for raw sewage were specific for all three samples. FRNA coliphages varied from 260,000 orgs/100mL at the top of the raw sewage container to 160,000 orgs/100mL at the bottom of the container.

Figures 4.1, 4.2 and 4.3 depict the variation of *E.coli*, enterococci and FRNA coliphages concentrations for raw sewage samples during S08, W08 and S09 experimental periods. As the actual values for *E.coli* and enterococci organisms during S08 are not reported, the values were not presented in Figures 4.1 and 4.2. As reported in Chapter 3 and Figure 4.3, due to low concentrations 24 hours after a spill during S08 and W08 experiments, the FRNA coliphage was not measured during S09. The raw sewage concentrations for indicator organisms during the three sampling periods are presented in Appendix C, Tables C1, C2 and C3 respectively.

There is a variation in concentration levels between the collected samples from top, middle and bottom layers of the container. Above figures depict that there is a magnitude difference between samples from different layers as well as between the two seasons, summer and winter. There was an approximate time difference of two hours between the collections of sample from each layer in the raw sewage container. The concentrations of microbes could vary due to the elapsed time of collecting samples.
Figure 4.1: Raw Sewage *E.coli* concentrations

Figure 4.2: Raw sewage enterococci concentrations
Nutrient concentrations of raw sewage samples are presented in Figures 4.4 and 4.5. Raw sewage samples collected had high nutrient values. Figure 4.4 depict that there was a variation in Total nitrogen (TN) levels between the two summer periods (S08 and S09). Concentration of TN for raw sewage was ranging from 90 mg/L to 100mg/L in S08 and 66 mg/L to 74 mg/L in W08 and S09 (Appendix C, Tables C1, C2 and C3).

Figure 4.5 illustrates the concentrations of Total phosphorus (TP) in raw sewage for the three experimental periods. There was no variation within collected samples from different layers, except the bottom layer sample (19 mg/L) during S08. Unlike for TN, TP levels, *E.coli* and enterococci concentrations within raw sewage during the two summer seasons were in the same range and during winter season it had dropped.
Figure 4.4: Concentrations of Total Nitrogen in raw sewage

Figure 4.5: Concentrations of Total Phosphorus in raw sewage
4.2.2 Control plot experiments

This section discusses on the results obtained from the control experimental surfaces (pervious and impervious) during S08, W08 and S09 experimental periods.

Pervious surfaces

Figures 4.6, 4.7, 4.8, 4.9, and 4.10 depict the variation in $E.\text{coli}$, enterococci, FRNA coliphages, TN and TP with time, from the pervious control plots during S08, W08 and S09 experimental periods. Although sewage was not applied on the control surfaces, the presence of $E.\text{coli}$ and enterococci organisms was evident on pervious surfaces (Figures 4.6 and 4.7). In addition, Tables C4, C5, C6 and C7 in Appendix C show the concentrations of $E.\text{coli}$ and enterococci organisms present on pervious surfaces during the three experimental periods. This may be due to the contamination of the surface before or during experimentation period as mentioned in Chapter 3. Byappanakalli and Fujioka (1998) reported that the faecal coliform and $E.\text{coli}$ are able to grow in tropical soil environments depending on the availability of nutrients. Furthermore, Davies et al. (1995) reported that $E.\text{coli}$ organisms can survive longer at low soil moisture levels. Soil moisture levels at the experimental site were low (5%) during all three experimental periods as stated in Table 3.1. The statement by above authors and the results from field experiments support the presence of $E.\text{coli}$ and enterococci on pervious control surfaces.

According to Figures 4.6 and 4.7 the concentrations of $E.\text{coli}$ and enterococci during the W08 experiments were higher compared to the data during S08 and S09 experiments. This may be due to the significant grass cover present on pervious surfaces during the winter season (Figure 4.11). This grass cover will help to protect the microbial stains present on the surface due to cow pats or kangaroo droppings from sunlight and ultraviolet rays and allow them to survive for a longer duration. In contrast, concentrations of FRNA coliphages were negligible on all pervious control surfaces (Figure 4.8).
Figure 4.6: Concentration of *E.coli* organisms on pervious control plots during three experimental periods

Figure 4.7: Concentration of enterococci on pervious control plots during three experimental periods
Collins (2002) and Reddy et al. (1981) reported that unlike *E. coli*, enterococci could be dormant for longer durations on a soil surface and could become active after a long time period. Comparatively high enterococci and nutrient values on control surfaces during winter could be due to significant grass cover on surface. As this grass cover acts as a protection cover from sunlight, ultraviolet rays and humidity allow microbes and nutrients to remain on the surface for longer periods. In addition, Reddy et al. (1981) reported that the lower soil moisture and pH values help enterococci stains to grow after a long time in the environment. Reddy et al. (1981) also reported that there is a significant relationship between the earlier faecal stains on soil surfaces and the concentrations of enterococci organisms observed on pervious control surfaces.

Tables C4 and C5 present the concentrations of FRNA coliphages. As seen in Figure 4.8, FRNA coliphages on control surfaces are negligible on all experimental days during S08 and W08. As such FRNA coliphages were not measured during S09 experiments as stated earlier in Chapter 3. Figures 4.9 and 4.10 (also in Tables C4, C5 and C8 in Appendix C) illustrate the TN and TP availability on control plots during experimental periods. The outcome indicates the presence of higher numbers of nutrients (higher than raw sewage values (Figures 4.4 and 4.5) from the plots used on the 7th and the 14th day during W08. Even if there was no raw sewage applied on control plots, reported values of nutrient concentration on the 7th and the 14th day for W08 suggests the possible contamination of these plots.

![Figure 4.8: Concentration of FRNA coliphages on pervious control plots during three experimental periods](image-url)
Figure 4.9: Concentration of Total N on pervious control plots during three experimental periods

Figure 4.10: Concentration Total P on pervious control plots during three experimental periods
Impervious surfaces
As mentioned earlier in Chapter 3, the experiments on impervious surfaces were not designed for the S09 experimental period. The concentrations of microbes on the impervious (cement) control surfaces were negligible compared to the pervious surfaces. Figures 4.12 and 4.13 depict the survival pattern of *E.coli* and enterococci respectively on impervious surfaces. The concentrations of *E.coli* (Figure 4.12) on all impervious (cement sheets) control surfaces were less than 10 orgs/100mL. Figure 4.13 show that there was a considerable amount of enterococci organisms on the impervious surface on the 1st day (410 enterococci organisms/100mL).

All the concentrations for *E.coli*, enterococci, TN and TP on impervious surfaces during S08 and W08 experiments are presented in Tables C9 and C10 in Appendix C. As seen in Figures 4.14 and 4.15, TN values vary between 0.5 mg/L and 7.6 mg/L. Total P values were very small and are in between 0.05 mg/L and 0.5 mg/L. Nutrient concentrations obtained from the impervious control surfaces were negligible.
Figure 4.12: Concentration of *E.coli* on impervious control plots during summer and winter 2008 experimental periods

Figure 4.13: Concentration of enterococci on impervious control plots during summer and winter 2008 experimental periods
Figure 4.14: Concentration of Total N on impervious control plots during summer and winter 2008 experimental periods

Figure 4.15: Concentration of Total N and Total P on impervious control plots during summer and winter 2008 experimental periods
4.2.3 Treated surfaces

As discussed in Chapter 3, the rainfall was simulated over randomly selected pervious and impervious surfaces at different time periods after applying sewage on each plot during all three sampling events (S08, W08 and S09). The layouts of the treated surfaces were depicted in Figures 3.15 - 3.17.

Pervious treated surfaces

Tables C11 to C24, depict the concentration levels obtained for *E.coli*, enterococci, FRNA coliphages, TN and TP from all pervious treated surfaces during the three experimental periods. Figures 4.16 to 4.21 depict the variation of concentrations of *E.coli* and enterococci with time on pervious surfaces during three different sampling events (S08, W08 and S09). It is important to note that at each time period, the samples were taken from the three new plots where sewage was applied as mentioned in Chapter 3 (Figures 3.16, 3.17 and 3.20). In addition to this, triplicate samples were collected for *E.coli* and enterococci from each experimental plot during S09 to observe the variation of microbes within the collected sample. The microbial levels obtained from the plots (three each day for S08 and W08, nine each day during S09) after a predetermined elapsed time period are presented (Figures 3.15, 3.18 and 3.19) as a range of values.

Similar to the microbial concentrations of raw sewage the actual concentration values (numbers) were not obtained from the laboratories for *E.coli* and enterococci for 2hr and 24hr (Day 1) elapsed time periods for the S08 experimental period, and hence these are not considered for any analysis. Instead it was stated that the observed *E.coli* and enterococci concentrations were higher than 24,000 orgs/100mL. There is a downward trend in concentration levels from Day 2 to Day 4 (Figure 4.16) during the S08 experimental period. However, during W08 and S09, the variation of concentration levels between the plots did not have a noticeable pattern. It is important to reiterate that the samples were taken from three new treated plots at each time period.

Tables C17 and C18 presents the concentrations of FRNA coliphages on pervious treated plots. Figure 4.22 depict the concentrations of FRNA coliphages present on pervious surfaces at different time intervals. However, during W08 FRNA coliphages were not present (0 pfu/100mL) on pervious surfaces throughout the experimental period (Table C18).
Figures 4.23 - 4.28 depict the concentrations of TN and TP on pervious treated plots at selected time intervals. The nutrient levels obtained from these treated plots are presented in Tables C19 to C24 and are similar to the values from pervious control surfaces (Tables C4, C5 and C8).

Figure 4.16: *E.coli* concentrations on soil plots during different time intervals (S08)

Figures 4.16 and 4.17 depict the variation of *E.coli* organisms from different pervious treated plots and show the concentration variations with time. Trevisan et al. (2002) studied faecal coliform survival on surface vegetation following manure spreading on pastures and found that the vegetation offered a protective effect limiting UV impacts and wetness to an extent. This statement supported higher concentration of microbes after 4 days on the pervious surfaces during W08 and confirms the relationship between vegetation and survival of microbes. In a long-term study by Sjogren (1995), a similar survival of *E.coli* on rye-grass field plots was measured, with an average survival time of 41 days. The decay and growth relation of the pathogens show complex relationships between growth and predation on the soil surface. The survival of *E.coli* on soil surface can only be possible if it is in the top 5 cm of soil as it cannot survive below that level (Desmarais et al., 2002). Muirhead et al. (2006) also reported that *E.coli* normally attach to soil particles and as a result is mainly found on the soil.
The above statements indicate that the vegetation cover has made the difference in the varying numbers of \textit{E.coli}, enterococci and nutrient levels on different plots at different time intervals. The survival of pathogens suggests the presence of resistant strains on the soil surface.

![Graph](image)

Figure 4.17: \textit{E.coli} concentrations on pervious treated surfaces during different time intervals (W08)

Figures 4.16 to 4.21 depict the range in variation of \textit{E.coli} and enterococci in all collected samples from different surfaces on a sample collection day. The variation within the samples collected on same day was high. This may be due to the complex relationships of microbes with soil properties (pH, soil moisture) or microbes bonding with soil particles. Furthermore, the microbes washed off with the stormwater depend on the application rate of rainfall.

Collins (2002) and Reddy et al. (1981) reported that the enterococci organisms have tendency to bond with soil particles, react with predators and survive with the help of nutrients present in the environment for longer duration. The variation in microbe’s concentrations from the same pool of water also could be high (WHO, 2003). To verify this statement samples were taken from the same pool of surface runoff and tested during S09. The results are presented later in section 4.3.
Figure 4.18: *E.coli* concentrations on pervious treated surfaces during different time intervals (S09)

Figure 4.19: Enterococci concentrations on pervious treated surfaces at different time intervals (S08)
Figure 4.20: Enterococci concentrations on pervious treated plots at different time intervals (W08)

Figure 4.21: Enterococci concentrations on pervious treated plots at different time intervals (S09)
Studies by Trevisan et al. (2002) and Sjogren (1995) supported the microbial variations obtained from field experiments during the three experimental periods. *E.coli* concentrations on Day 1 during S09 (Figure 4.18) are even higher than the raw sewage concentrations. This could be due to the variation of microbes within samples or contamination due to kangaroo waste present on marked plots before sampling event as stated in Section 3.5. On some instances the concentration values change by an order of magnitude (Figures 4.16 to 4.21).

Figure 4.22 depicts the concentrations of FRNA coliphages on pervious treated surfaces. The results illustrate that the die-off rate of FRNA coliphage stains is high in the natural environment. In addition, this suggests that these viruses can not survive for longer duration outside host’s (animal) body. As depicted in Figure 4.22 and Table C17, the concentrations of FRNA coliphages is in between 1 to 95 pfu/100mL and are negligible. Table C18 depict that FRNA coliphages could not survive on pervious treated surfaces during W08.

Similar to microbes, the nutrient values varied on different plots throughout the experimental period. The variations in nutrient values in Figures 4.23 to 4.28 from different pervious surfaces on the same day are presented. The results show that total nitrogen concentrations on pervious plots were high on the 14th day during winter 2008, 7th and 15th day during summer 2009 (Tables C19 to C24).

![Figure 4.22: Concentrations of FRNA coliphages during S08 experiments](image-url)
Figure 4.23: Concentration of TN on pervious treated plots (S08)

Figure 4.24: Concentration of TN on pervious treated plots (W08)
Figure 4.25: Concentration of TN on pervious treated plots at different time intervals (S09)

Figure 4.26: Concentration of TP on pervious treated plots at different time intervals (S08)
Figure 4.27: Concentration of TP on pervious treated plots at different time intervals (W08)

Figure 4.28: Concentration of Total P on pervious treated plots at different time intervals (S09)
Impervious treated surfaces

As mentioned earlier, the experiments on impervious surfaces were only designed for S08 and W08 experimental periods and not during the S09 experiments. Figures 4.29, 4.30 and 4.31 depict *E.coli* and enterococci concentrations from plots after spraying raw sewage on impervious surfaces during summer and winter 2008. *E.coli* organisms during S08 experiments were reported as >2400 (more than) orgs/100mL after 2 hours interval. This had dropped to <10 (less than) orgs/100mL at 24hrs for all samples taken during summer (Table C25). The concentrations on impervious plots were low compared to values on pervious surfaces.

Figure 4.29 and Table C26 depict the concentration of *E.coli* organisms on impervious surface during W08. These two indicates that there was a variation in the samples collected from different plots on the same day. The result shows (Table C26) a magnitude difference in between collected samples.

Figures 4.30 and 4.31 depict the concentrations of enterococci organisms on impervious plots during summer and winter 2008. This illustrate that the number of enterococci organisms present on impervious surface after 24 hours were negligible during the S08 experiments. Enterococci organisms present during W08 are higher than the S08 experimental period.
Figure 4.30: Concentration of enterococci on impervious treated plots (S08)

Figure 4.31: Concentration of enterococci on impervious treated plots (W08)
Tables C29 and C30 present the concentrations of FRNA coliphages on impervious treated surfaces. The concentration of FRNA coliphages during summer experiment was negligible (<1 pfu/100mL) on all occasions. Obtained results during W08 experiments (Table C30) illustrate that FRNA coliphages can not even survive on impervious surfaces. However, there was only exceptional value present on the 2nd day i.e. 41 pfu/100mL of sample collection. The numbers of FRNA coliphages present on impervious surfaces were negligible in comparison to the detected *E.coli* and enterococci organisms on impervious surfaces.

However, the nutrient levels from the treated impervious surfaces (raw sewage applied on cement sheets) were much lower (i.e. summer 1 to 2.8 N mg/L, 0.1 to 0.5 P mg/L and winter 5.8 to 15 N mg/L, 0.7 to 1.4 P mg/L) than from the pervious surfaces. The nutrients concentrations obtained during all three sets of experiments are presented in Tables C31 to C34.

### 4.3 Multiple Storm Event Experiment

Field experiments during S09 period consisted only pervious surfaces (Figure 3.20). Multiple storm event experiments were designed during this experimental period. As mentioned in Chapter 3, the main aim of these experiments was to examine the movement of microbes after a simulated storm event on pervious surface (multiple storm events on a single plot). One control plot and one treated plot were used for the experiments on the 1st day of the S09 experiments. Similar to all previous experiments, the rainfall was simulated and surface runoff samples were collected. The process was repeated on the same plot for another 3 hours on a hourly basis.

The results from this set of the experiments are presented in Tables 4.1 to 4.6. As shown in Table 4.1 and Figure 4.32, *E.coli* organisms on the control surface were negligible on all occasions (0 *E.coli* organisms/100mL to 41 *E.coli* organisms/100mL).
Table 4.1: *E.coli* on pervious control plot at different time intervals

<table>
<thead>
<tr>
<th>Sample</th>
<th>0 Hour</th>
<th>1 Hour</th>
<th>2 Hours</th>
<th>3 Hours</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample 1 (A)</td>
<td>22</td>
<td>0</td>
<td>2</td>
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<tr>
<td>Sample 1 (B)</td>
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<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Sample 1 (C)</td>
<td>18</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Sample 2</td>
<td>41</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Sample 3</td>
<td>20</td>
<td>0</td>
<td>8</td>
<td>0</td>
</tr>
</tbody>
</table>

Figure 4.32: Variation of *E.coli* on pervious control surface at different time intervals

However, Figure 4.33 shows that on the same control plot amount of enterococci organisms were considerably high (99 organisms/100mL to 53,000 organisms/100mL). This indicates the presence of enterococci stains on pervious plot prior to any raw sewage application. Enterococci organisms must have been present on the pervious surface in a dormant phase and was activated during the experimental period under
favourable conditions. It can also be seen in Table 4.2 that the concentration of enterococci organisms after an hour’s interval is higher than that during the previous rainfall event. The main reason for this can be the bonding between enterococci organisms and soil particles. Enterococci organisms were not washed off with the initial rainfall intensity of 60mm/hr applied for the duration of 15 minutes. This could be due to the strong bonding with soil particles or the intensity of the simulated rainfall as it was sprayed manually. The remaining concentration was washed off after an hour’s interval as the bonding between enterococci and soil particles get weaker with time.

Table 4.2: Enterococci on pervious control plot at different time intervals

<table>
<thead>
<tr>
<th>Sample</th>
<th>0 Hour</th>
<th>1 Hour</th>
<th>2 Hours</th>
<th>3 Hours</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample 1 (A)</td>
<td>99</td>
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<td>2600</td>
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<td>Sample 1 (B)</td>
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<td>42000</td>
<td>1900</td>
<td>5200</td>
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<tr>
<td>Sample 1 (C)</td>
<td>1600</td>
<td>50000</td>
<td>2600</td>
<td>2000</td>
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</tr>
<tr>
<td>Sample 3</td>
<td>5300</td>
<td>33000</td>
<td>5500</td>
<td>12000</td>
</tr>
</tbody>
</table>

Figure 4.33: Variation of enterococci on pervious control surface at different time intervals
Table 4.3 depicts the presence of nutrients on the soil control plots after rainfall events. The amount of TN (83 mg/L) present after the wash-off from the control plot during the initial storm event (0 Hour) was surprisingly, more than the raw sewage reading (66 to 74 mg/L).

On the other hand, the concentration of TP (10 mg/L) on the control plot was less than the raw sewage concentration at 0 time interval on the same surface indicating not having any kind of contamination of the experimental surface. Furthermore the TN concentration from the control plot was higher than the samples from the treated plots (Figure 4.34 and 4.38). TP values are almost the same from both (control and treated) surface, except on initial time (0 Hour) on the control surface.

Table 4.3: Concentration of nutrients on pervious control plot at different time intervals

<table>
<thead>
<tr>
<th>Time Interval (Hours)</th>
<th>0 Hour</th>
<th>1 Hour</th>
<th>2 Hours</th>
<th>3 Hours</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total N (mg/L)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>82</td>
<td>10</td>
<td>24</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td>Total P (mg/L)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>2.4</td>
<td>3.2</td>
<td>2</td>
<td></td>
</tr>
</tbody>
</table>

Figure 4.34: Total N on pervious control surface at different time intervals
Figures 4.36 and 4.37 depict the concentration of *E.coli* and enterococci on the pervious treated plots at different time intervals. The variations between the concentrations of *E.coli* and enterococci organisms (Tables 4.4 and 4.5) within the collected samples are high. However, Muirhead et al. (2006) supports the presence of microbial stains and the variation of microbes during the experimental period as the above author reported that the *E.coli* and enterococci are normally attach to soil particles for longer durations and hence they can be found on soil surface after a long elapsed period.

Table 4.4: *E.coli* on pervious treated plot at different time intervals

<table>
<thead>
<tr>
<th>Sample</th>
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<th>1 Hour</th>
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<td>Sample 1 (C)</td>
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<tr>
<td>control sample 3</td>
<td>3300000</td>
<td>16000000</td>
<td>910000</td>
<td>29000</td>
</tr>
</tbody>
</table>
Figure 4.36: Variation of *E.coli* on soil treated plots at different time intervals

Table 4.5: Enterococci on pervious treated plot at different time intervals

<table>
<thead>
<tr>
<th>Sample</th>
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<th>2 Hours</th>
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<td>Sample 1 (C)</td>
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<td>130000</td>
<td>18000</td>
<td>56000</td>
</tr>
<tr>
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<td>110000</td>
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<td>74000</td>
</tr>
<tr>
<td>control sample 3</td>
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<td>98000</td>
<td>14000</td>
<td>57000</td>
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</tbody>
</table>
Figure 4.37: Variation of enterococci on soil treated plots at different time intervals

Table 4.6: Concentration of nutrients on pervious treated plot at different time intervals

<table>
<thead>
<tr>
<th></th>
<th>0 Hour</th>
<th>1 Hour</th>
<th>2 Hours</th>
<th>3 Hours</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total N</td>
<td>22</td>
<td>13</td>
<td>10</td>
<td>13</td>
</tr>
<tr>
<td>Total P</td>
<td>5.3</td>
<td>4.1</td>
<td>3.2</td>
<td>2.7</td>
</tr>
</tbody>
</table>
Figure 4.38: Total N on soil treated plots at different time intervals

Figure 4.39: Total P on soil control plots at different time intervals
The results from these experiments suggest that not all the microbes and nutrients get washed off from a single storm event. This experiment has shown the ability of microbes to attach to soil particles and resist the force of water after a rainfall event. The microbes have an ability to hold on to soil particles and can survive on the soil surface after several storm events. However, McCarthy (2008) stated that the amount of microbes that gets washed off is proportional to rainfall intensity. Haydon (2006) also observed that the wash off concentration of microbes is dependent on the intensity of the rainfall. Based on the statements from above authors the results from the multiple storm experiments were consistent with the results obtained from the field experiments as all microbes did not get washed off after the rainfall event simulation. The relationship between rainfall intensity and washoff of microbes will be further analysed and discussed in Chapter 6.

4.4 Relationship between Microbes and Nutrients

Tunstall (2007) reported that there is a relationship between microbial survival and the availability of nutrients on the soil surface. Noble et al. (2004) and Shibata et al. (2004) also mentioned that microorganisms can flourish or survive longer with the favorable nutrient concentrations on soil surface or in water bodies. Fisher and Grimm (1985) reported environmental that nutrient and faecal bacterial sources must be reduced to achieve significant improvements in the watershed. In addition, Sharply et al. (1987) reported that the transport of nutrients (N and P) in surface runoff controls the biological productivity of surface water. Sharply et al. (1987) also stated that N and P are associated with accelerated eutrophication. Sawyer (1947), Vollenweider (1971) and Sharply et al. (1987) reported that the concentrations of P between 10 and 20 \( \mu gL^{-1} \) are critical for the environment as, excess amounts lead to eutrophication of lakes and water bodies.

Figures 4.40, 4.41, 4.42 and 4.43 below depict the relationships between microbes and nutrients in the surface water during the winter experimental period. The outcomes from field experiments suggest that there was no relationship between the survival of microbes and available nutrient concentration in the surface runoff from the pervious surface during the experimental periods.
Similar results were obtained from S09 results and presented in Appendix – D, Figures D1 to D4. This is due to nutrients getting washed off by attaching to sediment particles where as microbes get washed off with the surface runoff depending on the rainfall intensity (Edwards et al., 2000). As such the nutrient concentrations in the surface runoff will not give a correct representation of the nutrients in the pervious plot, especially if sediments do not get washed off.

The nutrient data obtained from the field experiments during W08 and S09 experiments will not be used for modeling and as such was not considered for any further analysis.

---

**Figure 4.40**: Relationship between *E.coli* and TN during W08 experiment

**Figure 4.41**: Relationship between enterococci and TN during W08 experiment

**Figure 4.42**: Relationship between *E.coli* and TP during W08 experiment

**Figure 4.43**: Relationship between enterococci and TP during W08 experiment
4.5 Removing Outliers of Collected Data

For this study experiments were conducted to obtain field data on the survival of *E.coli*, enterococci, FRNA coliphages and nutrients on pervious and impervious surfaces and how it varied with time. S08 data set was not considered for analysis as it did not report actual numerical numbers for most of the data microbes. It was reported as greater (<) or less (>) values due to the analytical technique used. The values obtained from Ecowise Environmental laboratories are reported in Tables C1, C11, C14, and C17. FRNA coliphages were measured on the pervious and impervious surfaces during S08 and W08 experiments. Obtained concentrations on pervious as well as impervious surfaces during both experiments (S08 and W08) were negligible. As a result of this, it was decided not to test for FRNA coliphages during the S09 experimental period. The results from S08 and W08 experiments (Tables C17 and C18) prove that FRNA coliphages can not survive even for a day after a spill event under natural conditions. As such FRNA coliphage data (similar to nutrients) will also not be considered for further analysis.

Tables 4.7 to 4.10 depict the variations of *E.coli* and enterococci concentrations between samples collected on the same day as well as on different days during W08 and S09 experiments. Tables also present mean, standard deviation and coefficient of variation (CV) within the samples collected on a particular day for *E.coli* and enterococci. Standard deviation is a measure of the variability or dispersion of a data set from the mean (DasGupta & Haff, 2006). Above authors also stated that high standard deviation indicates high variation in data set. The coefficient of variation (CV) is defined as the ratio of the standard deviation to the mean. On some days CV values are above 100% for *E.coli* and enterococci concentrations (Tables 4.7 to 4.10). Table 4.7 and 4.8 presents *E.coli* and enterococci values during winter experiments. High variations of CV on Day 2 and Day 4 for both organisms are due to high concentrations in Sample 2, Day 2 and Sample 3, Day 4 (same samples for both organisms). These suggest probable contamination of the samples and are considered as outliers. However, the CV of only enterococci is high on Day 7. This is due to concentration levels of Sample 2 (17,000 orgs/100mL).

Following concentrations were considered as outliers:

- Values outside the magnitude of difference from majority of points
- Concentrations more than raw sewage values
Highlighted values given in Tables 4.7 to 4.10 compared indicate the outliers within the samples collected on a particular day. Three samples each for *E.coli* and enterococci were collected during W08 experimental period whereas during S09 experiments 9 samples each were collected to check the variation of microbes on sample surface. Coefficient of variation is not high for raw sewage values during summer and winter.

Table 4.7: Daily mean E.coli concentrations, standard deviation and coefficient of variation during W08

<table>
<thead>
<tr>
<th>Time (days)</th>
<th>Raw sewage</th>
<th>1</th>
<th>2</th>
<th>4</th>
<th>7</th>
<th>14</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample 1</td>
<td>7500000</td>
<td>9900</td>
<td>2600</td>
<td>12000</td>
<td>74</td>
<td>850</td>
</tr>
<tr>
<td>Sample 2</td>
<td>4700000</td>
<td>11000</td>
<td><strong>29000</strong></td>
<td>3100</td>
<td>1500</td>
<td>630</td>
</tr>
<tr>
<td>Sample 3</td>
<td>7400000</td>
<td>14000</td>
<td>9900</td>
<td><strong>81000</strong></td>
<td>1500</td>
<td>3600</td>
</tr>
<tr>
<td>Mean</td>
<td><strong>6533333</strong></td>
<td><strong>11633</strong></td>
<td><strong>13833</strong></td>
<td><strong>32033</strong></td>
<td><strong>1025</strong></td>
<td>1693</td>
</tr>
<tr>
<td>σ</td>
<td>1588500</td>
<td>2122</td>
<td>13632</td>
<td>42639</td>
<td>823</td>
<td>1655</td>
</tr>
<tr>
<td>CV</td>
<td>24%</td>
<td>18%</td>
<td>99%</td>
<td>133%</td>
<td>80%</td>
<td>98%</td>
</tr>
</tbody>
</table>

σ = standard deviation; CV = coefficient of variation

Table 4.8: Daily mean enterococci concentrations, standard deviation and coefficient of variation during W08

<table>
<thead>
<tr>
<th>Time (days)</th>
<th>Raw sewage</th>
<th>1</th>
<th>2</th>
<th>4</th>
<th>7</th>
<th>14</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample 1</td>
<td>260000</td>
<td>200</td>
<td>380</td>
<td>640</td>
<td>790</td>
<td><strong>2000</strong></td>
</tr>
<tr>
<td>Sample 2</td>
<td>550000</td>
<td>850</td>
<td><strong>4200</strong></td>
<td>3600</td>
<td>17000</td>
<td>740</td>
</tr>
<tr>
<td>Sample 3</td>
<td>520000</td>
<td>520</td>
<td>840</td>
<td><strong>20000</strong></td>
<td>120</td>
<td>520</td>
</tr>
<tr>
<td>Mean</td>
<td><strong>4433333</strong></td>
<td><strong>523</strong></td>
<td><strong>1807</strong></td>
<td><strong>8080</strong></td>
<td><strong>5970</strong></td>
<td><strong>1087</strong></td>
</tr>
<tr>
<td>σ</td>
<td>159478</td>
<td>325</td>
<td>2085</td>
<td>10429</td>
<td>9558</td>
<td>799</td>
</tr>
<tr>
<td>CV</td>
<td>36%</td>
<td>62%</td>
<td>115%</td>
<td>129%</td>
<td>160%</td>
<td>73%</td>
</tr>
</tbody>
</table>

σ = standard deviation; CV = coefficient of variation

Tables 4.9 and 4.10 presented actual concentrations obtained from the field for *E.coli* and enterococci during S09 experiments. Above Tables indicate that there were few
reported values higher than raw sewage concentration on Days 1 and 15. These high values obtained from the same samples indicate the probable contamination of these samples. Samples 7, 8 and 9 on Day 1 and samples 1 to 6 on Day 15 are considered as outliers as these values are higher than raw sewage values. On the other hand, as seen in the tables (except for few samples), most of the values are in the same range within the collected triplicates on a particular day.

Table 4.9: Daily mean *E.coli* concentrations, standard deviation and coefficient of variation during S09

<table>
<thead>
<tr>
<th>Time (days)</th>
<th>Raw sewage</th>
<th>1</th>
<th>2</th>
<th>4</th>
<th>7</th>
<th>15</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample 1</td>
<td>9800000</td>
<td>360000</td>
<td>990</td>
<td>49000</td>
<td>5600</td>
<td>2000000</td>
</tr>
<tr>
<td>Sample 2</td>
<td>7700000</td>
<td>2000000</td>
<td>5200</td>
<td>46000</td>
<td>6800</td>
<td>2000000</td>
</tr>
<tr>
<td>Sample 3</td>
<td>11000000</td>
<td>3300000</td>
<td>3100</td>
<td>41000</td>
<td>6800</td>
<td>1700000</td>
</tr>
<tr>
<td>Sample 4</td>
<td>2600000</td>
<td>990</td>
<td>200000</td>
<td>34000</td>
<td>110000</td>
<td></td>
</tr>
<tr>
<td>Sample 5</td>
<td>6500000</td>
<td>7700</td>
<td>24000</td>
<td>28000</td>
<td>20222</td>
<td></td>
</tr>
<tr>
<td>Sample 6</td>
<td>16000000</td>
<td>9800</td>
<td>200000</td>
<td>25000</td>
<td>17000</td>
<td></td>
</tr>
<tr>
<td>Sample 7</td>
<td>24000000</td>
<td>3100</td>
<td>31000</td>
<td>740</td>
<td>1500</td>
<td></td>
</tr>
<tr>
<td>Sample 8</td>
<td>24000000</td>
<td>6300</td>
<td>33000</td>
<td>1600</td>
<td>2800</td>
<td></td>
</tr>
<tr>
<td>Sample 9</td>
<td>24000000</td>
<td>6300</td>
<td>41000</td>
<td>630</td>
<td>1900</td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>9500000</td>
<td>11418148</td>
<td>4831</td>
<td>73889</td>
<td>12130</td>
<td>650380</td>
</tr>
<tr>
<td>σ</td>
<td>1670329</td>
<td>10456248</td>
<td>3018</td>
<td>71910</td>
<td>13077</td>
<td>941798</td>
</tr>
<tr>
<td>CV</td>
<td>18%</td>
<td>92%</td>
<td>62%</td>
<td>97%</td>
<td>108%</td>
<td>145%</td>
</tr>
</tbody>
</table>

σ = standard deviation; CV = coefficient of variation
Table 4.10: Daily mean enterococci concentrations, standard deviation and coefficient of variation during S09

<table>
<thead>
<tr>
<th>Time (days)</th>
<th>Raw sewage</th>
<th>1</th>
<th>2</th>
<th>4</th>
<th>7</th>
<th>15</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample 1</td>
<td>7900000</td>
<td>39000</td>
<td>990</td>
<td>170000</td>
<td>300</td>
<td>580000</td>
</tr>
<tr>
<td>Sample 2</td>
<td>9900000</td>
<td>87000</td>
<td>5100</td>
<td>200000</td>
<td>400</td>
<td>300000</td>
</tr>
<tr>
<td>Sample 3</td>
<td>7500000</td>
<td>55000</td>
<td>3100</td>
<td>200000</td>
<td>1600</td>
<td>330000</td>
</tr>
<tr>
<td>Sample 4</td>
<td>170000</td>
<td>3100</td>
<td>98000</td>
<td>99000</td>
<td>99000</td>
<td>210000</td>
</tr>
<tr>
<td>Sample 5</td>
<td>40000</td>
<td>17000</td>
<td>92000</td>
<td>88000</td>
<td>88000</td>
<td>210000</td>
</tr>
<tr>
<td>Sample 6</td>
<td>73000</td>
<td>990</td>
<td>73000</td>
<td>88000</td>
<td>88000</td>
<td>330000</td>
</tr>
<tr>
<td>Sample 7</td>
<td>170000</td>
<td>990</td>
<td>13000</td>
<td>510</td>
<td>1100</td>
<td></td>
</tr>
<tr>
<td>Sample 8</td>
<td>200000</td>
<td>990</td>
<td>11000</td>
<td>410</td>
<td>1200</td>
<td></td>
</tr>
<tr>
<td>Sample 9</td>
<td>250000</td>
<td>1300</td>
<td>15000</td>
<td>200</td>
<td>980</td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>8400000</td>
<td>54400</td>
<td>2070</td>
<td>50333</td>
<td>570</td>
<td>1093</td>
</tr>
</tbody>
</table>

\[ \sigma = \text{standard deviation}; \quad CV = \text{coefficient of variation} \]

It is planned to use the data collected in the field to develop relationships between microbial survival rate, elapsed time and climate factors. Thus it is important to remove the outliers from the data set. The outliers from W08 and S09 data sets were removed before obtaining the mean daily readings to develop the above mentioned relationships. This will help in obtaining a better set of microbial data for predicting the microbial die-off. Tables 4.11 to 4.14 provide with the mean daily microbe concentrations, standard deviation, and CV values during W08 and S09 experimental periods after removing outliers for *E.coli* and enterococci. As seen in the above Tables after removal of outliers, there are days with more than 80% CV values. However, CV values on these days have improved considerably from the original data set (Tables 4.7 to 4.10). Table 4.10 shows that there was huge variation in the obtained values of enterococci on 15th day (17839%) during S09 experiments. This variation was reduced to 10% after removal of outliers (Table 4.14). Concentrations on Day 15 had reported values more than raw sewage concentrations and hence were considered as outliers. The removal of these values when developing statistical relationships resulted in improved CV values.
Table 4.11: Daily mean *E.coli* concentrations, standard deviation and coefficient of variation during W08 (excluding outliers)

<table>
<thead>
<tr>
<th>Time (days)</th>
<th>Raw sewage</th>
<th>1</th>
<th>2</th>
<th>4</th>
<th>7</th>
<th>14</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample 1</td>
<td>7500000</td>
<td>9900</td>
<td>2600</td>
<td>12000</td>
<td>850</td>
<td></td>
</tr>
<tr>
<td>Sample 2</td>
<td>4700000</td>
<td>11000</td>
<td>3100</td>
<td>1500</td>
<td>630</td>
<td></td>
</tr>
<tr>
<td>Sample 3</td>
<td>7400000</td>
<td>14000</td>
<td>9900</td>
<td>1500</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>6533333</td>
<td>11633</td>
<td>6250</td>
<td>7550</td>
<td>1500</td>
<td>740</td>
</tr>
<tr>
<td>σ</td>
<td>1588500</td>
<td>2122</td>
<td>5162</td>
<td>6293</td>
<td>0</td>
<td>156</td>
</tr>
<tr>
<td>CV</td>
<td>24%</td>
<td>18%</td>
<td>83%</td>
<td>83%</td>
<td>0%</td>
<td>21%</td>
</tr>
</tbody>
</table>

σ = standard deviation; CV = coefficient of variation

Figures 4.44 to 4.47 depict the microbial variations after the removal of outliers. Figures also provide primary and secondary water quality limits based on State Environmental Planning Policies, Victorian Government and Environmental Protection Agency Victoria guidelines. Figure 4.44 shows that most of the *E.coli* concentrations exceed secondary contact limits recommended by SEPP and EPA guidelines. In contrast, enterococci concentrations during the winter period (Figure 4.45) were below 1000 organisms/100mL (secondary contact limits) except during Day 4. The microbial concentrations between 200 to 1000 organisms/100mL suggest that the water would be acceptable for secondary contact recreational activities. It is important to note that these water quality concentrations will be at the inlet to the stormwater drain. Summer 2009 data (Figures 4.46 and 4.47) indicate that most of the *E.coli* and enterococci concentrations exceeds secondary water quality limit. These concentrations will get further diluted within the stormwater system before entering into receiving water body.
Figure 4.44: *E.coli* concentrations during winter period after removal of outliers with water quality limits (Primary and Secondary contact limits for recreational waters)

Table 4.12: Daily mean enterococci Values, standard deviation and coefficient of variation during W08 (excluding outliers)

<table>
<thead>
<tr>
<th>Time (days)</th>
<th>Raw sewage</th>
<th>1</th>
<th>2</th>
<th>4</th>
<th>7</th>
<th>14</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample 1</td>
<td>260000</td>
<td>200</td>
<td>380</td>
<td>640</td>
<td>790</td>
<td></td>
</tr>
<tr>
<td>Sample 2</td>
<td>550000</td>
<td>850</td>
<td>360</td>
<td>740</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sample 3</td>
<td>520000</td>
<td>520</td>
<td>840</td>
<td>120</td>
<td>520</td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>443333</td>
<td>523</td>
<td>610</td>
<td>2120</td>
<td>455</td>
<td>630</td>
</tr>
<tr>
<td>σ</td>
<td>159478</td>
<td>325</td>
<td>325</td>
<td>2093</td>
<td>474</td>
<td>156</td>
</tr>
<tr>
<td>CV</td>
<td>36%</td>
<td>62%</td>
<td>53%</td>
<td>99%</td>
<td>104%</td>
<td>25%</td>
</tr>
</tbody>
</table>

σ = standard deviation; CV = coefficient of variation
Figure 4.45: Enterococci concentrations during winter period after removal of outliers with water quality limits (Primary and Secondary contact limits for recreational waters)

Table 4.13: Daily mean *E.coli* Values, standard deviation and coefficient of variation during S09 (excluding outliers)

<table>
<thead>
<tr>
<th>Time (days)</th>
<th>Raw sewage</th>
<th>1</th>
<th>2</th>
<th>4</th>
<th>7</th>
<th>15</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample 1</td>
<td>9800000</td>
<td>990</td>
<td>49000</td>
<td>5600</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sample 2</td>
<td>7700000</td>
<td>2000000</td>
<td>5200</td>
<td>46000</td>
<td>6800</td>
<td></td>
</tr>
<tr>
<td>Sample 3</td>
<td>11000000</td>
<td>3300000</td>
<td>3100</td>
<td>41000</td>
<td>6800</td>
<td></td>
</tr>
<tr>
<td>Sample 4</td>
<td>2600000</td>
<td>990</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sample 5</td>
<td>6500000</td>
<td>7700</td>
<td>24000</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sample 6</td>
<td>9800</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sample 7</td>
<td>3100</td>
<td>3100</td>
<td>740</td>
<td>1500</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sample 8</td>
<td>6300</td>
<td>33000</td>
<td>1600</td>
<td>2800</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sample 9</td>
<td>6300</td>
<td>41000</td>
<td>630</td>
<td>1900</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>9500000</td>
<td>3600000</td>
<td>4831</td>
<td>37857</td>
<td>3695</td>
<td>2067</td>
</tr>
<tr>
<td>σ</td>
<td>1670329</td>
<td>2004994</td>
<td>3018</td>
<td>8877</td>
<td>3014</td>
<td>666</td>
</tr>
<tr>
<td>CV</td>
<td>18%</td>
<td>56%</td>
<td>62%</td>
<td>23%</td>
<td>82%</td>
<td>32%</td>
</tr>
</tbody>
</table>

σ = standard deviation; CV = coefficient of variation
Figure 4.46: *E.coli* concentrations during summer period after removal of outliers with water quality limits (Primary and Secondary contact limits for recreational waters)

Table 4.14: Daily mean enterococci Values, standard deviation and coefficient of variation during S09 (excluding outliers)

<table>
<thead>
<tr>
<th>Time (days)</th>
<th>Enterococci (organisms/100mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample 1</td>
<td>Raw sewage 1 2 4 7 15</td>
</tr>
<tr>
<td>Sample 2</td>
<td>9900000 87000 5100 400</td>
</tr>
<tr>
<td>Sample 3</td>
<td>7500000 55000 3100 1600</td>
</tr>
<tr>
<td>Sample 4</td>
<td>17000 3100 98000</td>
</tr>
<tr>
<td>Sample 5</td>
<td>40000 92000</td>
</tr>
<tr>
<td>Sample 6</td>
<td>73000 990 73000</td>
</tr>
<tr>
<td>Sample 7</td>
<td>990 13000 510 1100</td>
</tr>
<tr>
<td>Sample 8</td>
<td>990 11000 410 1200</td>
</tr>
<tr>
<td>Sample 9</td>
<td>1300 15000 200 980</td>
</tr>
<tr>
<td>Mean</td>
<td>8400000 54400 2070 50333 570 1093</td>
</tr>
<tr>
<td>σ</td>
<td>1285820 27455 1538 41740 516 110</td>
</tr>
<tr>
<td>CV</td>
<td>15% 50% 74% 83% 90% 10%</td>
</tr>
</tbody>
</table>

σ = standard deviation; CV = coefficient of variation
4.6 Summary and Conclusions

This chapter reported the outcomes from field experiments carried out during three experimental periods (summer 2008, winter 2008 and summer 2009). Field experiments clearly demonstrated that the die-off rate of \textit{E.coli} and enterococci is different on pervious and impervious surfaces. The data from the impervious surface was collected only during the S08 and W08 experiments which depict higher die-off rate of \textit{E.coli} and enterococci organisms.

The raw sewage concentration levels between the samples collected from the top, middle and bottom layers varied. In addition, there was a difference in obtained concentration values during the winter and summer. The concentrations obtained for microbes during the winter period were lower than the summer periods. Summer and winter 2008 experiments reported that FRNA coliphages did not survive on pervious and impervious surfaces for more than 24 hours after a spill event. As a result the survival rate of FRNA coliphages will not be analyzed and reported further in this study.
During the W08 experiments *E. coli* concentrations were low on the pervious (<170) and impervious (<2) control surfaces. This suggests that *E. coli* organisms cannot be present on surfaces in natural environment without any contamination. In contrast, on some days the enterococci concentrations were high on pervious control surfaces. As literature suggests (Reddy et al., 1981) enterococci organisms can be dormant for long periods and can be found on the soil surface without any spill or contamination. In addition, experiments on control surfaces show that enterococci organisms can survive longer than *E. coli* organisms under natural environments.

Summer 2009 experiments had a different sampling plan where three samples were collected from each plot for microbes testing and a single sample was collected for nutrient from each pervious surface. Collected sub-samples on pervious surfaces during S09 experiments showed similar values with little variation in concentration of microbes’ in-between the same pool of water.

Multiple storm event experiments carried out during S09 experimental period suggests that all microbes did not get washed off from the initial rainfall simulation. This suggests that the washoff rate of microbes during surface runoff is dependent on several factors such as rainfall intensity and the bonding between microbes and soil particles.

Concentrations obtained during winter 08 and summer 09 exceeds the primary and secondary contact limits set by the SEPP and the EPA for recreational waters within the collected samples during the experimental periods. *E. coli* concentrations obtained during both summer and winter experiments were ≥1000 orgs/100mL (secondary contact limits). Enterococci concentrations during winter were suitable for secondary recreational activities as it was between 200 – 1000 orgs/100mL except on day 4. However, these concentrations are at the entrance to the drainage system and will get further diluted within the drainage system reducing the concentrations in receiving waters.

Winter 08 and summer 09 data obtained from these field experiments were used for developing the prediction model of microbes with time and climate factors after a spill event. Summer 08 data will not be used as it did not provide specific values for microbes on some days. No relationships will be developed between microbes with time and climate factors on impervious surface as microbes did not survive at least 24 hours after the spill event on impervious surface.

The following chapter will present the technical development of the microbes prediction model with time and climate factors on a pervious surface after a spill event.
Chapter 5

Development of Microbes Prediction Model

5.1 Introduction

The water quality models have limited reliability when applied to large catchments due to behaviour of microbes under natural conditions (Haydon 2006; Novotny and Olem 1994). Haydon (2006) investigated microbial surface storage, die-off rate of microbes and their transport using the surface run-off experiments under different climatic conditions. This was followed by a study to determine survival pattern of microbes on different surfaces and under different climatic factors. Haydon (2006) reported that hydrology plays most important part in pathogen transport; especially the transport of pathogens is highly dependant on the intensity of storm events. Variations of microbes with and within events are also considered significant in terms of pathogen modelling and are considered to produce larger source of errors in terms of predicting microbial survival rate (Haydon 2006; McCarthy 2008). The above authors also stated that the actual and predicted microbial values even with an order of magnitude error are acceptable.

One of the main objectives of the current study is to estimate the survival rate of microbes with time after a sewer spill in urban catchments and to estimate the microbial concentrations that will get washed off. In addition, urban catchments consist of complex stormwater systems, sewer structures and open channel systems. As discussed above, climate conditions and storm intensity will play a major role in microbial survival and pathogen transport. Chapter 4 presented field data displaying variations in observed microbe concentrations on a particular day from different plots as well as from the same plot. This chapter will analyse the relationships between microbes survival rates, time and climate data. This chapter presents the development of the pathogen survival model, and concludes discussing the developed models to predict the microbes with time.
5.2 Relationship between Survival Rate of Microbes, Time and Climate Variables

Whitman et al. (2004) reported that the abiotic (e.g., salinity, sunlight and temperature etc.) and biotic (predation and competition for survival) factors influence the survival of faecal indicator bacteria (*E. coli* and enterococci). Fujioka et al. (1981) reported solar radiation to be the most important parameter contributing towards the inactivation of *E. coli* and enterococci organisms in water. The above statement was made based on many studies carried out in marine waters (Jimenez, 1994).

As discussed in Chapter 2, survival of microbes is dependent on various parameters such as time (*t*), temperature (*T*), relative humidity (RH), vapour pressure (VP), pH of the soil and soil moisture. Therefore it is necessary to obtain a relationship with climate variables and time to predict the concentration of microbes on the pervious surface at different elapsed time after a dry weather spill. The field data was used to develop relationships between microbe survival, climate variables and time. Estimating microbe die-off rate after a spill event will provide a good understanding of die-off or survival of microbes and its relationship to antecedent conditions before a storm event. For modelling purpose it was decided to consider that the occurrence of spill is not continuous and faecal deposition occurs only once. This will provide the opportunity for a realistic scenario of microbial transport after single spill event on surface.

The climate variables temperature, RH and VP were selected to develop relationships with survival rate of microbes and time. These values were obtained from the Frankston weather station and presented in Appendix A in Tables A2, A3 and A4. Total nitrogen and total phosphorus were not taken into consideration as there were no relationships between microbes and nutrients as shown in Section 4.4. Daily pH and soil moisture data were not available from the experimental plots throughout the experimental period (till 15 days). Climate variables such as temperature and relative humidity changed noticeably between experimental seasons. Field data showed that the concentrations of microbes varied by orders of magnitude between different days. Concentrations of microbes and climate variables were utilised for estimating cross correlation coefficients between climate variables (independent variables) and concentrations of indicator microbe (dependent variables). It is important to ensure that parameters are not interdependent to each other before regression relationships are developed. Tables 5.1 and 5.2 present the relationships between *E. coli*, enterococci and climate variables during winter 08 and summer 09 seasons. Summer 2008 data were not considered to obtain any kind of relationships as most of the obtained values
were not specific. Five data points were available on pervious surfaces during each season to develop the cross correlation matrix from W08 and S09 experiments. Raw sewage values were not considered when developing cross correlation matrix as these values are not climate dependent.

Table 5.1: Cross correlation matrix between climate variables and microbe concentrations for Winter 08 data

<table>
<thead>
<tr>
<th></th>
<th>E.coli</th>
<th>Enterococci</th>
<th>Time</th>
<th>RH</th>
<th>Temperature</th>
<th>VP</th>
</tr>
</thead>
<tbody>
<tr>
<td>E.coli</td>
<td>1.00</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Enterococci</td>
<td>0.24</td>
<td>1.00</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time</td>
<td>-0.85</td>
<td>-0.14</td>
<td>1.00</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RH</td>
<td>0.53</td>
<td>-0.27</td>
<td>-0.52</td>
<td>1.00</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Temperature</td>
<td>-0.71</td>
<td>0.28</td>
<td>0.91</td>
<td>-0.59</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>VP</td>
<td>0.94</td>
<td>0.10</td>
<td>-0.95</td>
<td>0.57</td>
<td>-0.91</td>
<td>1.00</td>
</tr>
</tbody>
</table>

Table 5.2: Cross correlation matrix between climate variables and microbe concentrations for Summer 09 data

<table>
<thead>
<tr>
<th></th>
<th>E.coli</th>
<th>Enterococci</th>
<th>Time</th>
<th>RH</th>
<th>Temperature</th>
<th>VP</th>
</tr>
</thead>
<tbody>
<tr>
<td>E.coli</td>
<td>1.00</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Enterococci</td>
<td>0.66</td>
<td>1.00</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time</td>
<td>-0.48</td>
<td>-0.55</td>
<td>1.00</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RH</td>
<td>-0.97</td>
<td>-0.66</td>
<td>0.67</td>
<td>1.00</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Temperature</td>
<td>-0.91</td>
<td>-0.44</td>
<td>0.34</td>
<td>0.89</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>VP</td>
<td>-0.77</td>
<td>-0.54</td>
<td>0.86</td>
<td>0.90</td>
<td>0.76</td>
<td>1.00</td>
</tr>
</tbody>
</table>

Shibata et al. (2004) suggested that cross correlation coefficients greater than 0.50 represent strong correlations between microbes and climate variables. Cross correlation coefficient of 0.66 between E.coli and enterococci organisms during summer (Table 5.2) showed that these indicator organisms have strong bonding and are highly dependent on each other. However, during winter (Table 5.1) these organisms have not shown any bonding (0.24). Table 5.1 showed that E.coli organisms have a significant correlation with time (-0.85), relative humidity (0.53), temperature (-0.71) and vapour pressure (0.94) during winter season. On the other hand enterococci organisms showed no correlations with any of the variables during winter. In addition, it also depict that there are significant correlations (>0.50) between time, temperature, relative humidity and vapour pressure. Table 5.2 showed that the concentrations of E.coli strongly correlated with relative humidity (-0.97), temperature (-0.91) and vapour pressure (-0.77) during summer 2009 period. Although enterococci organisms did not
show any correlation during winter, during summer, enterococci organisms have correlation coefficients of -0.55, -0.66, and -0.54 with time, relative humidity and vapour pressure respectively.

This data was further coupled with time factor and the concentrations of microbes to model a dry weather spill event under natural conditions.

5.2.1 Estimation of missing microbial data

Microbial data was taken on days 1, 2, 4, 7 and 14 (for winter) and 15 (for summer) during experimental periods. During W08 and S09 experiments, microbial concentrations for in-between days were not available. This missing microbial data (E.coli and enterococci values) for in-between days during experimental period was estimated before developing regression relationships. Based on the literature explained in Chapter 2, E.coli and enterococci show exponential decay curve (parabolic). Exponential decay curve is a straight line in the log domain and hence the linear interpolation of log microbe values between two observed values was used to estimate the missing microbial values during (1 to 15 days) experimental days. Table 5.3 presents the estimated microbial data during winter 08 and summer 09 experimental periods.

Table 5.3: Estimated microbial data during W08 and S09

<table>
<thead>
<tr>
<th>Days</th>
<th>Summer 2009</th>
<th>Winter 2008</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>E.coli</td>
<td>Enterococci</td>
</tr>
<tr>
<td>0</td>
<td>9500000</td>
<td>8400000</td>
</tr>
<tr>
<td>1</td>
<td>3600000</td>
<td>54400</td>
</tr>
<tr>
<td>2</td>
<td>4831</td>
<td>2070</td>
</tr>
<tr>
<td>3</td>
<td>13524</td>
<td>26202</td>
</tr>
<tr>
<td>4</td>
<td>37857</td>
<td>50333</td>
</tr>
<tr>
<td>5</td>
<td>17403</td>
<td>33746</td>
</tr>
<tr>
<td>6</td>
<td>7955</td>
<td>17158</td>
</tr>
<tr>
<td>7</td>
<td>3631</td>
<td>570</td>
</tr>
<tr>
<td>8</td>
<td>3388</td>
<td>635</td>
</tr>
<tr>
<td>9</td>
<td>3162</td>
<td>701</td>
</tr>
<tr>
<td>10</td>
<td>2951</td>
<td>766</td>
</tr>
<tr>
<td>11</td>
<td>2754</td>
<td>832</td>
</tr>
<tr>
<td>12</td>
<td>2570</td>
<td>897</td>
</tr>
<tr>
<td>13</td>
<td>2399</td>
<td>963</td>
</tr>
<tr>
<td>14</td>
<td>2239</td>
<td>1028</td>
</tr>
<tr>
<td>15</td>
<td>2089</td>
<td>1093</td>
</tr>
</tbody>
</table>

**The highlighted values presented are the actual concentrations collected from the field experiments.**
5.2.2 Data analysis

Multiple regression analysis between microbes (*E. coli* and enterococci), time and climate data (relative humidity, temperature) with an addition of potential evapotranspiration was carried out using Microsoft Excel. Consideration of antecedent climate conditions and time before a storm with decay rate of microbes will enhance the understanding of the decay rates of microbes under natural conditions in an urban catchment. Vapour pressure was not considered as the correlation matrix showed high correlation with relative humidity and temperature values during summer and winter. Relative humidity and temperature values for any location are easy to obtain from the Bureau of Meteorology (BOM) and hence it was decided to proceed with relative humidity and temperature instead of vapour pressure to estimate microbial concentration with time. Haydon (2006) considered potential evapotranspiration (PET) and time to estimate the die-off rate of microbes as PET is dependent on RH, T and vapour pressure. As such, it was decided to select PET as well when developing regression relationships for the current study. Each variable was tried separately to select the climatic variable which gives the best regression relationship,

Reed (2004), Haydon (2006), Bell et al. (2009) and Brookes et al. (2003) reported that the microbes follow an exponential decay function which is similar to power regressions. Based on this, a power regression relationship similar to the format given by Equation 5.1 was used. This equation could be rewritten in the form of Equation 5.2.

\[
y = ax_1^b x_2^c \\
\log y = \log a + b \log x_1 + c \log x_2
\]

where, \(a\), \(b\) and \(c\) are empirical constants,

\(y\) = concentration of *E. coli* or enterococci organisms on a given day (orgs/100mL),
\(x_1\) = elapsed time (days),
\(x_2\) = average value of the climate variable between the spill occurrences and storm event.

Multiple linear regression equations were developed between the logarithmic values of microbes, time and climate variables to obtain \(a\), \(b\) and \(c\) variables given in Equation 5.2. The concentration of microbes on a particular day will depend on the climate conditions between the day of the spill and the day of storm event. As such an average
value for the climatic variable for that particular period \((x_3)\) was taken when developing regression relationships.

Raw sewage concentrations were also taken into account during the development of the regression relationship. The following combinations have been used to develop regression relationships between microbes, time and climate variables.

- Microbes \((E. coli\) and enterococci\) with time and relative humidity
- Microbes \((E. coli\) and enterococci\) with time and temperature
- Microbes \((E. coli\) and enterococci\) with time and PET

The obtained regression equations with the above mentioned combinations are provided in Tables 5.4, 5.5, 5.6 and 5.7 below. Predicted concentrations of \(E. coli\) and enterococci with time during summer and winter periods were also calculated with the developed equations.

Table 5.4: Relationship between \(E. coli\), time and different climate variables during summer

<table>
<thead>
<tr>
<th>S09</th>
<th>(R^2)</th>
<th>SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>(E. coli = 10^{-26.3} \times \text{Time}^{-2.3} \times \text{RH}^{-11.3})</td>
<td>83%</td>
<td>50%</td>
</tr>
<tr>
<td>(E. coli = 10^{-6.4} \times \text{Time}^{-2.1} \times \text{Temp}^{8.9})</td>
<td>82%</td>
<td>51%</td>
</tr>
<tr>
<td>(E. coli = 10^{6.26} \times \text{Time}^{-2.79} \times \text{PET}^{0.26})</td>
<td>79%</td>
<td>55%</td>
</tr>
</tbody>
</table>

\(*\text{RH} = \text{average relative humidity between the time of spill and the elapsed time; Temp} = \text{average temperature between the time of spill and the elapsed time; PET} = \text{average potential evapotranspiration between the time of spill and the elapsed time; } R^2 = \text{correlation coefficient for the fitted equation; SE} = \text{standard error}\)

Table 5.5: Relationship between enterococci, time and different climate variables during summer

<table>
<thead>
<tr>
<th>S09</th>
<th>(R^2)</th>
<th>SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enterococci = 10^{-4.85} \times \text{Time}^{-2.9} \times \text{RH}^{10.71})</td>
<td>73%</td>
<td>64%</td>
</tr>
<tr>
<td>Enterococci = 10^{-18.12} \times \text{Time}^{-1.38} \times \text{Temp}^{15.92})</td>
<td>82%</td>
<td>51%</td>
</tr>
<tr>
<td>Enterococci = 10^{5.87} \times \text{Time}^{-2.75} \times \text{PET}^{0.26})</td>
<td>73%</td>
<td>64%</td>
</tr>
</tbody>
</table>
Table 5.6: Relationship between *E. coli*, time and different climate variables during winter

<table>
<thead>
<tr>
<th>W08</th>
<th>$R^2$</th>
<th>SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>$E. coli = 10^{27.01}$ $\times$ Time$^{-2.78}$ $\times$ RH$^{-11.57}$</td>
<td>83%</td>
<td>43%</td>
</tr>
<tr>
<td>$E. coli = 10^{16.64}$ $\times$ Time$^{-3.53}$ $\times$ Temp$^{19.04}$</td>
<td>87%</td>
<td>37%</td>
</tr>
<tr>
<td>$E. coli = 10^{8.66}$ $\times$ Time$^{2.88}$ $\times$ PET$^{0.86}$</td>
<td>83%</td>
<td>44%</td>
</tr>
</tbody>
</table>

Table 5.7: Relationship between enterococci, time and different climate variables during winter

<table>
<thead>
<tr>
<th>W08</th>
<th>$R^2$</th>
<th>SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enterococci $= 10^{32.82}$ $\times$ Time$^{1.82}$ $\times$ RH$^{-15.5}$</td>
<td>59%</td>
<td>52%</td>
</tr>
<tr>
<td>Enterococci $= 10^{24.65}$ $\times$ Time$^{-2.81}$ $\times$ Temp$^{24.69}$</td>
<td>71%</td>
<td>44%</td>
</tr>
<tr>
<td>Enterococci $= 10^{4.27}$ $\times$ Time$^{-1.83}$ $\times$ PET$^{0.91}$</td>
<td>54%</td>
<td>55%</td>
</tr>
</tbody>
</table>

Tables 5.4, 5.5, 5.6 and 5.7 depict the regression relationships, coefficient of determination ($R^2$) and standard error between actual and the fitted relationship for each curve between microbes and independent variables. From the above tables it can be observed that $R^2$ values obtained for *E. coli* and enterococci are greater than 70% during summer and winter except enterococci values during the winter period which provides 59% with relative humidity and 54% with PET. Table 5.4 presents the coefficient of determination ($R^2$) for *E. coli* developed with relative humidity (83%), temperature (82%) and PET (79%). In addition, it also provides a standard error of 50%, 51%, and 55% for equations developed with relative humidity, temperature and PET respectively. Enterococci concentrations predicted with the developed equations are also suggesting a good fit ($R^2$) between actual values during the summer period to be 70 to 80%. Standard error between actual and predicted values was 64% with relative humidity and PET and 51% with temperature.

Figures 5.1 and 5.2 depict the relationships between the actual and predicted data for *E. coli* and enterococci during the summer and winter experimental periods on the pervious surfaces. 45% line was plotted along with +1 magnitude line and -1 magnitude line to identify the association between actual and predicted microbes data. Haydon (2006) reported that the microbial modelling is not a simple process and may result in
many errors due to analytical techniques; hence an order of magnitude difference is acceptable for modelled microbial data within the actual and predicted values. The results preceded in Chapter 4 confirm Haydon’s (2006) observation. Figures 5.1 and 5.2 depict the efficacy of the developed equation for *E.coli* and enterococci during S09 and W08. Most of the predicted values are within the + or – one magnitude line. Figure 5.2 depict the efficacy of the developed equation for *E.coli* and enterococci during the winter period.

Enterococci values presented in above Figure 5.2 with relative humidity, temperature and PET show reasonable fit between actual and predicted values. Although there was a large variation in enterococci data during experimental periods, predicted values are within + or – magnitude line. Table 5.6 shows that the $R^2$ for *E.coli* organisms was above 80% during the winter period, with low standard errors (43% with relative humidity, 37% with temperature and 44% with PET). Table 5.7 provided with $R^2$ values for enterococci organisms suggesting reasonable fit. $R^2$ values obtained from equations were above 50% and standard error was below 55%.
Figure 5.1: Comparison between the actual and predicted values for *E. coli*, enterococci during summer 2009 with different independent variables (relative humidity, temperature and PET)
Figure 5.2: Comparison between the actual and predicted values for *E. coli*, enterococci during winter 2008 with different independent variables (relative humidity, temperature and PET)
5.3 Verification of the developed model

Developed regression equations require verification on an independent set of data. The developed exponential regression equations for E.coli and enterococci during S09 on pervious surface were used to predict the same during W08 and vice versa. Figure 5.3 represents the application of developed exponential decay equations during S09 for E.coli and enterococci to predict W08 data. 1:1 line was plotted along with +1 magnitude line and -1 magnitude line to identify the resemblance between actual and predicted data. However, as seen in Figure 5.3(c) the developed equation for PET during S09 is not effective to estimate the W08 values as all the predicted values are negative. Figures 5.3c and 5.3e show the ineffectiveness of the developed S09 equations to predict E.coli and enterococci organisms during W08 with respective climate variable.

As can be seen in Figure 5.3, most of the concentrations are near to the 1:1 line and few are outside the + or – magnitude line. Except the regression equations developed with S09 data for E.coli and enterococci with RH and temperature, the other equations did not give a good fit when predicting concentration of microbes with time during winter. Figure 5.3 shows that the equation developed with PET for E.coli and with temperature for enterococci did not give a good fit when predicting E.coli and enterococci concentrations during winter.

Figure 5.4 shows the application of developed regression equations using data collected during W08 for E.coli and enterococci to predict S09 data.
Figure 5.3: Verifications of *E.coli* and enterococci relationships developed with S09 data on W08 data
Figure 5.4: Verifications of *E. coli* and enterococci relationships developed with W08 data on S09 data
Most of the *E. coli* values predicted with the developed regression models with relative humidity and PET are within + or – one magnitude difference values. This suggests the efficacy of the developed equation to predict *E. coli* organisms. As seen in Figures 5.4b and 5.4e the developed equations to predict *E. coli* and enterococci with temperature using field data collected during W08 are not effective to estimate the microbial concentrations during S09 as all the predicted values are greater than one magnitude.

It was considered to be good to obtain one equation for *E. coli* and enterococci which will predict microbes after a spill event at any given time interval.

The regression relationships developed with RH and PET with data collected during winter gave acceptable results. Daily relative humidity data are easily available from BOM or can be measured. Whereas, daily PET values need to be calculated from the relative humidity, temperature, vapour pressure and radiation data. As such it was decided to use the equations developed with RH for further investigation. Equations 5.3 to 5.6 presented below developed with RH during summer and winter to estimate the microbes with time (Tables 5.4 to 5.6).

S09 data:

\[
E. coli = 10^{26.30} \text{Time}^{-2.3} \text{RH}^{-11.31} \quad R^2 = 83\% \quad (5.3)
\]

\[
\text{Enterococci} = 10^{4.85} \text{Time}^{-2.91} \text{RH}^{0.71} \quad R^2 = 73\% \quad (5.4)
\]

W08 data:

\[
E. coli = 10^{27.01} \text{Time}^{-2.70} \text{RH}^{-11.57} \quad R^2 = 83\% \quad (5.5)
\]

\[
\text{Enterococci} = 10^{32.85} \text{Time}^{-1.82} \text{RH}^{-15.5} \quad R^2 = 59\% \quad (5.6)
\]

where,

*E. coli* or enterococci = Concentration of survived organisms after a spill (orgs/100mL)

Time = Elapsed time after a dry weather spill (days)

RH = Relative humidity between the time of spill and the elapsed time (%)

Above equations could be written in the general format as give in Equation 5.7.

\[
\text{Microbes} = 10^{a} \text{Time}^{-b} \text{RH}^{-c} \quad (5.7)
\]

The definitions of microbes, time and relative humidity are as given above.
The equations developed for *E.coli* (Equations 5.4 and 5.6) consists of a, b and c of the same order. As such equation for *E.coli* will be given as in Equation 5.8. (Note: a, b and c are taken as round numbers).

\[
\text{Microbes} = 10^{27} \, \text{Time}^{-2.5} \, \text{RH}^{-11.5} \tag{5.8}
\]

The verification of the equation developed for enterococci with S09 data (Equation 5.4) did not give good results when verified on the W08 data. However, the equation developed for enterococci with W08 data (Equation 5.6) did give a good fit when verified on S09 data. As a result it was decided to further investigate on the applicability of Equation 5.6 to estimate the enterococci concentrations.

It was considered good if one equation could be obtained for both microbes (*E.coli* and enterococci) in predicting microbial concentrations after a spill. a, b and c coefficients in Equation 5.6 is very close to the coefficients taken in Equation 5.8. As a result Equation 5.8 was applied to enterococci data obtained in W08 and S09. The results were compared with the results obtained with equations 5.4 and 5.6.

Figures 5.5 and 5.6 present the comparison between the predicted values with the developed equation (Equation 5.8) and predicted values with Equations 5.4 and 5.6 for winter08 and summer09 respectively and the actual observed concentrations.
Figure 5.5: Comparison between estimated enterococci concentrations obtained from Equation 5.6 and Equation 5.8 with actual values.

Figure 5.6: Comparison between estimated enterococci concentrations obtained from Equation 5.4 and Equation 5.8 with actual values.
The results show that most of the predicted values with Equation 5.8 overestimate the enterococci survival rate compared to values obtained from Equations 5.4 and 5.6. However, these values are in-between the +/- Magnitude line which is acceptable. Given the uncertainties associated with the behaviour of enterococci organisms, Equation 5.8 could be used with cautions.

Based on the above information the developed Equation 5.8 will be used to estimate the concentration of microbes (E.coli and enterococci) with time after a dry weather spill event. The coefficient ‘a’ in Equation 5.7 partly accounts for the raw sewage concentration. Based on the field data and literature (Zhang and Farahbakhsh, 2007), 1E+07 organisms/100mL represents the concentration of raw sewage.

Figures 5.7 and 5.8 present the comparison between the field data and model estimated values during winter and summer respectively. Based on Figures 5.7(a) and 5.8(a) although the predicted E.coli concentrations overestimate the actual values, they are within a magnitude range. However, the predicted enterococci concentrations [Figures 5.7(b) and 5.8(b)] are not close to the concentrations obtained from field experiments. As mentioned earlier the developed equation needs to be used with caution when estimating the survival rate of enterococci with elapsed time after a dry weather spill.

a) E.coli

b) Enterococci

Figure 5.7: Comparison of model estimated concentrations and field data for E.coli and enterococci during winter 2008
Figure 5.8: Comparison of model estimated concentrations and field data for *E.coli* and enterococci during summer 2009

Table 5.8 presents the predicted *E.coli* and enterococci values with the above developed equation during winter and summer period. It is important to note that the predicted concentrations with the developed equation for *E.coli* and enterococci are the same as one equation was recommended from the study. When applying the Equation 5.8 to estimate microbes, if the microbes concentration on the following day (t+1) is greater than the previous day (t) then it was assumed that the microbial concentration on the 't+1' th day is the same as on the previous day.

The above results certainly indicate efficacy of the developed equation which can be invariably used to predict the loss of microbes with time on a pervious surface. On the other hand, there are some limitations to some extent on the estimation of enterococci organisms during both seasons due to the variation in the field data.
Table 5.8: *E. coli* and enterococci values with the developed model

<table>
<thead>
<tr>
<th>Days</th>
<th>Field data during summer 2009</th>
<th>Predicted values during winter 2008</th>
<th>Field data during winter 2008</th>
<th>Predicted values during winter</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
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<td>6250</td>
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<td>-</td>
<td>-</td>
<td>89812</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>37857</td>
<td>50333</td>
<td>56773</td>
<td>7550</td>
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</tr>
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<td>-</td>
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<td>18334</td>
<td>-</td>
</tr>
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<td>570</td>
<td>13022</td>
<td>1500</td>
</tr>
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<td>8</td>
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<td>15</td>
<td>2067</td>
<td>1093</td>
<td>1540</td>
<td>-</td>
</tr>
</tbody>
</table>

5.4 Summary and Conclusions

This chapter has discussed development of a microbial survival prediction model. Microbe prediction model was developed using Microsoft Excel statistical package. Multiple exponential regression analysis has been carried out with winter 08 and summer 09 data sets to develop the relationships between microbes, time and relative humidity.

The cross correlation matrix suggests high cross correlations between *E. coli* and enterococci during summer whereas it shows poor relationship during winter period. The cross correlation matrix between microbial data and climate parameters suggest high cross correlations between *E. coli*, relative humidity, temperature and vapour pressure during summer and winter periods. In addition, relative humidity, temperature and vapour pressure are also correlated with each other during both experimental periods. Whereas, enterococci show high correlation with time, relative humidity and vapour pressure during summer experiments, there was no correlation obtained between enterococci and climate variables during the winter period.
The study showed that by using multiple exponential regressions it is possible to develop a model to calculate concentrations of *E.coli* and enterococci with time and average relative humidity after a dry weather spill event. The developed model is presented below:

\[
\text{Microbes} = 10^{27} \text{Time}^{-2.5} \text{RH}^{-11.5}
\]

where,
- **Microbes** = Concentration of survived microbes after a spill (orgs/100mL);
- **Time** = Elapsed time after a dry weather spill (days);
- **RH** = Average relative humidity between the time of spill and the elapsed time (%).

The above equation was verified with an independent set of data obtained during W08 and S09 experimental periods. This suggests the efficacy of the developed model for predicting the concentration of microbes after a dry weather sewer spill. However, prediction of enterococci organisms with this model has limitations due to the uncertainties of enterococci survival pattern on pervious surface.

The developed model is then coupled with simple wash-off equation to predict the concentration of microbes at stormwater drain in the following chapter (Chapter 6).
Chapter 6

Impact of Dry Weather Sewer Spills on Water Quality in Stormwater Drains

6.1 Introduction

Preceding chapter presented the development of a microbe prediction model for a pervious surface after a dry weather spill event. The data sets from the field experiments were used for this purpose. The primary aim of this study is to estimate the microbial inflow into stormwater drains after a dry weather sewer spill.

This Chapter will discuss the dry weather sewage spill data collected throughout year 2007 (Phase II of the data collection), analysis of the data, effects of rainfall intensity on the washoff of microbes towards stormwater drains and the contribution of microbes from selected catchments towards waterways. This analysis plays a vital role in determining the impact of dry weather sewer spills on stormwater quality within the catchment. This chapter explains the selection of catchments, actual sewer spill data collection procedure, estimation of microbes at the stormwater drain, the development of a relationship between the storm event and percent of microbes washed into drain and annual contribution of microbes into the Yarra due to dry weather spills. As reported in Chapter 1, the actual dry weather sewage spill data collection was carried out at South East Water with the assistance of an affiliated company, Utility Services. Usually if a dry weather blockage (or sewer collapse) occurs outside a household property, the public will inform the water retail company. However, if the spills occur within a household property the blockages go unrecorded as it is the responsibility of the owner to get the problem fixed. Prahran Main Drain and Gardiners Creek which falls in South East Waters region were selected for the study. The selection of the catchments was carried out on the basis of the availability of historical dry weather sewer spill data in the main catchment area. Dry weather spill data on catchments’ reticulation system was extracted from the South East Water data base for year 2007.

At initial stage of the study, it was proposed to contact the local plumbers in the area within and nearby catchment and gather spill data and additional information on spills (if any) to obtain the greater knowledge about the reported spill events by water retail companies or property owners. However, it was not possible to organize the data collection from local plumbers during this period. It was decided to collect the reported
number of spills within South East Water during the year 2007, the information provided with the number of spills, number of sampling locations and spill event season for the analysis.

Figure 6.1 below demonstrates the transport of microbes after a dry weather spill towards stormwater drain. Haydon (2006) reported that the surface and sub-surface flows are two major components of water flow in a large catchment. In an urban catchment the stormwater drains and sewer lines are almost parallel to each other. If a sewage spill occurs it will be relatively close to an inlet of a stormwater drain. Furthermore, the sub-surface flow could be ignored as this flow could not enter into the stormwater system. Surface flow and intensity of the storm event are the most important factors contributing towards the transport of microbes to the stormwater drain after a spill event.

![Diagram of transport of microbes towards the stormwater drain after a dry weather spill](image)

Figure 6.1: Transport of microbes towards the stormwater drain after a dry weather spill

The washoff of microbes is directly proportional to rainfall intensity and hence, the rainfall intensity is an important factor. Sub-surface flow will not affect the concentration of microbes at the stormwater drains because of the distance between the spills and drain as seen in Figure 6.1. As a result, the concentration from sub-surface flow was not considered while estimating the amount of microbes contributed to the stormwater drain.
6.2 Selection of Catchments

Pollard et al. (2004) reported that dry weather sewer spills contribute significantly to pollutant loads in stormwater systems within catchments. Robinson et al. (2007) showed that *E.coli* contamination is high in the lower Yarra section. *E.coli* loads vary from 2000 to 160000 organisms/100ml at Elizabeth Street Main drain and Princess Bridge council drain in dry weather as well as wet weather season, where as the acceptable standard is set at 1000 organisms/100ml (Yarra Watch 2007). Robinson et al. (2007) suggested detailed follow-up investigations of 12 identified stormwater drainage networks as a priority action to locate and remove the source of human faecal contamination in these drains. Historical data at the Prahran Main Drain has highly variable levels of *E.coli* indicator bacteria (i.e. 31 to 52000 organisms/100ml). EPA Victoria in 1990s declared the Prahran Main Drain as a leading human faecal source to the Yarra River (Rooney, 2007). Data provided by South East Water showed that average faecal contamination in Prahran Main Drain catchment with an area of 7.55 km$^2$ is 24,700 *E.coli* organisms/100ml. The catchment hosts 115.22 km of stormwater infrastructure and 143.3 km of sewerage infrastructure (mainly drains). Based on Robinson et al. (2007) study, the Prahran main drain has been selected for the current study as it is the most significant faecal contributor from the South East Water’s area.

Three most contributing tributaries have also been identified in the above study by EPA Victoria, which are Gardiners Creek, Koonung Creek and Darebin Creek (Figure 6.2). The levels of *E.coli* contamination were higher in these tributaries (i.e. 913 to 1984 *E.coli* organisms/100 ml) than the standards set for *E.coli* (550 *E.coli* organisms/100ml). Koonung Creek contributed large amounts of pollutant loads as the loads of *E.coli* varied from 1048 to 3091 organisms/100ml. Unfortunately Koonung Creek and Darebin Creek tributaries are out of South East Water’s authorised catchment area and as a result, there is no access to dry weather spill data required for this study. Most of the Gardiners Creek catchments fall under South East Water and EPA Victoria’s authority. Gardiners Creek catchment was selected as a second catchment for this study as it was convenient to access and collect the actual dry weather sewer spill data for this study.

The selection of catchments was carried out based on the *E.coli* levels in Yarra River as well as on the basis of historical dry weather sewer spill data in the main catchment area.
If a dry weather blockage occurs the water retail company (in this case South East Water) clears the blockages and records the reasons for the sewage spill throughout the sewer reticulation system. However, if the spills occur in a household property the blockage goes unrecorded as it is the responsibility of the owner to get the problem fixed. Dry weather spill data on catchments’ reticulation system (i.e. Prahran Main Drain and Gardiners Creek which falls in South East Waters region) was decided to be extracted from the South East Water data base for year 2007. It was planned to contact the local plumbers to obtain spill data from households to obtain greater knowledge about the reported spill events by water retail companies or property owners. Frequency of the number of spills occurring in the past, number of sampling locations, spill event season and some other information were required to analyse the dry weather spill data.

The catchments selected for the study purpose ensures a superior representation of the types of uniqueness present in urban catchments such as population density, domestic animals, wildlife, commercial and industrial establishments etc. Two catchments selected for the study (Prahran Main Drain and Gardiners Creek) represents an urban stormwater system as the areas are densely populated as seen in satellite image Figure 6.3 below. In addition, as these catchments consist of considerable pollutant loads, their contribution towards pollutant loads in the Yarra River is considered significant as mentioned in Chapter 2.
Figure 6.3: Selected catchments for the current study (Prahran Main Drain and Gardiners Creek) [Source: Melbourne Water]
6.3 Catchment Description

Both wet weather sewer overflows and dry weather sewer spills within catchments significantly contribute towards the contamination of waterways within its boundary limits (Yarra Watch, 2007). The main selection criteria of the selected catchments were:

1. Within the boundaries of South East Water
2. Typical representation of urban catchments (population densities)
3. Urban catchment portrays better picture of different types of wastewater systems such as sewered, unsewered, and combined systems
4. Water quality issues
5. Land use patterns of these selected catchments give an idea of the urban catchment. Land use pattern reflects the load of microorganisms within the catchment (McCarthy 2008 and Bannerman et al. 1993).

6.3.1 Gardiners Creek

Figure 6.4 depicts the map of Gardiners Creek catchment. Gardiners Creek catchment is located 10 km south east of the City of Melbourne. This is an urban catchment and contains densely populated areas of mainly residential and commercial establishments. Gardiners catchment covers 115 km² of the eastern suburbs (Sokolov, 1996).

Gardiners Creek, a tributary of Yarra River flows in between the municipal boundary of Boroondara and Stonnington councils. Population of the surrounding area (Glen Iris) is 23,270 according to 2006 census. This catchment consists of a number of human activities in day to day life. Many schools are located in this area. This stretch of the Yarra River (Middle Yarra) is used for a number of recreational and sporting activities as mentioned in Chapter 1.
The river path within the catchment region has a poor water quality from an environmental outlook. The creek contains freshwater species such as native fish, water rats and the growling grass frog.

Urban and industrial developments around Melbourne metropolitan region are intensive. However, these industries have minute impact on water quality in the river because of their process effluents are discharged in sewers as observed by Sokolov (1996). The entire catchment has a population density of 2000-3000 persons/km².

### 6.3.2 Prahran Main Drain Catchment

This catchment is located 5 km south-east from city of Melbourne, Victoria, Australia. An area of 9.55 km² is covered by Prahran city. The local city council for this region is the City of Stonnington. Population within the catchment area as per 2001 census was 54,141. This area is mainly residential and commercial; landscape mainly consists of impervious surface. The city of Prahran is known for its parks, many shops, restaurants
and cafes indicating many recreational and social activities. Figure 6.5 shows Prahran Main Drain catchment marked with boundaries with the marked sub-catchments.

Figure 6.5: Prahran Main Drain Catchment, marked with the catchment boundary and the Main drain connected to Yarra River (Melbourne Water)

6.4 Catchment Dry Weather Spill Data for 2007

The contribution of the Prahran Main Drain and the Gardiners Creek catchment is significant towards the faecal contamination in the Yarra River, as described earlier in Section 6.3. *E.coli* levels in the river water within the two selected catchments are significant. The levels of *E.coli* organisms exceeded the primary and secondary limits (Prahran Main Drain 31 to 52,000 organisms/100mL and Gardiners Creek 913 to 1984 organisms/100mL). It is necessary to check the impact of dry weather sewer spills within these catchments on the waterways. The total number of spills within these catchments throughout year 2007 will assist to isolate the faecal contribution. Actual dry weather spill data from the Prahran Main Drain and the Gardiners Creek catchment
were obtained from the South East Water’s database. Further analysis was carried out to understand the contribution or significance of dry weather spills towards the Yarra River faecal contamination. The analysed actual dry weather sewer spill data is presented in Tables 6.1 and 6.2 below. On the other hand, all the spills inside the household property would have not been recorded as the owner of the property could contact the local plumber instead of SEW services to fix the blockages and these spills may go unrecorded.

Table 6.1 indicates that there had been 80 dry weather sewer spills during the year 2007 in the Prahran Main Drain catchment. As seen in Table 6.1, Caulfield North region contributed 50 spills towards the pollutant loads throughout the year. The actual spill data indicated that this catchment contributed high pollutant loads towards the Yarra River and also supports the observations (i.e. 31 to 52000 organisms/100 mL, Prahran Main Drain as a leading human faecal source to the Yarra River) from the studies on Yarra Catchment, (Yarra Watch, 2007). The occurrences of spill events were more during January 2007 to April 2007 in the Prahran Main Drain catchment.

Table 6.1: Reported dry weather spills in Prahran Main Drain catchment during 2007

<table>
<thead>
<tr>
<th>Month</th>
<th>Albert Park</th>
<th>Caulfield</th>
<th>Caulfield North</th>
<th>Armadale</th>
<th>Total</th>
</tr>
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<tr>
<td>January</td>
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<tr>
<td>February</td>
<td>3</td>
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<td>6</td>
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<td>March</td>
<td>-</td>
<td>-</td>
<td>9</td>
<td>1</td>
<td>10</td>
</tr>
<tr>
<td>April</td>
<td>2</td>
<td>-</td>
<td>6</td>
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<td>9</td>
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<td>May</td>
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<td>July</td>
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<td>November</td>
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<td>1</td>
</tr>
<tr>
<td>December</td>
<td>2</td>
<td>-</td>
<td>-</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Total</td>
<td>13</td>
<td>2</td>
<td>50</td>
<td>15</td>
<td>80</td>
</tr>
</tbody>
</table>
On the other hand, the total numbers of reported dry weather sewer spills within Gardiners Creek catchment are 15 and are lower than the Prahran Main Drain catchment. Table 6.2 depicts that the Glen Iris region contributed more towards the pollutant loads within Gardiners Creek catchment (Total number of spills 7). The data suggest that contribution of faecal microbes from Prahran Main Drain and Gardiners Creek catchment is considerable, and they carry a considerable load towards overall microbial pollution in the Yarra River. Section 6.7 will describe the estimation of microbial contribution to the river from the total load from selected catchments.

Table 6.2: Reported dry weather spills in Gardiners Creek catchment during 2007

<table>
<thead>
<tr>
<th>Month</th>
<th>Balwyn</th>
<th>Hawthorn</th>
<th>Malvern East</th>
<th>Malvern</th>
<th>Glen Iris</th>
<th>Kew</th>
<th>Total</th>
</tr>
</thead>
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<td>-</td>
<td>1</td>
<td>-</td>
<td>2</td>
<td>-</td>
<td>3</td>
</tr>
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<td>February</td>
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<td>-</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1</td>
</tr>
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<td>-</td>
<td>-</td>
<td>-</td>
<td>1</td>
</tr>
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<td>1</td>
</tr>
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<td>May</td>
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<td>-</td>
<td>-</td>
<td>-</td>
<td>1</td>
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</tr>
<tr>
<td>June</td>
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<td>-</td>
<td>2</td>
<td>1</td>
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<td>-</td>
<td>-</td>
<td>-</td>
<td>1</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td>August</td>
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<td>-</td>
<td>-</td>
<td>-</td>
<td>1</td>
<td>-</td>
<td>2</td>
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<td>September</td>
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<td>-</td>
<td>-</td>
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<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Total</td>
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<td>1</td>
<td>3</td>
<td>2</td>
<td>7</td>
<td>1</td>
<td>15</td>
</tr>
</tbody>
</table>

6.5 Computation of Concentration of Microbes at Stormwater Drain Inlet

Chapter 5 presented the estimation of microbe concentration with and time depending on the climatic conditions after a dry weather spill on pervious surface. A spill on impervious surfaces (roads or pedestrian surface outside the household properties) contains less number of microbes due to higher die-off rate of microbes with time on impervious surface. Thus the prediction model was developed to estimate of microbes
retained only on pervious surface after a dry weather spill. Microorganisms remained at a given time on surface after a dry weather sewer spill gets washed off with the surface run-off. The current section focuses on the estimation of the amount of survived microbes transported into the drain after a rainfall event.

One of the main aims of this research is to estimate the impact of dry weather sewer spills on the stormwater system. The above said objective can be achieved by calculating the amount of faecal microbes (E.coli and enterococci) transported towards a stormwater drain from a dry weather sewer spill.

Figure 6.6 presents the conceptual microbe transport model used for the current study. Main assumptions made to develop the conceptual microbial movement model are as follows:

- The location of spill is near the stormwater drain inlet.
- Volume of each dry weather spill is 10L.
- There is no rainfall loss. All rain water flows as surface runoff.
- Only the surface runoff component is considered in predicting the transport of microbes into a stormwater drain due to a dry weather spill.
- There is no sub-surface flow on impervious surface. Hence, all the survived microbes would be transported into stormwater drains during a storm event.
Equation 6.1 (same as Equation 5.8) was used to estimate the concentration of *E.coli* and enterococci at different time intervals after a dry weather spill event.

\[
\text{Microbes} = 10^{27} \times \text{Time}^{2.5} \times \text{RH}^{11.5} \quad \text{.................................................. (6.1)}
\]

where,

- Microbes = concentration of survived microbes after a spill (orgs/100mL);
- Time = Elapsed time after a dry weather spill (days);
- RH = Average relative humidity during the elapsed time period (%).

Estimated values for *E.coli* and enterococci were then used with washoff Equation 6.2 developed by McCarthy (2008) to predict the amount of microbes at a stormwater drain inlet which will eventually determine the significance of the spill on stormwater quality in a drain. As stated in Chapter 2; McCarthy (2008) and Haydon (2006) stated that rainfall intensity plays a major part in generating washoff of the microbes with generated flow of surface runoff. During field experiments, rainfall intensity of 60mm/hr was used to mimic 1 in 10 years (15 minutes duration) rainfall event on pervious surfaces over the study region.
Simple wash-off equation used by McCarthy (2008) is given in Equation 6.2.

\[ C_s(t) = P_s(t) \times RI(t)^{0.293} \] \hspace{2cm} (6.2)

where,

- \( C_s(t) \) = concentration of microbes at the stormwater outlet (orgs/L)
- \( P_s(t) \) = microorganism levels in surface store (orgs/L)
- \( RI(t) \) = routed and translated rainfall intensity (mm/min)
- \( t \) = time after a spill event (minutes)

The above author used routed and translated rainfall intensity \( RI(t) \) for his study to calculate the concentration of microbes at stormwater outlet \( C_s(t) \). McCarthy (2008) used the routed rainfall intensity as it reflects the time period and the attenuation effects in stormwater drains when calculating the microbes at the drain outlet.

Current study concentrates on estimating the concentration of microbes at the inlet of the stormwater drain after a dry weather sewer spill, and the movement of microbes on land surface. As a result of this, rainfall intensity was not routed for the estimation of microbes transported. Equation 6.2 was simplified for the current study and given in Equation 6.3.

\[ C_s(t) = P_s(t) \times RI^{0.293} \] \hspace{2cm} (6.3)

where,

- \( C_s(t) \) = concentration of microbes at the stormwater drain inlet (orgs/L)
- \( P_s(t) \) = number of microbes present on surface before storm event (orgs/L)
- \( RI \) = the average rainfall intensity of the storm event (mm/min)
- \( t \) = elapsed time after a spill event (days)

Table 6.3 presents the estimated concentration of \textit{E.coli} at the stormwater drain inlet using the surface washoff equation (Equation 6.3). \textit{E.coli} values estimated with the developed model (Equation 6.1) were used to calculate the microbial concentrations on the surface after a certain elapsed time period following a spill event. It was assumed that the spill size was equivalent to 10L (Personal Communication Kristy Bebend, SEW). The concentration of microbes in 10L spill was calculated and reported in Column 3 of Table 6.3. 60mm/hr rainfall intensity was used to calculate the
concentration of microbes at the stormwater inlet using Equation 6.3. Table 6.3 shows that with 60mm/hr rainfall intensity, irrespective of the antecedent time period, all the available concentration of microbes get washed off from the location of the spill (i.e. 100% concentration).

Table 6.3: Application of surface washoff equation on the predicted E.coli values by exponential decay equation

<table>
<thead>
<tr>
<th>Time (days)</th>
<th>Concentration of E.coli (Orgs/100mL)</th>
<th>Concentration of E.coli organisms in a 10L spill (Orgs/10L)</th>
<th>Concentration at stormwater inlet Cs(t) Orgs/10L</th>
<th>% Contribution to the drain</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5.0E+06</td>
<td>5.0E+08</td>
<td>5.0E+08</td>
<td>100</td>
</tr>
<tr>
<td>2</td>
<td>4.0E+05</td>
<td>4.0E+07</td>
<td>4.0E+07</td>
<td>100</td>
</tr>
<tr>
<td>3</td>
<td>9.0E+04</td>
<td>9.0E+06</td>
<td>9.0E+06</td>
<td>100</td>
</tr>
<tr>
<td>4</td>
<td>6.0E+04</td>
<td>6.0E+06</td>
<td>6.0E+06</td>
<td>100</td>
</tr>
<tr>
<td>5</td>
<td>3.0E+04</td>
<td>3.0E+06</td>
<td>3.0E+06</td>
<td>100</td>
</tr>
<tr>
<td>6</td>
<td>2.0E+04</td>
<td>2.0E+06</td>
<td>2.0E+06</td>
<td>100</td>
</tr>
<tr>
<td>7</td>
<td>1.0E+04</td>
<td>1.0E+06</td>
<td>1.0E+06</td>
<td>100</td>
</tr>
<tr>
<td>8</td>
<td>1.0E+04</td>
<td>1.0E+06</td>
<td>1.0E+06</td>
<td>100</td>
</tr>
<tr>
<td>9</td>
<td>9.0E+03</td>
<td>9.0E+05</td>
<td>9.0E+05</td>
<td>100</td>
</tr>
<tr>
<td>10</td>
<td>7.0E+03</td>
<td>7.0E+05</td>
<td>7.0E+05</td>
<td>100</td>
</tr>
<tr>
<td>11</td>
<td>5.0E+03</td>
<td>5.0E+05</td>
<td>5.0E+05</td>
<td>100</td>
</tr>
<tr>
<td>12</td>
<td>4.0E+03</td>
<td>4.0E+05</td>
<td>4.0E+05</td>
<td>100</td>
</tr>
<tr>
<td>13</td>
<td>3.0E+03</td>
<td>3.0E+05</td>
<td>3.0E+05</td>
<td>100</td>
</tr>
<tr>
<td>14</td>
<td>2.0E+03</td>
<td>2.0E+05</td>
<td>2.0E+05</td>
<td>100</td>
</tr>
<tr>
<td>15</td>
<td>2.0E+03</td>
<td>2.0E+05</td>
<td>2.0E+05</td>
<td>100</td>
</tr>
</tbody>
</table>

Application of Equation 6.3 with a rainfall intensity of 60mm/hr indicates that all the available microbes’ washoff to the stormwater drain after a storm event. This confirms the assumption used in Chapter 5 when developing the regression relationships between microbes, time and climate factor, where all survived microbes were washed off with the stormwater while simulating the rain event at an intensity of 60mm/hr.

Zhang and Farahbakhsh (2007) reported that the raw sewage concentration of faecal microbes can not exceed the limit of $10^7$ orgs/100 mL. The values for E.coli organisms obtained from raw sewage samples collected during winter and summer experiments were 8.0E+06 E.coli orgs/100mL and 6.5E+06 E.coli orgs/100mL respectively. These concentrations are close to the reported value of $10^7$ orgs/100 mL for raw sewage concentrations. Hence, the value of 1.0E+07 E.coli orgs/100mL was used to estimate the contribution on the initial day after a dry weather spill event.
Different rainfall intensities were further examined with an assumed raw sewage value of $1E+07$ organisms/100mL. Average rainfall intensities for Melbourne, Australia were calculated using the software named, “AUS-IFD Version 2.0” (Jenkins, 2004). This software follows the procedures described in Australian Rainfall and Runoff, (1987) and is shown in Figure 6.7.

Figure 6.7: Rainfall intensities for Melbourne, Victoria

1 in 1 year ARI with different rainfall intensities for different storm durations were used with a raw sewage value of $1E+07$ to calculate percent contributed to stormwater drain after a spill event (Table 6.4). Equation 6.3 was used to calculate the concentration of microbes at the stormwater drain inlet $C_s(t)$ after a specific rainfall intensity $RI$. 
Table 6.4: Wash-off of microbes (%) calculated with different rainfall intensities for 1 in 1 year storm (mm/hr) for Melbourne

<table>
<thead>
<tr>
<th>Duration (minutes)</th>
<th>Rainfall intensity (mm/hr)</th>
<th>Raw sewage values (orgs/100mL)</th>
<th>Cs(t) concentration of microbes at stormwater inlet (orgs/100mL)</th>
<th>Contribution to stormwater drain</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>47</td>
<td>1E+07</td>
<td>9309500</td>
<td>93%</td>
</tr>
<tr>
<td>10</td>
<td>35.8</td>
<td>1E+07</td>
<td>8595862</td>
<td>86%</td>
</tr>
<tr>
<td>15</td>
<td>29.7</td>
<td>1E+07</td>
<td>8138031</td>
<td>81%</td>
</tr>
<tr>
<td>20</td>
<td>25.8</td>
<td>1E+07</td>
<td>7809195</td>
<td>78%</td>
</tr>
<tr>
<td>30</td>
<td>20.8</td>
<td>1E+07</td>
<td>7331524</td>
<td>73%</td>
</tr>
<tr>
<td>40</td>
<td>17.7</td>
<td>1E+07</td>
<td>6992909</td>
<td>70%</td>
</tr>
<tr>
<td>50</td>
<td>15.6</td>
<td>1E+07</td>
<td>6738872</td>
<td>67%</td>
</tr>
<tr>
<td>60</td>
<td>14</td>
<td>1E+07</td>
<td>6528557</td>
<td>65%</td>
</tr>
<tr>
<td>90</td>
<td>10.9</td>
<td>1E+07</td>
<td>6066911</td>
<td>61%</td>
</tr>
<tr>
<td>120</td>
<td>9.09</td>
<td>1E+07</td>
<td>5752557</td>
<td>58%</td>
</tr>
<tr>
<td>180</td>
<td>7.01</td>
<td>1E+07</td>
<td>5330857</td>
<td>53%</td>
</tr>
<tr>
<td>240</td>
<td>5.83</td>
<td>1E+07</td>
<td>5050595</td>
<td>51%</td>
</tr>
<tr>
<td>300</td>
<td>5.05</td>
<td>1E+07</td>
<td>4842460</td>
<td>48%</td>
</tr>
</tbody>
</table>

The washoff rate of micro-organisms declines with the rainfall intensities and is equal to \( R^{0.293} \) (47mm/hr gives 93% washoff and 5mm/hr provides with 48% washoff). In addition, the washoff percentage was calculated for different rainfall intensities and various storm durations. The results explaining relationship between storm event (rainfall intensity) and the concentration of microbes are tabulated in Tables E1, E2, E3, E4, E5 and E6.

Table 6.5 depicts the relationship between the rainfall intensities and the % of microbes transported to the drain after a spill. Figure 6.8 graphically represents the relationship between the storm event and the percent of microbes transported to the drain. This indicates that the microbes transported to the drain will decrease with the rainfall intensity.
Table 6.5: Relationship of rainfall intensity and % of microbes transported to the drain

<table>
<thead>
<tr>
<th>Rainfall intensity (mm/hr)</th>
<th>% Contribution of microbes</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt;60</td>
<td>100</td>
</tr>
<tr>
<td>40-60</td>
<td>90-100</td>
</tr>
<tr>
<td>30-40</td>
<td>80-90</td>
</tr>
<tr>
<td>20-30</td>
<td>70-80</td>
</tr>
<tr>
<td>10-20</td>
<td>60-70</td>
</tr>
<tr>
<td>5-10</td>
<td>50-60</td>
</tr>
<tr>
<td>&lt;5</td>
<td>&lt;50</td>
</tr>
</tbody>
</table>

Figure 6.8: Relationships of storm events and percent of microbes transported to the drain inlet

Above analysis demonstrates the relationship of microbes transported from the location of the sewer spill towards the stormwater drain and rainfall intensity of a storm event.
6.6 Application of the Developed Model to Prahran Main Drain and Gardiners Catchment

As reported in Tables 6.1 and 6.2, there were 80 and 15 spills in year 2007 from Prahran Main Drain and Gardiners Creek catchments respectively. It is important to estimate the contribution from these spills into Yarra River due to subsequent rain. It was planned to apply the model developed in Chapter 5 (Equation 6.1) to the spill data from above 2 catchments. The climate variable relative humidity (RH) is a parameter in the developed model. It was decided to investigate the difference between the RH values in Frankston (experimental site) and Melbourne as well as the sensitivity of RH before applying the developed equation to Prahran Main Drain and Gardiners Creek catchments.

6.6.1 Comparison of relative humidity in Melbourne and Frankston

The monthly averages of relative humidity values in Melbourne metropolitan region were collected from the Bureau of Meteorology website to investigate its impact on the survival of microbes after a spill event. Figure 6.9 presents the monthly mean values for relative humidity in Melbourne and Frankston for the period of 1855 – 2000 and 1992 – 2008 respectively (http://www.bom.gov.au). Figure 6.9 shows that the relative humidity in both stations varies in between 60% – 80%. As can be seen in Figure 6.9, the relative humidity values in both stations are within 10% variation.
6.6.2 Sensitivity of relative humidity on microbial survival rate

The sensitivity of relative humidity on predicting microbial survival rate was further investigated. Based on Figure 6.9 the average relative humidity in Melbourne varies between 60 – 80%. Table 6.6 presents the estimated values of microbes with the developed Equation 6.1 for relative humidity of 60%, 70% and 80%. The outcome shows that there is a magnitude difference in concentrations of microbes with the different relative humidity values.
Table 6.6: Relationship between relative humidity and survival of microbial concentrations with time

<table>
<thead>
<tr>
<th>Time (days)</th>
<th>RH 60%</th>
<th>RH 70%</th>
<th>RH 80%</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>6.3E+05</td>
<td>1.1E+05</td>
<td>2.3E+04</td>
</tr>
<tr>
<td>2</td>
<td>2.3E+05</td>
<td>3.9E+04</td>
<td>8.3E+03</td>
</tr>
<tr>
<td>3</td>
<td>1.1E+05</td>
<td>1.9E+04</td>
<td>4.1E+03</td>
</tr>
<tr>
<td>4</td>
<td>6.4E+05</td>
<td>1.1E+04</td>
<td>2.3E+03</td>
</tr>
<tr>
<td>5</td>
<td>4.0E+05</td>
<td>6.9E+03</td>
<td>1.5E+03</td>
</tr>
<tr>
<td>6</td>
<td>2.7E+04</td>
<td>4.7E+03</td>
<td>1.0E+03</td>
</tr>
<tr>
<td>7</td>
<td>2.0E+04</td>
<td>3.3E+03</td>
<td>7.2E+02</td>
</tr>
<tr>
<td>8</td>
<td>1.5E+04</td>
<td>2.5E+03</td>
<td>5.4E+02</td>
</tr>
<tr>
<td>9</td>
<td>1.1E+04</td>
<td>1.9E+03</td>
<td>4.1E+02</td>
</tr>
<tr>
<td>10</td>
<td>8.9E+03</td>
<td>1.5E+03</td>
<td>3.2E+02</td>
</tr>
<tr>
<td>11</td>
<td>7.1E+03</td>
<td>1.2E+03</td>
<td>2.6E+02</td>
</tr>
<tr>
<td>12</td>
<td>5.8E+03</td>
<td>9.9E+02</td>
<td>2.1E+02</td>
</tr>
<tr>
<td>13</td>
<td>4.9E+03</td>
<td>8.2E+02</td>
<td>1.8E+02</td>
</tr>
<tr>
<td>14</td>
<td>4.1E+03</td>
<td>6.9E+02</td>
<td>1.5E+02</td>
</tr>
<tr>
<td>15</td>
<td>4.1E+03</td>
<td>6.9E+02</td>
<td>1.5E+02</td>
</tr>
</tbody>
</table>

As mentioned earlier the Prahran Main Drain and the Gardiners Creek catchments have had 80 and 15 spill respectively in 2007. It was decided to analyse the microbial contribution from these spills to Yarra River with different elapsed times after a dry weather spill.

6.6.3 Estimation of faecal loads from selected catchments

A 70% RH was used to estimate the amount of microbes for different elapsed times within 15 days in the Yarra catchment which drains the Prahran Main drain and Gardiners Creek. This RH value was selected as the average RH value based on the historical data gathered from BOM for Melbourne region (Figure 6.9). By applying Equation 6.1 the microbial survival rates on each day after the spill with a relative humidity of 70% are given in Table 6.6. These values together with Equation 6.3 were used to calculate the concentrations of microbes with different elapsed time after a dry weather spill in the catchment. Rainfall intensities for different storm events were considered. Figures 6.10, 6.11, and F1 to F14 in Appendix F depict the relationship between storm events and concentrations of microbes transported towards stormwater drain when a storm occurs after different elapsed times. The concentrations are estimated for different ARI values and storm durations for Melbourne. A raw sewage
microbial concentration of $1 \times 10^7 \text{ orgs/100mL}$ was used as the base on the day of spill to estimate the effect of the storm events on the transport of the microbes.

Outcomes from the analysis suggest that considerable amount of microbes washoff towards the stormwater drains even with storm events of smaller duration. In addition, this analysis shows that the intensity of rainfall has a significant impact on washoff of microbes from the location of the spill.

Figure 6.10: Relationship between the storm event and concentrations of microbes transported towards stormwater drain for different ARI values for Yarra catchment on the initial day of spill event
The total numbers of dry weather spill events in the Prahran Main Drain and Gardiners Creek catchments are 80 and 15 respectively. The quantity of each spill was assumed to be 10 L as discussed earlier in this chapter. This data was further used to estimate the contribution of these dry weather sewer spills towards the microbial contribution in the waterways. The Equation 6.1 together with relative humidity 70% were used with 47mm/hr rainfall intensity (ARI = 1in 1 year) to estimate the contribution from the total number of spills in each catchment towards the stormwater system. Table 6.7 depicts the amount of microbes transported to the stormwater system at specific time intervals with total number of dry weather spills from the Prahran Main Drain and the Gardiners Creek catchment during 2007.
Table 6.7: The contribution of microbes at each elapsed time interval for each spill event from a catchment with a rainfall intensity of 47mm/hr and relative humidity 70% (Assume spill volume is 10L)

<table>
<thead>
<tr>
<th>Time (days)</th>
<th>Microbial concentrations (organisms/100mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.1E+05</td>
</tr>
<tr>
<td>2</td>
<td>3.9E+04</td>
</tr>
<tr>
<td>3</td>
<td>1.9E+04</td>
</tr>
<tr>
<td>4</td>
<td>1.1E+04</td>
</tr>
<tr>
<td>5</td>
<td>6.9E+03</td>
</tr>
<tr>
<td>6</td>
<td>4.7E+03</td>
</tr>
<tr>
<td>7</td>
<td>3.3E+03</td>
</tr>
<tr>
<td>8</td>
<td>2.5E+03</td>
</tr>
<tr>
<td>9</td>
<td>1.9E+03</td>
</tr>
<tr>
<td>10</td>
<td>1.5E+03</td>
</tr>
<tr>
<td>11</td>
<td>1.2E+03</td>
</tr>
<tr>
<td>12</td>
<td>9.9E+02</td>
</tr>
<tr>
<td>13</td>
<td>8.2E+02</td>
</tr>
<tr>
<td>14</td>
<td>6.9E+02</td>
</tr>
<tr>
<td>15</td>
<td>6.9E+02</td>
</tr>
</tbody>
</table>

The above analysis demonstrated in a limited way the contribution of dry weather sewer spill towards the microbial levels in the waterways from two selected catchments, adversely impacting the biological health of the waterways.

6.7 Summary and Conclusion

This Chapter considered actual dry weather sewage spill data throughout year 2007 for the Prahran Main Drain and the Gardiners Creek catchment draining to the Yarra River, estimated the % contribution of microbes after a dry weather spill event and contribution of microbes from selected catchments towards water quality in the waterways. This section indicated the importance of the second phase of the reported research in determining the significance of dry weather sewer spills within catchments.

Chapter 6 explained the selection of catchments based on historical data, estimated microbes at stormwater drain, modeled washoff of microbes with time after a spill event.
and demonstrated the importance of rainfall intensities in microbes transport process. Rooney (2007) identified the Prahran Main Drain and the Gardiners Creek catchments as the leading human faecal contamination source to the Yarra River based on the concentrations obtained from this part of the Yarra catchment. These two catchments were selected for the current study and the actual dry weather spill data from the Prahran Main Drain and Gardiners Creek catchments during year 2007 was analysed further. It has been noted that frequency of dry weather spills is high in the Prahran Main Drain catchment (total number of 80 spills). This indicated considerable faecal contribution in Yarra River from this catchment. In addition, contribution from Gardiners Creek catchment was also examined. There were 15 reported spills in Gardiners Creek catchment throughout 2007.

Further analysis showed that the Prahran Main Drain catchment and the Gardiners Creek contributed considerable amount of microbial concentrations through the stormwater system to the Yarra River during the year 2007.

Current chapter also developed a relationship between storm events and microbial movement towards stormwater drains by simulating the movement of microbes from location of the sewer spill towards the stormwater drain after a storm event. The analysis also demonstrated that all the microbial concentration available on surface (pervious or impervious) gets washed off during the rainfall event, if the intensity of rainfall is above 60mm/hr (rainfall intensity of 1 in 1 year ARI, 15 minutes duration storm).

Main conclusions derived from this chapter are as follows:

- The relationship between storm event (rainfall intensity) and microbial washoff has been confirmed and can be modeled using Equations 6.1 and 6.3.

- Rainfall intensity above 60mm/hr will wash-off all the microbes present on the surface towards the stormwater drain. If the rainfall intensity is below 60mm/hr the wash-off rate is $R_{I^{0.293}}$ 100%.

- Relationships were successfully developed between rainfall intensities and wash-off rate of microbes for different storm events.

- The Prahran Main Drain and the Gardiners Creek catchments dry weather spills contributed considerable amount of microbes to those present in the Yarra River during 2007 test year.
Chapter 7

Summary, Conclusions and Recommendations

7.1 Summary

The Yarra River is considered an important environmental and recreational asset by the Melbourne community. The upper reaches of the Yarra provides drinking water for 3.9 Million Melburnians and some percent is used for an agricultural purpose. The lower Yarra is mainly utilized for recreational purposes. Water quality of the Yarra River in its lower sections is relatively poor as compared to the upper sections of Yarra. This may be due to urbanisation. Faecal coliform levels have been observed to be high in lower sections of the Yarra River during both wet and dry weather periods. Faecal contamination during dry weather occurs due to the sewer spills generated by structural collapses (sewage infrastructure) or blockages from tree roots or fat blocks.

High amounts of nutrients, pathogens, organic toxicants and heavy metals enter the waterways during spill events. At a representative site in Lower Yarra, the E.coli loads were found to vary from 2000 to 160000 organisms/100ml. These values exceed the primary and secondary contact levels recommended by the National Health and Medical Research Council (NHMRC, 2000).

The main objective of the study is to investigate the effects of dry weather sewer spills on river water quality. The number of faecal microbes carried to the waterways will depend on the amount of spill, magnitude of the storm as well as on the antecedent rainfall period. Based on the objective of the current study, two phases of data collection were assigned for the current research:

1. Field Experiments were carried out at the Mount Martha treatment plant during the summer 2008 (S08), winter 2008 (W08) and summer 2009 (S09) to determine relationships between microbes survival rate with elapse time after the spill and climate factors; and
2. Actual dry weather spill data collected from the Prahran Main Drain and Gardiners Creek Catchment during 2007.
These two phases of data collection were carried out concurrently. Field experiments were carried out under controlled conditions. Literature suggested strong relationships between nutrients and microbes on soil surface. As a result, nutrients were also monitored to investigate the above mentioned relationship.

The results obtained from field data for S08, W08 and S09 were scrutinized to estimate the survival rate of microbes with time. The results from field experiments demonstrate that the \textit{E.coli} and enterococci organisms have considerable variation between the collected samples on the same day. \textit{E.coli} organisms on the control surfaces were negligible, whereas the enterococci stains were present on pervious control surface. However, the nutrient concentration levels had dropped significantly with time. The results also indicate that the \textit{E.coli} and enterococci have survived for longer duration (15\textsuperscript{th} day) in spite of the weather conditions and low moisture conditions on pervious surfaces. On the other hand, FRNA coliphages on pervious and impervious surface were negligible after selected time intervals, indicating that FRNA coliphages can not survive for long durations under natural conditions. Multiple storm event experiments simulated during summer 2009 experimental period demonstrated that not all the microbes get washed off after the initial rainfall event. In addition, these experiments depict that the number of microbes vary within the same pool of collected sample.

The results from field experiments were used to predict the concentration of microbes with time. Obtained data sets from winter 2008 and summer 2009 field experiments were used to develop the relationship between microbes, climate variables (relative humidity, temperature and vapour pressure) and time. The daily mean concentrations of microbes (after removal of outliers) were used with climate variables and time to predict the concentration of microbes at different time intervals. The inclusion of climate factor enhanced the modelling results. A relationship between microbes and time on impervious surface was not estimated due to low survival rate of microbes on the impervious surface.

A microbe prediction model was developed with elapsed time and relative humidity using data collected during summer, and verified with the data collected during winter. Strength of the developed model is to predict the concentrations of \textit{E.coli} with time after a spill event. The developed model should be used with caution when estimating the survival of Enterococci organisms with elapsed time due to the unusual behaviour of organisms on soil surface.
The results obtained with the developed models for *E.coli* and enterococci were further coupled with the simple washoff equation (McCarthy, 2008) to estimate the potential effects of dry weather spills on river water quality. Also, the analysis of actual dry weather spill data supported the discussion of the potential effects on river water quality due to dry weather spills.

### 7.2 Conclusions

#### 7.2.1 Findings from previous studies on faecal contamination on Yarra River water quality

- Melbourne Water and EPA Victoria have reported significant contribution of microbes from urban stormwater systems entering waterways.
- Considerable amount of microbes were present in the tributaries of Yarra catchment during the dry weather period especially in the Prahran Main Drain and the Gardiners Creek.
- Dry weather spills contribute significant amount of microbes to waterways.
- The life span of microbes on the pervious and impervious surfaces varies after a spill event with time and antecedent conditions.
- Minimising dry weather sewer spills is key to protecting water quality for recreation and in general, the critical factor for maintaining river health.

#### 7.2.2 Post – spill variation of microbes and nutrients with elapsed time on pervious and impervious surfaces during field experiments

- The raw sewage concentration levels between the collected samples from top, middle and bottom layers of the container varied significantly.
- The obtained concentrations for microbes during the winter experimental period were lower than for the summer experimental periods.
- Field experiments reported that FRNA coliphages did not survive on pervious and impervious surfaces for more than 24 hours after the spill event.
- The presence of enterococci organisms on the control surface was significant during all three experimental periods. This suggests that enterococci stains can possibly be present on the soil surface in the natural environment for longer periods. Unlike Enterococci, *E.coli* organisms were not present on the control surface suggesting that *E.coli* organisms can not survive on the soil surface in the natural environment for a long period of time.
• The concentration of microbes on impervious surface after the spill was negligible, suggesting higher die-off rate than pervious surface.

• *E. coli* and enterococci survived on the pervious surface even on the 15th day despite hot weather conditions during summer.

• The variation of microbes within a same pool of collected runoff sample is high. On some instances the variation of microbes was more than an order of magnitude. The coefficient of variation of microbes within a single sample is also as high as 170% for *E. coli*, 140% for enterococci.

• Nutrients and microbes did not show any relationship during the field experiments.

• The results from the multiple storm event experiment illustrate that all the microbes did not get washed off from the initial rainfall simulation. As literature indicates and confirmed by this study, the washoff rate of microbes during surface runoff is dependent on rainfall intensity.

• Microbe concentrations at the end of winter and summer experimental periods in the Yarra River exceed the primary and secondary contact limits set by SEPP and NHMRC for recreational waters.

### 7.2.3 Developing microbes prediction model

• Cross correlation matrix express that the average temperature, vapour pressure and relative humidity have an influence on die-off rate of *E. coli* and enterococci organisms on both (pervious and impervious) surfaces. In addition, it presents the interdependency of *E. coli* and enterococci during summer. In contrast, there was no relationship between *E. coli* and enterococci during the winter season.

• The following microbial prediction model was successfully developed to model its survival time and relative humidity data:

\[
\text{Microbes} = 10^{27 \cdot \text{Time}^{-2.5} \cdot \text{RH}^{-11.5}}
\]

where,

- \(\text{Microbes}\) = Concentration of survived organisms after a spill (orgs/100mL)
- \(\text{Time}\) = Elapsed time after a dry weather spill (days)
- \(\text{RH}\) = Average relative humidity between the time of spill and the elapsed time (%)

• The developed model should be used with caution when estimating the survival of Enterococci organisms with elapsed time.
• The comparison between the field data and estimated values with the developed model depict that the model is overestimating the microbial concentrations.
• The survival of microbes with time on pervious surface is inversely proportional to the prevailing relative humidity.
• The variation of relative humidity (60 to 80%) illustrates an indication of a magnitude difference while the analysis was carried out for 1 to 15 days.

7.2.4 Relationship between storm events and microbe washoff concentrations

• The Prahran Main Drain and Gardiners Creek catchments were selected for the study based on actual spill data.
• The Prahran Main Drain and Gardiners Creek catchments contributed to polluting the waterways with a total number of 80 and 15 dry weather spills in 2007 respectively.
• The amount of faecal coliform that will be carried to the stream after a dry weather sewer spill will depend on the magnitude of the storm as well as on antecedent rainfall conditions that determine surface run-off and the weather during the period between the dry weather spill and the wash off event.
• Rainfall intensity above 60mm/hr will wash-off all the microbes present on the surface towards the stormwater drain.
• Relationships were successfully developed between different design storm events and wash-off rate of microbes for Melbourne after a dry weather spill event.

\[
\% \text{ of microbes washed off to the stormwater drain} = RI^{0.293} \times 100\%
\]

where,
\[
RI = \text{Rainfall intensity (mm/hr)}
\]
7.3 Recommendations

- Surface run-off experiments should be carried out on daily basis during summer and winter for obtaining the data sets of *E.coli* and enterococci on pervious surface with daily readings. The data obtained with the current study can be analysed together with a new set of data to refine the current model.

- The experiments have to be designed for impervious surface for shorter durations as the die-off rate of microbes on impervious surface is higher than pervious surface. The sampling should be carried out after each 2 or 3 hours to understand the survival mechanism of microbes on impervious surface.

- Testing of the developed model on the actual spill location would be beneficial for estimating the concentration of microbes after a spill event. This would assists in determining the limitations and benefits of the developed microbial prediction model.


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Appendix A: Climate Data

Table A1: Daily rainfall data during experimental period (Measured on experimental site and data from BOM website for Frankston weather observation station)

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**T = Temperature (Degree Celsius)**
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Table A5: Daily potential evapotranspiration (PET) during W08 experimental period (Calculated with Penman – Monteith equation based on Frankston weather details)

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<th>Min T</th>
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<th>Relative humidity (Min)</th>
<th>Actual vapour pressure Hpa</th>
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Table A6: Daily potential evapotranspiration (PET) during S09 experimental period (Calculated with Penman – Monteith equation based on Frankston weather details)

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<th>Relative humidity (Min)</th>
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Appendix B: Experimental Setup

Figure B1: Mt. Martha WWTP staff fencing experimental site to prevent intrusion of cows during summer 2008 experiments

Figure B2: Marked pervious surface (soil plot) for summer 2008 experiments
Figure B3: Plastic sheet fixed in trench before rainfall simulation on pervious plot

Figure B4: Simulation of rainfall on pervious plot after 2 hours of applied raw sewage
Figure B5: collection of surface runoff from sample collection trench

Figure B6: Collected surface runoff from pervious treated plot
Figure B7: Sampling details entered on microbial sample collection bottle (500mL) before collecting the samples

Figure B8: Transferring collected surface runoff sample to sampling bottle (250 mL for nutrient analysis)
Figure B9: Transferring collected surface runoff sample to sampling bottle (500 mL for microbial analysis)

Figure B10: Storage of collected sample bottles in an ice box (To maintain the standards of samples by keeping low temperatures during transportation of samples to Ecowise laboratories)
Figure B11: Pervious experimental plot during winter 2008 with significant grass cover

Figure B12: Experimental plots covered with transparent plastic sheet between 4 to 7th and 7 to 14th day to protect the wash off from natural rainfall event on site during winter
Table C1: Concentration of Microbes and nutrients in raw sewage during S08

<table>
<thead>
<tr>
<th></th>
<th>Escherichia coli (orgs/100mL)</th>
<th>Enterococci (orgs/100mL)</th>
<th>FRNA Coliphages (pfu/100mL)</th>
<th>Total Nitrogen mg N/ L</th>
<th>Total Phosphorus mg P/ L</th>
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<td>(Sample taken from top of container)</td>
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Table C2: Concentration of Microbes and nutrients in Raw Sewage during W08

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<th>FRNA Coliphages (pfu/100mL)</th>
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Table C3: Concentration of Microbes and nutrients in Raw Sewage during S09

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<th>Total Nitrogen mg N/ L</th>
<th>Total Phosphorus mg P/ L</th>
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Table C4: Concentration of microbes and nutrients on pervious control plots on different time intervals during S08

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<td>&lt;1</td>
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<td>30</td>
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<td><strong>Total P</strong></td>
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Table C5: Concentration of microbes and nutrients on pervious control plots on different time intervals during W08

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<th>Day 4</th>
<th>Day 7</th>
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Table C6: Concentration of *E. coli* on soil control plots on different time intervals during S09

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<th>Day 4</th>
<th>Day 7</th>
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Table C7: Concentration of enterococci on soil control plots on different time intervals during S09

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Table C8: Concentration of nutrients on soil control plots on different time intervals during S09

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Table C9: Concentration of microbes and nutrients on impervious control plots on different time intervals during S08

<table>
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<tr>
<th>2nd Hour</th>
<th>2nd Hour</th>
<th>2nd Hour</th>
<th>2nd Hour</th>
</tr>
</thead>
<tbody>
<tr>
<td>E.coli</td>
<td>0</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Enterococci</td>
<td>3</td>
<td>6</td>
<td>8</td>
</tr>
<tr>
<td>F-RNA coliphage</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Total N</td>
<td>0.7</td>
<td>0.9</td>
<td>0.9</td>
</tr>
<tr>
<td>Total P</td>
<td>0.06</td>
<td>0.07</td>
<td>0.07</td>
</tr>
</tbody>
</table>

Table C10: Concentration of microbes and nutrients on impervious control plots on different time intervals during W08

<table>
<thead>
<tr>
<th></th>
<th>Day 1</th>
<th>Day 2</th>
<th>Day 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>E.coli</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Enterococci</td>
<td>410</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>F-RNA coliphage</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total N</td>
<td>7.6</td>
<td>6.1</td>
<td>5.2</td>
</tr>
<tr>
<td>Total P</td>
<td>0.45</td>
<td>0.14</td>
<td>0.22</td>
</tr>
</tbody>
</table>
Table C11: Concentration of *E.coli* on pervious treated plots on different time intervals (days) after application of sewage during S08

<table>
<thead>
<tr>
<th>Sample</th>
<th>2nd Hour</th>
<th>Day 1</th>
<th>Day 2</th>
<th>Day 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample 1</td>
<td>&gt;24000</td>
<td>&gt;24000</td>
<td>29000</td>
<td>100000</td>
</tr>
<tr>
<td>Sample 2</td>
<td>&gt;24000</td>
<td>&gt;24000</td>
<td>140000</td>
<td>160000</td>
</tr>
<tr>
<td>Sample 3</td>
<td>&gt;24000</td>
<td>&gt;24000</td>
<td>220000</td>
<td>27000</td>
</tr>
</tbody>
</table>

Table C12: Concentration of *E.coli* on pervious treated plots on different time intervals (days) after application of sewage during W08

<table>
<thead>
<tr>
<th>Sample</th>
<th>Day 1</th>
<th>Day 2</th>
<th>Day 4</th>
<th>Day 7</th>
<th>Day 14</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample 1</td>
<td>9900</td>
<td>2600</td>
<td>12000</td>
<td>74</td>
<td>850</td>
</tr>
<tr>
<td>Sample 2</td>
<td>11000</td>
<td>29000</td>
<td>3100</td>
<td>1500</td>
<td>630</td>
</tr>
<tr>
<td>Sample 3</td>
<td>14000</td>
<td>9900</td>
<td>81000</td>
<td>1500</td>
<td>3600</td>
</tr>
</tbody>
</table>

Table C13: Concentration of *E.coli* on pervious treated plots on different time intervals (days) after application of sewage during S09

<table>
<thead>
<tr>
<th>Soil plot 1 – sample 1</th>
<th>Day 1</th>
<th>Day 2</th>
<th>Day 4</th>
<th>Day 7</th>
<th>Day 15</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soil plot 1 – sample 2</td>
<td>360000</td>
<td>990</td>
<td>49000</td>
<td>5600</td>
<td>2000000</td>
</tr>
<tr>
<td>Soil plot 1 – sample 3</td>
<td>2000000</td>
<td>5200</td>
<td>46000</td>
<td>6800</td>
<td>2000000</td>
</tr>
<tr>
<td>Soil plot 2 – sample 1</td>
<td>3300000</td>
<td>3100</td>
<td>41000</td>
<td>6800</td>
<td>1700000</td>
</tr>
<tr>
<td>Soil plot 2 – sample 2</td>
<td>2600000</td>
<td>990</td>
<td>200000</td>
<td>34000</td>
<td>110000</td>
</tr>
<tr>
<td>Soil plot 2 – sample 3</td>
<td>6500000</td>
<td>7700</td>
<td>24000</td>
<td>28000</td>
<td>20222</td>
</tr>
<tr>
<td>Soil plot 3 – sample 1</td>
<td>16000000</td>
<td>9800</td>
<td>200000</td>
<td>25000</td>
<td>170000</td>
</tr>
<tr>
<td>Soil plot 3 – sample 2</td>
<td>24000000</td>
<td>3100</td>
<td>31000</td>
<td>740</td>
<td>1500</td>
</tr>
<tr>
<td>Soil plot 3 – sample 3</td>
<td>24000000</td>
<td>6300</td>
<td>33000</td>
<td>1600</td>
<td>2800</td>
</tr>
<tr>
<td>Soil plot 3 – sample 3</td>
<td>24000000</td>
<td>6300</td>
<td>41000</td>
<td>630</td>
<td>1900</td>
</tr>
</tbody>
</table>

(Note: each day samples were taken from different soil plots)
Table C14: Concentration of Enterococci on pervious treated plots on different time intervals (days) after application of sewage during S08

<table>
<thead>
<tr>
<th></th>
<th>2nd Hour</th>
<th>Day 1</th>
<th>Day 2</th>
<th>Day 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>sample 1</td>
<td>&gt;24000</td>
<td>7700</td>
<td>3100</td>
<td>&lt;1000</td>
</tr>
<tr>
<td>sample 2</td>
<td>&gt;24000</td>
<td>6900</td>
<td>49000</td>
<td>17000</td>
</tr>
<tr>
<td>sample 3</td>
<td>&gt;24000</td>
<td>&gt;2400</td>
<td>14000</td>
<td>4100</td>
</tr>
</tbody>
</table>

Table C15: Concentration of Enterococci on pervious treated plots on different time intervals (days) after application of sewage during W08

<table>
<thead>
<tr>
<th></th>
<th>Day 1</th>
<th>Day 2</th>
<th>Day 4</th>
<th>Day 7</th>
<th>Day 14</th>
</tr>
</thead>
<tbody>
<tr>
<td>sample 1</td>
<td>200</td>
<td>380</td>
<td>640</td>
<td>790</td>
<td>2000</td>
</tr>
<tr>
<td>sample 2</td>
<td>850</td>
<td>4200</td>
<td>3600</td>
<td>17000</td>
<td>740</td>
</tr>
<tr>
<td>sample 3</td>
<td>520</td>
<td>840</td>
<td>20000</td>
<td>120</td>
<td>520</td>
</tr>
</tbody>
</table>

Table C16: Concentration of Enterococci on pervious treated plots on different time intervals (days) after application of sewage S09

<table>
<thead>
<tr>
<th></th>
<th>Day 1</th>
<th>Day 2</th>
<th>Day 4</th>
<th>Day 7</th>
<th>Day 15</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soil plot 1 – sample 1</td>
<td>40000</td>
<td>990</td>
<td>17000</td>
<td>300</td>
<td>580000</td>
</tr>
<tr>
<td>Soil plot 1 – sample 2</td>
<td>87000</td>
<td>5100</td>
<td>20000</td>
<td>400</td>
<td>300000</td>
</tr>
<tr>
<td>Soil plot 1 – sample 3</td>
<td>55000</td>
<td>3100</td>
<td>20000</td>
<td>1600</td>
<td>330000</td>
</tr>
<tr>
<td>Soil plot 2 – sample 1</td>
<td>17000</td>
<td>3100</td>
<td>98000</td>
<td>9900</td>
<td>210000</td>
</tr>
<tr>
<td>Soil plot 2 – sample 2</td>
<td>40000</td>
<td>17000</td>
<td>92000</td>
<td>8800</td>
<td>210000</td>
</tr>
<tr>
<td>Soil plot 2 – sample 3</td>
<td>73000</td>
<td>990</td>
<td>73000</td>
<td>8800</td>
<td>330000</td>
</tr>
<tr>
<td>Soil plot 3 – sample 1</td>
<td>170000</td>
<td>990</td>
<td>13000</td>
<td>510</td>
<td>1100</td>
</tr>
<tr>
<td>Soil plot 3 – sample 2</td>
<td>200000</td>
<td>990</td>
<td>11000</td>
<td>410</td>
<td>1200</td>
</tr>
<tr>
<td>Soil plot 3 – sample 3</td>
<td>250000</td>
<td>1300</td>
<td>15000</td>
<td>200</td>
<td>980</td>
</tr>
</tbody>
</table>
Table C17: Concentration of FRNA coliphages on pervious treated plots on different time intervals (days) after application of sewage during S08

<table>
<thead>
<tr>
<th>Sample</th>
<th>2nd Hour</th>
<th>Day 1</th>
<th>Day 2</th>
<th>Day 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample 1</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>95</td>
<td>44</td>
</tr>
<tr>
<td>Sample 2</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>64</td>
<td>76</td>
</tr>
<tr>
<td>Sample 3</td>
<td>17</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>&lt;1</td>
</tr>
</tbody>
</table>

Table C18: Concentration of FRNA coliphages on pervious treated plots on different time intervals (days) after application of sewage during W08

<table>
<thead>
<tr>
<th>Sample</th>
<th>Day 1</th>
<th>Day 2</th>
<th>Day 4</th>
<th>Day 7</th>
<th>Day 14</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample 1</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Sample 2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Sample 3</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Table C19: Concentration of Total N on pervious treated plots on different time intervals (days) after application of sewage during S08

<table>
<thead>
<tr>
<th>Sample</th>
<th>2nd Hour</th>
<th>Day 1</th>
<th>Day 2</th>
<th>Day 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample 1</td>
<td>24</td>
<td>11</td>
<td>16</td>
<td>18</td>
</tr>
<tr>
<td>Sample 2</td>
<td>27</td>
<td>15</td>
<td>18</td>
<td>11</td>
</tr>
<tr>
<td>Sample 3</td>
<td>20</td>
<td>21</td>
<td>23</td>
<td>15</td>
</tr>
</tbody>
</table>
Table C20: Concentration of Total N on pervious treated plots on different time intervals (days) after application of sewage during W08

<table>
<thead>
<tr>
<th></th>
<th>Day 1</th>
<th>Day 2</th>
<th>Day 4</th>
<th>Day 7</th>
<th>Day 14</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample 1</td>
<td>11</td>
<td>21</td>
<td>13</td>
<td>24</td>
<td>36</td>
</tr>
<tr>
<td>Sample 2</td>
<td>17</td>
<td>33</td>
<td>41</td>
<td>17</td>
<td>110</td>
</tr>
<tr>
<td>Sample 3</td>
<td>29</td>
<td>14</td>
<td>19</td>
<td>17</td>
<td>11</td>
</tr>
</tbody>
</table>

Table C21: Concentration of Total N on pervious treated plots on different time intervals (days) after application of sewage during S09

<table>
<thead>
<tr>
<th></th>
<th>Day 1</th>
<th>Day 2</th>
<th>Day 4</th>
<th>Day 7</th>
<th>Day 15</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample 1</td>
<td>22</td>
<td>13</td>
<td>12</td>
<td>16</td>
<td>11</td>
</tr>
<tr>
<td>Sample 2</td>
<td>20</td>
<td>28</td>
<td>16</td>
<td>38</td>
<td>12</td>
</tr>
<tr>
<td>Sample 3</td>
<td>32</td>
<td>40</td>
<td>37</td>
<td>120</td>
<td>62</td>
</tr>
</tbody>
</table>

Table C22: Concentration of Total P on pervious treated plots on different time intervals (days) after application of sewage during S08

<table>
<thead>
<tr>
<th></th>
<th>2nd Hour</th>
<th>Day 1</th>
<th>Day 2</th>
<th>Day 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample 1</td>
<td>2.5</td>
<td>1.4</td>
<td>2.2</td>
<td>2.5</td>
</tr>
<tr>
<td>Sample 2</td>
<td>3.2</td>
<td>2.5</td>
<td>2.5</td>
<td>1.44</td>
</tr>
<tr>
<td>Sample 3</td>
<td>2.3</td>
<td>2.8</td>
<td>2.0</td>
<td>1.9</td>
</tr>
</tbody>
</table>
Table C23: Concentration of Total P on pervious treated plots on different time intervals (days) after application of sewage during W08

<table>
<thead>
<tr>
<th>Sample</th>
<th>Day 1</th>
<th>Day 2</th>
<th>Day 4</th>
<th>Day 7</th>
<th>Day 14</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample 1</td>
<td>1.5</td>
<td>1.8</td>
<td>1.4</td>
<td>2.4</td>
<td>4.0</td>
</tr>
<tr>
<td>Sample 2</td>
<td>1.9</td>
<td>3.1</td>
<td>4.0</td>
<td>1.8</td>
<td>12</td>
</tr>
<tr>
<td>Sample 3</td>
<td>3.0</td>
<td>1.3</td>
<td>2.0</td>
<td>1.6</td>
<td>1.3</td>
</tr>
</tbody>
</table>

Table C24: Concentration of Total P on pervious treated plots on different time intervals (days) after application of sewage during S09

<table>
<thead>
<tr>
<th>Sample</th>
<th>Day 1</th>
<th>Day 2</th>
<th>Day 4</th>
<th>Day 7</th>
<th>Day 15</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample 1</td>
<td>5.3</td>
<td>2.2</td>
<td>2.7</td>
<td>2.8</td>
<td>2.1</td>
</tr>
<tr>
<td>Sample 2</td>
<td>5.2</td>
<td>4.2</td>
<td>3.8</td>
<td>6.1</td>
<td>1.8</td>
</tr>
<tr>
<td>Sample 3</td>
<td>7.4</td>
<td>5.3</td>
<td>6.4</td>
<td>19</td>
<td>9.6</td>
</tr>
</tbody>
</table>

Table C25: Concentration of *E.coli* on impervious treated plots on different time intervals (days) after application of sewage during S08

<table>
<thead>
<tr>
<th>Sample</th>
<th>2nd Hour</th>
<th>Day 1</th>
<th>Day 2</th>
<th>Day 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample 1</td>
<td>&gt;2400</td>
<td>0</td>
<td>&lt;10</td>
<td>&lt;10</td>
</tr>
<tr>
<td>Sample 2</td>
<td>&gt;2400</td>
<td>2</td>
<td>&lt;10</td>
<td>&lt;10</td>
</tr>
<tr>
<td>Sample 3</td>
<td>260</td>
<td>0</td>
<td>&lt;10</td>
<td>&lt;10</td>
</tr>
</tbody>
</table>

Table C26: Concentration of *E.coli* on impervious treated plots on different time intervals (days) after application of sewage during W08

<table>
<thead>
<tr>
<th>Sample</th>
<th>Day 1</th>
<th>Day 2</th>
<th>Day 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample 1</td>
<td>98</td>
<td>1600</td>
<td>1100</td>
</tr>
<tr>
<td>Sample 2</td>
<td>1600</td>
<td>3000</td>
<td>440</td>
</tr>
<tr>
<td>Sample 3</td>
<td>1400</td>
<td>1400</td>
<td>730</td>
</tr>
</tbody>
</table>
Table C27: Concentration of Enterococci on impervious treated plots on different time intervals (days) after application of sewage during S08

<table>
<thead>
<tr>
<th></th>
<th>2nd Hour</th>
<th>Day 1</th>
<th>Day 2</th>
<th>Day 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample 1</td>
<td>920</td>
<td>6</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Sample 2</td>
<td>&gt;2400</td>
<td>17</td>
<td>10</td>
<td>&lt;10</td>
</tr>
<tr>
<td>Sample 3</td>
<td>40</td>
<td>6</td>
<td>&lt;10</td>
<td>&lt;10</td>
</tr>
</tbody>
</table>

Table C28: Concentration of Enterococci on impervious treated plots on different time intervals (days) after application of sewage during W08

<table>
<thead>
<tr>
<th></th>
<th>Day 1</th>
<th>Day 2</th>
<th>Day 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample 1</td>
<td>200</td>
<td>100</td>
<td>410</td>
</tr>
<tr>
<td>Sample 2</td>
<td>100</td>
<td>100</td>
<td>310</td>
</tr>
<tr>
<td>Sample 3</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

Table C29: Concentration of F-RNA coliphages on impervious treated plots on different time intervals (days) after application of sewage during S08

<table>
<thead>
<tr>
<th></th>
<th>2nd Hour</th>
<th>Day 1</th>
<th>Day 2</th>
<th>Day 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample 1</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Sample 2</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Sample 3</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>&lt;1</td>
</tr>
</tbody>
</table>
Table C30: Concentration of F-RNA coliphages on impervious treated plots on different time intervals (days) after application of sewage during W08

<table>
<thead>
<tr>
<th></th>
<th>Day 1</th>
<th>Day 2</th>
<th>Day 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample 1</td>
<td>0</td>
<td>41</td>
<td>0</td>
</tr>
<tr>
<td>Sample 2</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Sample 3</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Table C31: Concentration of Total N on impervious treated plots on different time intervals (days) after application of sewage during S08

<table>
<thead>
<tr>
<th></th>
<th>2nd Hour</th>
<th>Day 1</th>
<th>Day 2</th>
<th>Day 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample 1</td>
<td>1.7</td>
<td>1.0</td>
<td>1.1</td>
<td>1.3</td>
</tr>
<tr>
<td>Sample 2</td>
<td>2.8</td>
<td>1.6</td>
<td>1.2</td>
<td>1.3</td>
</tr>
<tr>
<td>Sample 3</td>
<td>1.8</td>
<td>2.2</td>
<td>1.1</td>
<td>2.0</td>
</tr>
</tbody>
</table>

Table C32: Concentration of Total N on impervious treated plots on different time intervals (days) after application of sewage during W08

<table>
<thead>
<tr>
<th></th>
<th>Day 1</th>
<th>Day 2</th>
<th>Day 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample 1</td>
<td>5.8</td>
<td>9.8</td>
<td>14</td>
</tr>
<tr>
<td>Sample 2</td>
<td>7.4</td>
<td>12</td>
<td>9.4</td>
</tr>
<tr>
<td>Sample 3</td>
<td>9.6</td>
<td>12</td>
<td>15</td>
</tr>
</tbody>
</table>
Table C33: Concentration of Total P on impervious treated plots on different time intervals (days) after application of sewage during S08

<table>
<thead>
<tr>
<th>Sample</th>
<th>2nd Hour</th>
<th>Day 1</th>
<th>Day 2</th>
<th>Day 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample 1</td>
<td>0.34</td>
<td>0.12</td>
<td>0.12</td>
<td>0.12</td>
</tr>
<tr>
<td>Sample 2</td>
<td>0.52</td>
<td>0.17</td>
<td>0.16</td>
<td>0.12</td>
</tr>
<tr>
<td>Sample 3</td>
<td>0.34</td>
<td>0.18</td>
<td>0.13</td>
<td>0.22</td>
</tr>
</tbody>
</table>

Table C34: Concentration of Total P on impervious treated plots on different time intervals (days) after application of sewage during W08

<table>
<thead>
<tr>
<th>Sample</th>
<th>Day 1</th>
<th>Day 2</th>
<th>Day 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample 1</td>
<td>0.66</td>
<td>0.87</td>
<td>1.2</td>
</tr>
<tr>
<td>Sample 2</td>
<td>1.0</td>
<td>1.0</td>
<td>0.93</td>
</tr>
<tr>
<td>Sample 3</td>
<td>1.1</td>
<td>0.69</td>
<td>1.4</td>
</tr>
</tbody>
</table>
Appendix D: Relationships between Microbes and Nutrients during Summer Experiment

Figure D1: Relationship between *E.coli* and Total Nitrogen during S09 experiment

Figure D2: Relationship between enterococci and Total Nitrogen during S09 experiment
Figure D3: Relationship between *E.coli* and Total Phosphorus during S09 experiment

Figure D4: Relationship between enterococci and Total Phosphorus during S09 experiment
### Table E1: Wash-off of microbes (%) calculated with different storm durations for 1 in 2 years ARI

<table>
<thead>
<tr>
<th>Duration (minutes)</th>
<th>Rainfall intensity (mm/hr)</th>
<th>Raw sewage values (orgs/100mL)</th>
<th>RI (mm/min)</th>
<th>RI^0.293</th>
<th>Cs(t) orgs/100mL</th>
<th>Ps(t) orgs/100mL</th>
<th>% loss</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>63</td>
<td>1E+07</td>
<td>1.05</td>
<td>1.01</td>
<td>10143982</td>
<td>-143982</td>
<td>100%</td>
</tr>
<tr>
<td>10</td>
<td>47.6</td>
<td>1E+07</td>
<td>0.79</td>
<td>0.93</td>
<td>9344165</td>
<td>655835</td>
<td>93%</td>
</tr>
<tr>
<td>15</td>
<td>39.5</td>
<td>1E+07</td>
<td>0.66</td>
<td>0.88</td>
<td>8847175</td>
<td>1152825</td>
<td>88%</td>
</tr>
<tr>
<td>20</td>
<td>34.2</td>
<td>1E+07</td>
<td>0.57</td>
<td>0.85</td>
<td>8481474</td>
<td>1518526</td>
<td>85%</td>
</tr>
<tr>
<td>30</td>
<td>27.5</td>
<td>1E+07</td>
<td>0.46</td>
<td>0.80</td>
<td>7956575</td>
<td>2043425</td>
<td>80%</td>
</tr>
<tr>
<td>40</td>
<td>23.4</td>
<td>1E+07</td>
<td>0.39</td>
<td>0.76</td>
<td>7588954</td>
<td>2411046</td>
<td>76%</td>
</tr>
<tr>
<td>50</td>
<td>20.6</td>
<td>1E+07</td>
<td>0.34</td>
<td>0.73</td>
<td>7310798</td>
<td>2689202</td>
<td>73%</td>
</tr>
<tr>
<td>60</td>
<td>18.4</td>
<td>1E+07</td>
<td>0.31</td>
<td>0.71</td>
<td>7072831</td>
<td>2927169</td>
<td>71%</td>
</tr>
<tr>
<td>90</td>
<td>14.3</td>
<td>1E+07</td>
<td>0.24</td>
<td>0.66</td>
<td>6569240</td>
<td>3430760</td>
<td>66%</td>
</tr>
<tr>
<td>120</td>
<td>11.9</td>
<td>1E+07</td>
<td>0.20</td>
<td>0.62</td>
<td>6224965</td>
<td>3775035</td>
<td>62%</td>
</tr>
<tr>
<td>180</td>
<td>9.17</td>
<td>1E+07</td>
<td>0.15</td>
<td>0.58</td>
<td>5767345</td>
<td>4232655</td>
<td>58%</td>
</tr>
<tr>
<td>240</td>
<td>7.61</td>
<td>1E+07</td>
<td>0.13</td>
<td>0.55</td>
<td>5460688</td>
<td>4539312</td>
<td>55%</td>
</tr>
<tr>
<td>300</td>
<td>6.58</td>
<td>1E+07</td>
<td>0.11</td>
<td>0.52</td>
<td>5232893</td>
<td>4767107</td>
<td>52%</td>
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</tbody>
</table>

RI – Rainfall Intensity in mm/min
Table E2: Wash-off of microbes (%) calculated with different storm durations for 1 in 5 years ARI

<table>
<thead>
<tr>
<th>Duration (minutes)</th>
<th>Rainfall intensity (mm/hr)</th>
<th>Raw sewage values (orgs/100mL)</th>
<th>RI (mm/min)</th>
<th>RI^0.293</th>
<th>Cs(t) orgs/100mL</th>
<th>Ps(t) orgs/100mL</th>
<th>% loss</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>87</td>
<td>1E+07</td>
<td>1.45</td>
<td>1.12</td>
<td>11150153</td>
<td>-1150153</td>
<td>100%</td>
</tr>
<tr>
<td>10</td>
<td>65</td>
<td>1E+07</td>
<td>1.08</td>
<td>1.02</td>
<td>10237297</td>
<td>-237297</td>
<td>100%</td>
</tr>
<tr>
<td>15</td>
<td>54</td>
<td>1E+07</td>
<td>0.90</td>
<td>0.97</td>
<td>9696010</td>
<td>303990</td>
<td>97%</td>
</tr>
<tr>
<td>20</td>
<td>46.4</td>
<td>1E+07</td>
<td>0.77</td>
<td>0.93</td>
<td>9274520</td>
<td>725480</td>
<td>93%</td>
</tr>
<tr>
<td>30</td>
<td>37.1</td>
<td>1E+07</td>
<td>0.62</td>
<td>0.87</td>
<td>8686168</td>
<td>1313832</td>
<td>87%</td>
</tr>
<tr>
<td>40</td>
<td>31.4</td>
<td>1E+07</td>
<td>0.52</td>
<td>0.83</td>
<td>8271839</td>
<td>1728161</td>
<td>83%</td>
</tr>
<tr>
<td>50</td>
<td>27.4</td>
<td>1E+07</td>
<td>0.46</td>
<td>0.79</td>
<td>7948087</td>
<td>2051913</td>
<td>79%</td>
</tr>
<tr>
<td>60</td>
<td>24.5</td>
<td>1E+07</td>
<td>0.41</td>
<td>0.77</td>
<td>7691789</td>
<td>2308211</td>
<td>77%</td>
</tr>
<tr>
<td>90</td>
<td>18.9</td>
<td>1E+07</td>
<td>0.32</td>
<td>0.71</td>
<td>7128612</td>
<td>2871388</td>
<td>71%</td>
</tr>
<tr>
<td>120</td>
<td>15.7</td>
<td>1E+07</td>
<td>0.26</td>
<td>0.68</td>
<td>6751500</td>
<td>3248500</td>
<td>68%</td>
</tr>
<tr>
<td>180</td>
<td>12</td>
<td>1E+07</td>
<td>0.20</td>
<td>0.62</td>
<td>6240247</td>
<td>3759753</td>
<td>62%</td>
</tr>
<tr>
<td>240</td>
<td>9.89</td>
<td>1E+07</td>
<td>0.16</td>
<td>0.59</td>
<td>5896499</td>
<td>4103501</td>
<td>59%</td>
</tr>
<tr>
<td>300</td>
<td>8.52</td>
<td>1E+07</td>
<td>0.14</td>
<td>0.56</td>
<td>5644435</td>
<td>4355565</td>
<td>56%</td>
</tr>
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</table>

RI – Rainfall Intensity in mm/min
Table E3: Wash-off of microbes (%) calculated with different storm durations for 1 in 10 years ARI

<table>
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<th>Duration (minutes)</th>
<th>Rainfall intensity (mm/hr)</th>
<th>Raw sewage values (orgs/100mL)</th>
<th>RI (mm/min)</th>
<th>RI^0.293</th>
<th>Cs(t) orgs/100mL</th>
<th>Ps(t) orgs/100mL</th>
<th>% loss</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>103</td>
<td>1E+07</td>
<td>1.72</td>
<td>1.17</td>
<td>11715558</td>
<td>-1715558</td>
<td>100%</td>
</tr>
<tr>
<td>10</td>
<td>77</td>
<td>1E+07</td>
<td>1.28</td>
<td>1.08</td>
<td>10758295</td>
<td>-758295</td>
<td>100%</td>
</tr>
<tr>
<td>15</td>
<td>63</td>
<td>1E+07</td>
<td>1.05</td>
<td>1.01</td>
<td>10143982</td>
<td>-143982</td>
<td>100%</td>
</tr>
<tr>
<td>20</td>
<td>55</td>
<td>1E+07</td>
<td>0.92</td>
<td>0.97</td>
<td>9748279</td>
<td>251721</td>
<td>97%</td>
</tr>
<tr>
<td>30</td>
<td>43.5</td>
<td>1E+07</td>
<td>0.73</td>
<td>0.91</td>
<td>9100789</td>
<td>899211</td>
<td>91%</td>
</tr>
<tr>
<td>40</td>
<td>36.7</td>
<td>1E+07</td>
<td>0.61</td>
<td>0.87</td>
<td>8658623</td>
<td>1341377</td>
<td>87%</td>
</tr>
<tr>
<td>50</td>
<td>32</td>
<td>1E+07</td>
<td>0.53</td>
<td>0.83</td>
<td>8317841</td>
<td>1682159</td>
<td>83%</td>
</tr>
<tr>
<td>60</td>
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<td>1E+07</td>
<td>0.48</td>
<td>0.80</td>
<td>8048537</td>
<td>1951463</td>
<td>80%</td>
</tr>
<tr>
<td>90</td>
<td>22</td>
<td>1E+07</td>
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<td>0.75</td>
<td>7453007</td>
<td>2546993</td>
<td>75%</td>
</tr>
<tr>
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<td>1E+07</td>
<td>0.30</td>
<td>0.70</td>
<td>7038847</td>
<td>2961153</td>
<td>70%</td>
</tr>
<tr>
<td>180</td>
<td>13.8</td>
<td>1E+07</td>
<td>0.23</td>
<td>0.65</td>
<td>6501091</td>
<td>3498909</td>
<td>65%</td>
</tr>
<tr>
<td>240</td>
<td>11.4</td>
<td>1E+07</td>
<td>0.19</td>
<td>0.61</td>
<td>6147164</td>
<td>3852836</td>
<td>61%</td>
</tr>
<tr>
<td>300</td>
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<td>0.16</td>
<td>0.59</td>
<td>5893002</td>
<td>4106998</td>
<td>59%</td>
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</table>

RI – Rainfall Intensity in mm/min
Table E4: Wash-off of microbes (%) calculated with different storm durations for 1 in 20 years ARI

<table>
<thead>
<tr>
<th>Duration (minutes)</th>
<th>Rainfall intensity (mm/hr)</th>
<th>Raw sewage values (orgs/100mL)</th>
<th>RI (mm/min)</th>
<th>RI^0.293</th>
<th>Cs(t) orgs/100mL</th>
<th>Ps(t) orgs/100mL</th>
<th>% loss</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
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<td>1E+07</td>
<td>2.08</td>
<td>1.24</td>
<td>12399276</td>
<td>-2399276</td>
<td>100%</td>
</tr>
<tr>
<td>10</td>
<td>93</td>
<td>1E+07</td>
<td>1.55</td>
<td>1.14</td>
<td>11370176</td>
<td>-1370176</td>
<td>100%</td>
</tr>
<tr>
<td>15</td>
<td>76</td>
<td>1E+07</td>
<td>1.27</td>
<td>1.07</td>
<td>10717169</td>
<td>-7117169</td>
<td>100%</td>
</tr>
<tr>
<td>20</td>
<td>66</td>
<td>1E+07</td>
<td>1.10</td>
<td>1.03</td>
<td>10283195</td>
<td>-283195</td>
<td>100%</td>
</tr>
<tr>
<td>30</td>
<td>52</td>
<td>1E+07</td>
<td>0.87</td>
<td>0.96</td>
<td>9589383</td>
<td>410617</td>
<td>96%</td>
</tr>
<tr>
<td>40</td>
<td>43.9</td>
<td>1E+07</td>
<td>0.73</td>
<td>0.91</td>
<td>9125229</td>
<td>874771</td>
<td>91%</td>
</tr>
<tr>
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<td>1E+07</td>
<td>0.64</td>
<td>0.88</td>
<td>8760850</td>
<td>1239150</td>
<td>88%</td>
</tr>
<tr>
<td>60</td>
<td>34</td>
<td>1E+07</td>
<td>0.57</td>
<td>0.85</td>
<td>8466911</td>
<td>1533089</td>
<td>85%</td>
</tr>
<tr>
<td>90</td>
<td>26.1</td>
<td>1E+07</td>
<td>0.44</td>
<td>0.78</td>
<td>7835692</td>
<td>2164308</td>
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</tr>
<tr>
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<td>7402973</td>
<td>2597027</td>
<td>74%</td>
</tr>
<tr>
<td>180</td>
<td>16.3</td>
<td>1E+07</td>
<td>0.27</td>
<td>0.68</td>
<td>6826100</td>
<td>3173900</td>
<td>68%</td>
</tr>
<tr>
<td>240</td>
<td>13.4</td>
<td>1E+07</td>
<td>0.22</td>
<td>0.64</td>
<td>6445304</td>
<td>3554696</td>
<td>64%</td>
</tr>
<tr>
<td>300</td>
<td>11.5</td>
<td>1E+07</td>
<td>0.19</td>
<td>0.62</td>
<td>6162915</td>
<td>3837085</td>
<td>62%</td>
</tr>
</tbody>
</table>

RI – Rainfall Intensity in mm/min
Table E5: Wash-off of microbes (%) calculated with different storm durations for 1 in 50 years ARI

<table>
<thead>
<tr>
<th>Duration (minutes)</th>
<th>Rainfall intensity (mm/hr)</th>
<th>Raw sewage values (orgs/100mL)</th>
<th>RI (mm/min)</th>
<th>RI^0.293</th>
<th>Cs(t) orgs/100mL</th>
<th>Ps(t) orgs/100mL</th>
<th>% loss</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>157</td>
<td>1E+07</td>
<td>2.62</td>
<td>1.33</td>
<td>13255627</td>
<td>-3255627</td>
<td>100%</td>
</tr>
<tr>
<td>10</td>
<td>116</td>
<td>1E+07</td>
<td>1.93</td>
<td>1.21</td>
<td>12130756</td>
<td>-2130756</td>
<td>100%</td>
</tr>
<tr>
<td>15</td>
<td>95</td>
<td>1E+07</td>
<td>1.27</td>
<td>1.07</td>
<td>10717169</td>
<td>-717169</td>
<td>100%</td>
</tr>
<tr>
<td>20</td>
<td>81</td>
<td>1E+07</td>
<td>1.35</td>
<td>1.09</td>
<td>10919124</td>
<td>-919124</td>
<td>100%</td>
</tr>
<tr>
<td>30</td>
<td>64</td>
<td>1E+07</td>
<td>1.07</td>
<td>1.02</td>
<td>10190897</td>
<td>-190897</td>
<td>100%</td>
</tr>
<tr>
<td>40</td>
<td>54</td>
<td>1E+07</td>
<td>0.90</td>
<td>0.97</td>
<td>9696010</td>
<td>303990</td>
<td>97%</td>
</tr>
<tr>
<td>50</td>
<td>47</td>
<td>1E+07</td>
<td>0.78</td>
<td>0.93</td>
<td>9309500</td>
<td>690500</td>
<td>93%</td>
</tr>
<tr>
<td>60</td>
<td>41.8</td>
<td>1E+07</td>
<td>0.70</td>
<td>0.90</td>
<td>8995107</td>
<td>1004893</td>
<td>90%</td>
</tr>
<tr>
<td>90</td>
<td>31.9</td>
<td>1E+07</td>
<td>0.53</td>
<td>0.83</td>
<td>8310217</td>
<td>169783</td>
<td>83%</td>
</tr>
<tr>
<td>120</td>
<td>26.2</td>
<td>1E+07</td>
<td>0.44</td>
<td>0.78</td>
<td>7844476</td>
<td>2155524</td>
<td>78%</td>
</tr>
<tr>
<td>180</td>
<td>19.8</td>
<td>1E+07</td>
<td>0.33</td>
<td>0.72</td>
<td>7226443</td>
<td>2773557</td>
<td>72%</td>
</tr>
<tr>
<td>240</td>
<td>16.2</td>
<td>1E+07</td>
<td>0.27</td>
<td>0.68</td>
<td>6813803</td>
<td>3186197</td>
<td>68%</td>
</tr>
<tr>
<td>300</td>
<td>13.9</td>
<td>1E+07</td>
<td>0.23</td>
<td>0.65</td>
<td>6514859</td>
<td>3485141</td>
<td>65%</td>
</tr>
</tbody>
</table>

RI – Rainfall Intensity in mm/min
<table>
<thead>
<tr>
<th>Duration (minutes)</th>
<th>Rainfall intensity (mm/hr)</th>
<th>Raw sewage values (orgs/100mL)</th>
<th>Cs(t) orgs/L (concentration to stormwater outlet)</th>
<th>Ps(t) (Surface store) orgs/L</th>
<th>% loss</th>
</tr>
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<tbody>
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<td>3.05</td>
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</tr>
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</tr>
</tbody>
</table>

**Table E6: Wash-off of microbes (%) calculated with different storm durations for 1 in 100 years ARI**

RI – Rainfall Intensity in mm/min
Appendix F: Relationships between Rainfall Durations and Washoff of Microbes towards Stormwater Drain

Figure F1: Relationship between rainfall duration and concentrations of microbes transported towards stormwater drain for different ARI values for Yarra catchment on 2\textsuperscript{nd} day after a spill event.

Figure F2: Relationship between rainfall duration and concentrations of microbes transported towards stormwater drain for different ARI values for Yarra catchment on 3\textsuperscript{rd} day after a spill event.
Figure F3: Relationship between rainfall duration and concentrations of microbes transported towards stormwater drain for different ARI values for Yarra catchment on 4th day after a spill event.

Figure F4: Relationship between rainfall duration and concentrations of microbes transported towards stormwater drain for different ARI values for Yarra catchment on 5th day after a spill event.
Figure F5: Relationship between rainfall duration and concentrations of microbes transported towards stormwater drain for different ARI values for Yarra catchment on 6\textsuperscript{th} day after a spill event.

Figure F6: Relationship between rainfall duration and concentrations of microbes transported towards stormwater drain for different ARI values for Yarra catchment on 7\textsuperscript{th} day after a spill event.
Figure F7: Relationship between rainfall duration and concentrations of microbes transported towards stormwater drain for different ARI values for Yarra catchment on 8\textsuperscript{th} day after a spill event.

Figure F8: Relationship between rainfall duration and concentrations of microbes transported towards stormwater drain for different ARI values for Yarra catchment on 9\textsuperscript{th} day after a spill event.
Figure F9: Relationship between rainfall duration and concentrations of microbes transported towards stormwater drain for different ARI values for Yarra catchment on 10\textsuperscript{th} day after a spill event.

Figure F10: Relationship between rainfall duration and concentrations of microbes transported towards stormwater drain for different ARI values for Yarra catchment on 11\textsuperscript{th} day after a spill event.
Figure F11: Relationship between rainfall duration and concentrations of microbes transported towards stormwater drain for different ARI values for Yarra catchment on 12th day after a spill event.

Figure F12: Relationship between rainfall duration and concentrations of microbes transported towards stormwater drain for different ARI values for Yarra catchment on 13th day after a spill event.
Figure F13: Relationship between rainfall duration and concentrations of microbes transported towards stormwater drain for different ARI values for Yarra catchment on 14th day after a spill event.

Figure F14: Relationship between rainfall duration and concentrations of microbes transported towards stormwater drain for different ARI values for Yarra catchment on 15th day after a spill event.